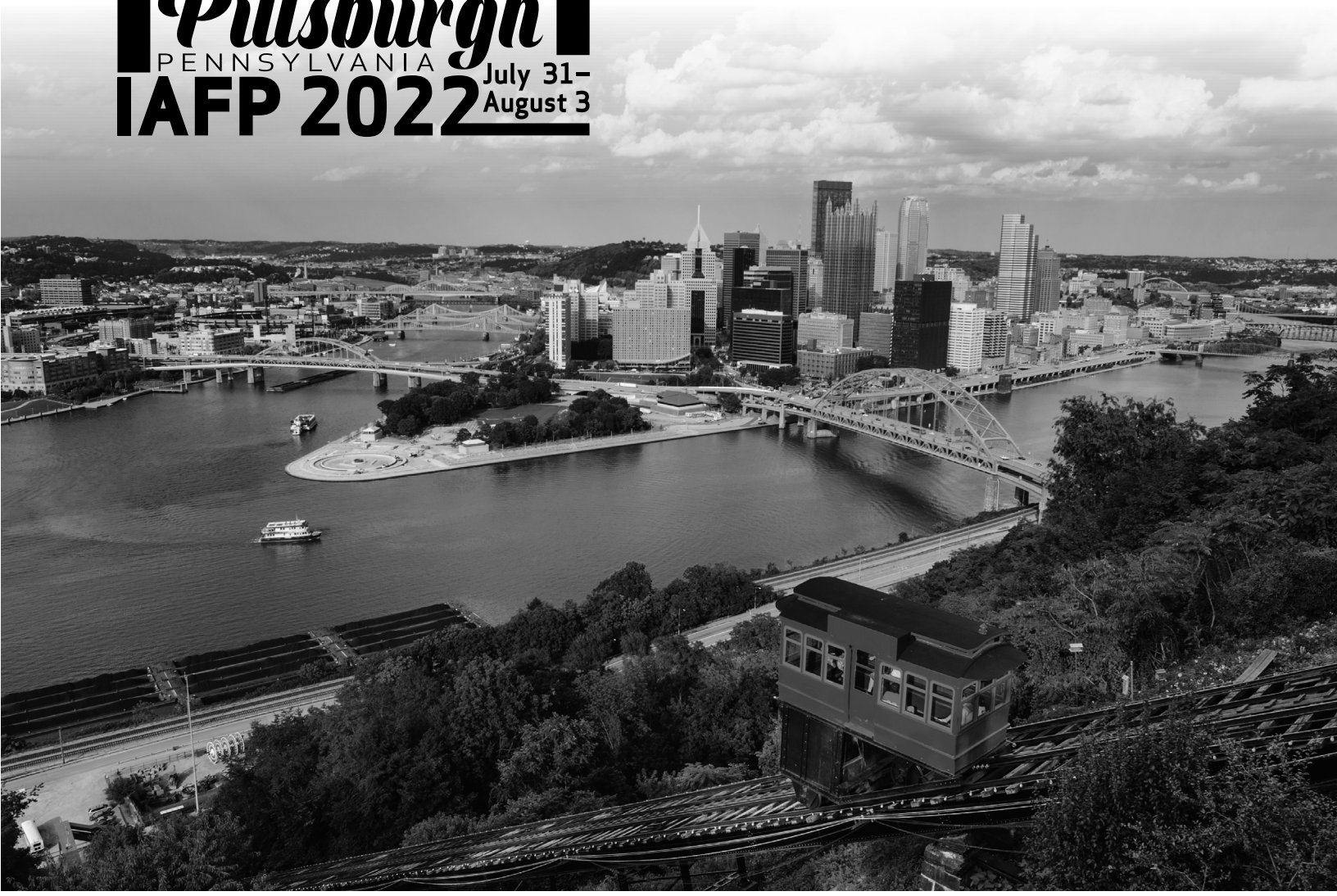
Pittsburgh
PENNSYLVANIA
IAFP 2022
July 31–
August 3



Abstracts

A collection of abstracts
from the IAFP 2022 Annual Meeting.

International Association for Food Protection®

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Table of Contents

IVAN PARKIN LECTURE

SUNDAY, JULY 31
OPENING SESSION
6:00 P.M. – 7:30 P.M.

OUT OF AFRICA

LUCIA ANELICH, PH.D.

Anelich Consulting
Pretoria, South Africa

SUNDAY, JULY 31
OPENING SESSION
6:00 P.M. – 7:30 P.M.

Dr. Lucia Anelich established Anelich Consulting, a successful national and international food safety consulting and training business in South Africa, in 2011. Dr. Anelich was previously with the Consumer Goods Council of South Africa, beginning in 2005, where she established a food safety body for the food industry, a first for the country. She spent the prior 25 years at the Tshwane University of Technology in South Africa, later becoming Head of the Department of Biotechnology and Food Technology and Associate Professor, the first female head of such a department in South Africa.

Dr. Anelich was instrumental in providing guidance to the food industry on *Listeria monocytogenes* during the 2018 listeriosis outbreak and on SARS-CoV-2 related to food safety during the COVID-19 pandemic, beginning in January 2020. On both these and other food safety-related topics, she is interviewed regularly by print media, radio, and TV. She spoke at several national and international events on the listeriosis outbreak and on SARS-CoV-2 and the safety of food and food packaging.

In July 2020, through the President of South Africa's office, Dr. Anelich was appointed to be part of a group of 80 authors to develop a Country Report on the Impact of Government's Decisions on the COVID-19 Pandemic. As part of this group, she convened and co-authored the chapter on "Agriculture and the Food Supply Chain."

In September 2021, Dr. Anelich received the prestigious SAAFoST President's Award as its first female recipient for her significant contributions toward advancing Food Safety Technology for the provision of safe and wholesome food.

Dr. Anelich's career achievements include serving as Adjunct Professor at Central University of Technology in South Africa; membership in ICMSF since 2005; Past President of the South African Association for Food Science and Technology (SAAFoST); member of the African Union Advisory Group developing a Food Safety Strategy for the African continent and the newly-developed AU Food Safety Authority; first South African Chair of the Scientific Council of IUFoST; Fellow of the International Academy of Food Science and Technology; technical expert and consultant for FAO and UNIDO; and co-editor of two books on food safety. Dr. Anelich serves on several committees related to regulations and standards development in the food safety space. She has authored/co-authored numerous publications and book chapters and presented more than 180 talks nationally and internationally.

An IAFP Member since 2012, Dr. Anelich served on the organizing committee of the African Continental Association for Food Protection's inaugural ACAFP Food Safety Conference for Africa, held virtually 10–11 November 2021.

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JOHN H. SILLIKER LECTURE

WEDNESDAY, AUGUST 3
CLOSING SESSION
4:00 P.M. – 4:45 P.M.

THE POWER OF DIVERSE PERSPECTIVES FOR EFFECTIVE FOOD SAFETY MANAGEMENT

KATHERINE M.J. SWANSON, PH.D.

Retired, KMJ Swanson Food Safety Inc.
Mendota Heights, Minnesota, USA

WEDNESDAY, AUGUST 3
CLOSING SESSION
4:00 P.M. – 4:45 P.M.

Dr. Katherine "Katie" Swanson is retired from a 40-year food safety career that began with investigating "new" technology (microwave inactivation of *Salmonella* in eggs) while earning her bachelor's degree with distinction at the University of Delaware. Her studies continued at the University of Minnesota researching the fate of aflatoxin during single cell protein production while attaining her master's degree.

Dr. Swanson gained industry experience at Economics Laboratory (Ecolab) as a Microbiology Consultant for food and medical device manufacturers before returning to Dr. Frank Busta's lab at the University of Minnesota to conduct research on *Bacillus cereus* growth and inactivation models during completion of her Ph.D.

For a brief time, Dr. Swanson was an assistant professor of food microbiology at Cornell University and Senior Microbiologist for 3M ATP and Petrifilm™ applications before honing her prevention-focused food safety expertise at The Pillsbury Company. While there, she worked with food safety pioneers Howard Bauman, Bill Sperber, and an incredible team, progressing to the position of Global Product Safety Director at General Mills.

Dr. Swanson returned to Ecolab in 2004 as Vice President of Food Safety. In 2013, she joined the Food Safety Preventive Control Alliance as Program Manager for Curriculum Development and Executive Editor for the U.S. Food and Drug Administration's recognized standardized curriculum for meeting the Food Safety Preventive Controls for Human Foods regulation requirements.

Dr. Swanson served as IAFP President 2012–2013, after joining IAFP in 1979 as a Student Member and in 1980 as a Member. She served on the *Journal of Food Protection* Editorial Board for 12 years; on the *Food Protection Trends* Editorial Board for three years; and on numerous IAFP Award Selection Committees and organizing committees for meetings outside the U.S. She received the GMA Food Safety Award in 2003; the IAFP Fellow Award in 2015; and the Honorary Life Membership Award in 2017.

Dr. Swanson has written and edited numerous chapters, reports, and papers, and served on influential committees, including the International Commission on Microbiological Specifications for Foods (ICMSF) (as Past Secretary); the National Advisory Committee on Microbiological Criteria for Foods (NACMCF); and the National Academies of Science Committees (NAS).

In retirement, Dr. Swanson's interests include genealogy research, travel, and needlework ignored for many years.

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Symposium Abstracts

S1 *Salmonella* in Poultry: Issues and Solutions

JANELL KAUSE: *U.S. Department of Agriculture, Food Safety and Inspection Service, Manassas, VA, USA*
SHANICE KROMBEEN: *Pilgrim's Pride Corp, Westminster, SC, USA*
ALBERTO TORRES-RODRIGUES: *Cobb-Vantress, Siloam Springs, AR, USA*
XIANGYU DENG: *University of Georgia, Center for Food Safety, Griffin, GA, USA*
ELENA BEHNKE: *National Poultry Improvement Plan (NPIP) - USDA APHIS, Conyers, GA, USA*

Contamination of poultry with *Salmonella* continues to be one of the most pressing issues in food safety. *Salmonella* serovars Enteritidis and Typhimurium are responsible for multiple cases linked to poultry and in recent years *S*. Infantis is now considered a re-occurring, emerging and persistent serovar. Multiple efforts are currently being taken by industry, government, and academia to better understand *Salmonella* in the pre- and post-harvest environment, including the amount on poultry and more pathogenic subtypes, to develop effective interventions. The opening talk of this session talk will discuss federal efforts to better assess how the control of *Salmonella*, including levels and more pathogenic subtypes, in chicken and turkey may improve public health. The second presentation will summarize novel diagnostic approaches that can provide more robust testing data for *Salmonella* in poultry useful for risk assessment and enhancing monitoring control strategies. The last talk, presenters will shed light on the origins of the *Salmonella* Enteritidis pandemic in chicken populations and the recently formulated hypothesis tracking it upstream in the breeder supply chain.

S2 Recent Developments in Applications of Predictive Tools for Meat and Poultry Products

MARIEM ELLOUZE: *Nestlé Research Center, Lausanne, Switzerland, Switzerland*
PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece, Greece*
LIHAN HUANG: *USDA Agricultural Research Service, Wyndmoor, PA, USA*

Meat and poultry products are generally safe for consumption. The safety is largely attributed to the good manufacturing practices, processing guidelines, and performance standards established by the regulatory agencies and the diligent following of these guidelines and regulations by the manufacturers. While the processing, storage, and handling protocols for meat products are designed to eliminate, reduce, or prevent the growth of foodborne pathogens, there have been occurrences of product recalls and outbreaks of foodborne illnesses due to pathogen contamination. The majority of the recalls and outbreak of illnesses have been attributed to improper cooking, cooling, and handling of the products. Therefore, research has continually been conducted to explore additional control measurements for meat and poultry products to reduce the risks. One of the most frequent studied subjects is the development of predictive and risk-assessment tools that estimate the survival or growth potential of pathogens in products during processing, storage, and distribution. Many microbial and risk assessment models, databases, and software have been developed and are available for estimating the survival, growth probability, and potential risk of pathogens in meat products as affected by the product formulation and its physiochemical properties and cooking, cooling, and storage conditions. These tools have been proven to be useful, e.g., cooling models estimating the growth of Clostridium perfringens in cooked meats have been frequently used by the producers to determine the adequacy of a cooling process and the follow-up actions when a batch of product's cooling is deviated from the performance standards. The theme of this symposium is to present an update on the applications of microbiological and risk models and new methodologies for developing models for meat and poultry products that can be easily accessed by industrial and academic personnel. The purposes are (i) to demonstrate the applications of predictive models and risk assessment tools to enhance the safety of meat and poultry products and (ii) outline the recent developments in predictive modeling and (iii) provide guidance on formulating the proper safety assessment question(s) case-wise, that enable efficient use of predictive modelling tools.

S3 Challenges and Strategies in Implementing Food Safety Management Systems in Multinational Companies

BALA KOTTAPALLI: *Walmart, Omaha, NE, USA*
LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
VICKIE LEWANDOWSKI: *Barry Callebaut, Amery, WI, USA*

There are many challenges to developing and implementing food safety management systems uniformly across different markets in multinational companies. While the primary focus of the company is a business growth strategy, there is an expectation of consistency in product and service from that company, requiring a uniform food safety management system. Therefore, implementing science-based standards is essential to protect both consumer and the company's reputation from a food safety standpoint. Factors involving a complex mix of organizational and technical challenges act as barriers to the implementation of the food safety standards. The challenges vary from one country to another and across different business functions. Furthermore, local regulations, cultural nuances, differences in maturity of the educational systems, economic pressures and other socio-political factors create hurdles in the implementation of the food safety management systems. In this symposium, experts from industry will discuss the different challenges and opportunities associated with implementation of food safety management systems as well present case studies on strategic approaches and solutions to effectively design, communicate and implement science-based food safety standards to ensure safe food from a public health standpoint.

S4 Implementation of HACCP-Based Egg Product Inspection

KERRI GEHRING: *Texas A&M University, College Station, TX, USA*
KIMBERLY RICE: *Rose Acre Farms, Seymour, IN, USA*
OSCAR GARRISON: *United Egg Producers, Johns Creek, GA, USA*

On October 29, 2020, USDA's Food Safety and Inspection Service (FSIS) published the final rule on Egg Products Inspection Regulations (85 FR 68640). This regulatory change eliminated the prescriptive command-and-control requirements to align egg product regulations with the meat and poultry product regulations. The rule requires plants that produce egg products to develop and implement Hazard Analysis and Critical Control Point (HACCP) Systems and Sanitation Standard Operating Procedures (Sanitation SOPs). The implementation is occurring in two phases:

Sanitation SOPs / Sanitation Performance Standards (SPS) as per 9 CFR part 416 was implemented on October 29, 2021; and

Hazard Analysis and Critical Control Point (HACCP) Systems in 9 CFR part 417 will be implemented on October 31, 2022.

Under the old regulations, egg products plants had to comply with command-and-control regulations that prescribed the exact means by which egg product plants must pasteurize egg products and maintain sanitary conditions, hindering innovation. Under the new rule, plants are required to develop HACCP systems and Sanitation SOPs that ensure that pathogens cannot be detected in finished egg products. The HACCP-based system will improve the effectiveness of egg products production and inspection while allowing plants to be more efficient over the long-term and allowing them to innovate facility design, construction, and operations.

The purpose of this symposium is to provide information on how egg products plants can meet the new HACCP system requirements. We will discuss the new regulations, validation of HACCP systems, resources available to plants, and practical recommendations from the perspectives of the HACCP Alliance, industry, and an industry group.

S5 Non-Destructive Superior Sampling

TERRANCE ARTHUR: *U.S. Department of Agriculture – ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*
DANIEL DEMARCO: *Eurofins Microbiology Laboratories, Louisville, KY, USA*
ERIC WILHELMSEN: *FREMONTA, Fremont, CA, USA*

Destructive sampling of foodstuff is the traditional approach for microbial risk assessment. Samples are often made of multiple grabs to be more representative. Discussions regarding how to take these samples, the amount of product to collect, and how many grabs to collect, have sought to increase the accuracy and utility of grab-based assessments. Increasing the number and size of samples and increasing the number of grabs per sample provides limited improvement in representativeness, and detection of pathogens.

Aggregated sampling involves sampling the surface of a large percentage of the product, effectively increasing the amount of product sampled for the target of interest. Additionally, aggregated sampling assures more representative results while simplifying sample handling and reducing enrichment volumes, which reduces the time to results. These benefits can apply to many food industry sectors including protein foods, produce, low moisture powders, nutmeats and other products where surface contamination is likely. The specific benefit of a potentially reduced time to results for Shiga-toxigenic *Escherichia coli* (STEC) detection and less product loss will be presented and opportunities for applying these concepts to other highly perishable foods exposed to rare STEC risk, such as leafy greens post-harvest, will be explored.

The beef industry has claimed some of these benefits and moved away from sampling using sharp blades to excise meat tissues by shifting towards manual and continuous aggregated sampling. This approach is being explored for other protein foods where this should both simplify sample analysis and reduce the total time to result.

Fresh cut produce and leafy greens have faced notorious pathogen outbreaks. Aggregated sampling shows promise as a more sensitive risk assessment tool to drive root cause analysis and preventative-measure research. It presents a tool for enhanced screening efforts to meet the demands of the marketplace.

An examination of the efforts in the meat and produce industry will allow other practitioners to consider how aggregated sampling can improve sampling in their industries.

S6 Food Safety by Design

GALE PRINCE: *President, SAGE Food Safety Consultants, LLC., Cincinnati, OH, USA*
JOHN HOLAH: *Holchem/Kersia, FS&PH, Bury, United Kingdom, United Kingdom*
RICK HEIMAN: *3-A, McLean, VA, USA*
DEBRA SMITH: *Vikan, Swindon, United Kingdom, United Kingdom*
DIMITRI TAVERNARAKIS: *Mondelez International, Heraklio, Greece, Greece*
EDYTA MARGAS: *Global Head of Food Safety at Bühler Group, Uzwil, St Gallen, Switzerland, Switzerland*

Hygienic design of food processing facilities and equipment is a key factor in the maintenance of safe food production.

Despite this, awareness of hygienic design and its prevalence within the food industry is still limited. Its influence, however, is acute with many major food safety incidences and company enforcement notices having resulted from poor hygienic design.

Hygienic design of food manufacturing facilities and equipment:

- Enables hygienic manufacturing systems.
- Delivers more effective and efficient sanitation
- Prevention and reduction biological, chemical and physical hazards
- Achieve reliability for food safety controls
- Enhances sustainability

In the U.S., hygienic design has been championed since the 1920's by 3-A SSI, while in the EU, the European Hygienic Engineering Design Group (EHEDG) has been active since 1989. Both organizations have produced a wealth of standards and guidance to help equipment manufactures and facility constructors. Additionally, international and EU hygienic design standards are available and, in the EU, hygienic design of food processing machinery is a legal requirement.

This symposium addresses the benefits of food facility and equipment hygienic design for the everchanging requirements of the global food industry. The principles of hygienic design and current standards and guidance are described as well as the value to business owners and facilities operators. The timely development of the Global Food Safety Initiative (GFSI) scopes JI and JII in version 2020 of the Benchmarking Requirements is also reviewed to explore how GFSI approved CPOs (e.g. BRCGS, FSSC22000 and SQF) could develop standards to which hygienic design could be audited and standardized. Finally, it will take a look into the future to consider the drivers for improved design and the economic and environmental benefits of hygienically designed systems.

S7 Addressing the Global Threat of Antimicrobial Resistance Using One Health Approach

MOHAMMAD ISLAM: *Paul G. Allen School for Global Health, Washington State University, Pullman, WA, USA*
NUR HASAN: *EzBiome Inc., Gaithersburg, MD, USA*
MOHAMMED ABDUS SAMAD: *Bangladesh Livestock Research Institute, Savar, Bangladesh, Bangladesh*
RUBY SINGH: *U.S. Food and Drug Administration, Derwood, MD, USA*

The World Health Organization (WHO) has declared antimicrobial resistance (AMR) as one of the top 10 global public health threats facing humanity due to increased mortality, treatment complications, and health care costs. An estimated 700,000 annual deaths occur from antimicrobial-resistant organisms (AROs) globally. A significant proportion of deaths occur in low- and middle-income countries where AMR rates are much higher than high-income countries. Misuse and overuse of antibiotics in treating infectious diseases in humans as well as use of medically important antimicrobials in animal production and agriculture are the main drivers for the development of AROs that are subsequently exchanged between hosts via contaminated environments. As human health is so closely connected to the health of animals, agriculture, and the shared environments, a cross-sectoral, holistic approach like One Health is needed to adequately address the AMR globally. It is especially critical for resource poor countries, where health, sanitation, and environmental infrastructures are poor, and natural environments are conducive for continued AMR emergences. "One Health" approach with integrated actions across human, animal and environmental health sectors will not only help combat AMR locally, but also help restrict their transmission globally.

Tackling AMR is a Sustainable Development Agenda issue and will be critical in achieving the Sustainable Development Goals (SDGs). The WHO and a few countries including the United States have launched new guidelines on the use of medically important antimicrobials in food-producing animals. These guidelines aim to reduce their use in animals with the goal of ensuring their continued efficacy in human medicine. This symposium aims to bring together a pool of professionals from both developed and developing countries working in the fields of food safety and hygiene, water quality, public health, and antibiotic resistance to discuss the implementation of One Health approach. Items of discussion will include surveillance of AROs, community resistomes and the role of regulatory bodies in implementing antibiotic stewardship in interconnected sectors to address AMR from a One Health perspective.

S8 Continuing the Comanagement Conversation: Establishing a Conceptual Framework for Understanding Trade-Offs and Synergies between Food Safety and Conservation Aims

PATRICK BAUR: *University of Rhode Island, Kingston, RI, USA*
DANIEL WELLER: *Department of Statistics and Computational Biology, University of Rochester Medical Center, Rochester, NY, USA*
AARON ADALJA: *Cornell University, Ithaca, NY, USA*

Recent studies have raised concerns about the impact of food safety measures on ecosystem health in agricultural environments. For example, following the 2006 *E. coli* outbreak in spinach, buyers, auditors, and other groups increased pressure to minimize wildlife intrusion into produce fields (e.g., non-crop vegetation removal). However, as several papers published since 2006 have reported, many of these measures can negatively affect on-farm ecosystem health, including pollinator health and soil quality. This may force growers to choose between sustainability and food safety concerns, even though there is limited scientific data that would enable them to calculate the food safety and sustainability trade-offs associated with specific food safety practices. Thus, there is a specific need for research and discussion on the trade-offs between food safety and sustainability in preharvest environments, and how to comanage agricultural environments for multiple aims. This symposium follows-on the 2019 symposium that provided an overview of comanagement problem by showing, for a specific food safety practice (maintenance or removal of non-crop vegetation) and environmental outcome (microbial and physicochemical water quality), how synergies and trade-offs between food safety, and competing environmental and economic aims can be investigated, quantified, and used to develop guidance.

The three talks presented here are the product of a collaborative project by the speakers; as such, this symposium provides a conceptual framework for holistically examining the impacts of a specific food safety practice using sociological, ecological, and economics approaches, and using these data to balance food safety, environmental and economic aims. By examining trade-offs and synergies between food safety and environmental outcomes as they relate to a single food safety practice and specific set of environmental outcomes, this symposium will present a blueprint for how such analyses can be expanded to other practices of interest helping to reduce unexpected consequences from on-farm food safety decisions move us towards a safer, more sustainable food system.

S9 Infectious or Not Infectious? Advances in Virus Quantification and Translation to Health Risk

SAMANTHA WALES: *U.S. Food and Drug Administration, Laurel, MD, USA*
JAMES LOWTHER: *UK National Reference Laboratory for Foodborne Viruses, Dorset, United Kingdom, United Kingdom*
SUSANA GUIX ARNAU: *Dr., Barcelona, Spain, Spain*

Foodborne viruses such as human noroviruses are the leading cause of gastroenteritis and foodborne disease worldwide. The gold standard to detect these viruses in food, clinical, and environmental samples is via molecular assays (e.g., RT-PCR); however, molecular methods alone cannot address if the virus particle is infectious.

During the last decade, there have been major research advances in the development of cell culture systems and small animal models for estimating infectious norovirus titer. But how close are we to having a variety of tools that can be used broadly to accurately measure infectious virus and relate the titer to the actual health risk associated with it?

This question will be tackled by a group of international experts who each will cover a different approach to assessing virus infectivity and the associated advances and limitations. First, an overview of the most recent developments in the culture systems and small animal models for studying human noroviruses will be presented along with how these systems have been applied to measure infectious virus recovered from food and environmental samples. Second, the concept of indicator organisms (e.g., bacteriophages) as a model for human fecal contamination will be discussed as well as viability PCR as a way to identify infectious virus in naturally contaminated samples.

Overall, development and optimization of techniques to evaluate the level of infectious virus in food and environmental samples can assist in outbreak management, assessing viral prevalence, and implementation of more effective control of these agents in the food supply.

S10 Goodbye Old Friend: Best Practices for When and How to Replace, Restore, and Retire Food Processing Equipment

JOHN BUTTS: *Safety by Design, Eagen, MN, USA*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
APRIL BISHOP: *TreeHouse Foods, Oak Brook, IL, USA*

Poor hygienic conditions of facilities and equipment contribute to poor quality and shelf life, line stoppages, potential product contamination with particulates and pathogens, and to extending the time needed for cleaning and re-cleaning activities, and they may lead to many more inefficiencies that are harder to detect and solve. A potential solution to address these issues takes the form of equipment replacement and reconditioning. The challenge is how to go about making such capital expenditure decisions and when to do so. How and when do we say goodbye to our trusty older pieces of equipment? This symposium will begin with a journey through the past 50 years of equipment design, covering what has been learned so far and firsthand experiences of what has been, what is, and what should be in the future, presented by Dr. John Butts. Next, Dr. Jeff Kornacki will discuss zones 3, 2, and 1 on equipment structures, approaches to sampling them during crisis and non-crisis times, and discuss the relationship between these zones. April Bishop and Duane Grassmann will provide insights into how and when it is appropriate to recondition equipment instead of saying goodbye too soon. The panel will answer important questions related to decision making and engage in a discussion-based approach to address questions from the audience.

S11 Clean-Label Antimicrobial Innovations and Applications

FAITH CRITZER: *University of Georgia, Athens, GA, USA*
JOSHUA GURTLER: *U.S. Department of Agriculture – ARS, Wyndmoor, PA, USA*
DES FIELD: *University College Cork Ireland, Cork, Ireland, Ireland*

Antimicrobials are used in numerous foods for the inactivation, prevention or reduction of microbial growth, including bacteria, fungi, or viruses. Organic clean-label antimicrobials are increasing in demand by consumers and becoming more widely used. However, innovative compounds and processes are called for to meet today's challenges in food safety and preservation. Antimicrobial microemulsions have been successfully used to disperse essential oils in aqueous food products to maximize the surface area and contact with microorganisms, thus increasing the efficacy of the compounds. Thermal processes, when combined with antimicrobials, have shown synergistic activity. Mild heating (ca. 50°C) with antimicrobials is able to achieve significant log reductions of bacteria that would not be possible by applying either heat or antimicrobials individually. Finally, bacteriocins are naturally occurring compounds that can be used as clean-label antimicrobials, are widely used and new bacteriocins are being discovered, produced and marketed. The final topic will be addressed by a world bacteriocin expert from University College Cork, Cork, Ireland. This symposium will provide updates on the aforementioned antimicrobials and processes relevant to the food industry and should attract the attention of small and large food and beverage manufacturers, alike.

S12 Using Consumer Research to Inform Labeling Policy for Food Products

AARON LAVALLEE: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*
LISA SHELLEY: *Department of Agricultural and Human Sciences, North Carolina State University, Raleigh, NC, USA*
DONNA GARREN: *American Frozen Food Institute, Woodbridge, VA, USA*

Food labels are an important tool for communicating food safety information to consumers. Based on recent foodborne outbreaks and research, data shows that consumers do not always read and follow mandated safe-handling instructions and manufacturer's voluntary heating and/or cooking instructions, thus increasing risk of foodborne illness. Failure to follow food safety instructions is of particular concern for households and communities with immunocompromised individuals who are at increased risk for foodborne illness. To inform policy decisions regarding labeling of food products, better information is needed about how consumers use labeling information during food preparation and how their perceived risk of certain foods might influence adherence to food safety instructions. In this session, presenters will discuss findings from recent outbreaks and research focused on the gaps in understanding consumer attention to and use of food safety instructions on food products; discuss how risk perceptions of specific foods might affect adherence to food safety instructions; how regulators are using findings from consumer research to inform policy decisions; and how food industry and food safety educators are addressing the challenges identified in consumer research to mitigate food safety concerns.

S13 Metagenomics: Where Do Viruses and Parasites Fit in?

SOIZICK F. LE GUYADER: *Ifremer, Laboratoire de Microbiologie, Nantes, France, France*
BAS OUDE MUNNINK: *Erasmus University Medical Center, Rotterdam, MD, Netherlands, Netherlands*
SUSAN R. LEONARD: *Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*
MAURICIO DURIGAN: *U.S. Food and Drug Administration – CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, USA*

Metagenomics has shed light on bacterial profile and distribution in various food and environmental samples. Several studies have demonstrated bacterial pathogens ability to persist are influenced by the natural microbiota of a food and food-associated environment(s). However, very little is known about the role of the microbiome in identifying the occurrence and prevalence of foodborne viruses and parasites in food and food environments. For instance, even though viruses such as Norovirus are attributed to the highest number of foodborne illnesses per year, and the number of reported cases linked to *Cyclospora* has been rising in recent years, their relationship to food microbiomes remain unknown. Investigating these interactions will reveal potential and interesting relationships between viruses, parasites, and natural food microflora. Combining information about virus and parasite prevalence and microbial community composition provides a different perspective to microbiome data and will answer critical data gaps. Therefore, the goal of this symposium is to provide a framework for utilizing metagenomics in understanding the foodborne virus and parasite prevalence and dispersion in food-associated environments.

The symposium will begin with an overview of the detection and prevalence of foodborne viruses in food and food environments (with a focus on preharvest environments), identify current tools and diagnostic methods for virus and parasite diagnostics in different production systems, continue with the bioinformatic analysis and pipeline building which are utilized in meta-data analysis, and finish with insights on using complex metadata from microbiomes to generate foodborne virus and parasite profiles and interactions for food safety assessments.

S14 Getting Floured by E. coli: Risk Assessment and Mitigation

FRANCISCO DIEZ-GONZALEZ: *Center for Food Safety, Univ. of Georgia, Griffin, GA, USA*
ABHINAV MISHRA: *University of Georgia, Athens, GA, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

Starting as early as 1952, there have been more than thirty outbreaks of foodborne illness and recalls around the world linked to wheat flour and products that contain wheat flour, such as baking mixes. *Salmonella* has been well characterized as a pathogen of concern in low-moisture foods, but Shiga Toxin- producing *Escherichia coli* (STEC) has recently emerged as a concern to the safety of these products.

One reason pathogens like STEC are such a problem in flour and its subsidiary products like baking mixes is that neither the flour milling process, nor the baking mix manufacturing process are designed to reduce the microbial load of the product. While the water activities of baking mixes are too low to support the microbial growth, STEC possesses molecular mechanisms for surviving in dry environments and can persist for long periods of time. When these mixes are re-hydrated to form a batter, optimum conditions could be created for STEC to increase in population. At this point, responsibility falls on the consumer to apply a lethality step by baking the batter prior to consumption. However, relying on consumers to do this is not an effective strategy for mitigating the risk posed by uncooked batters. Such batters may encounter time and temperature abuse if a consumer leaves it sitting out too long before baking or tries to refrigerate it for later use, which could allow contaminating bacteria time to proliferate. Further, consumers have been known to taste raw batter and dough prior to baking, or they may undercook these products prior to consumption

Determining the survival and growth of wheat outbreak associated STEC serogroups in flour, mixes and batters is essential for risk assessment. Further, the evaluation of intervention strategies to reduce the risk of STEC contamination in wheat flour and cake mixes would result in improved safety for consumers. The symposium is proposed to address these topics of relevance.

S15 Recent Advances in Phage-Based Systems for Food and Water Analysis

JOEY TALBERT: *Iowa State University, Ames, IA, USA*
SAM NUGEN: *Cornell University, Ithaca, NY, USA*
JUHONG CHEN: *Virginia Tech, Blacksburg, VA, USA*

The ability of bacteriophage (phage) to select and replicate in a host has led to the development of novel approaches that employ phage to detect bacteria. Recent advancements in phage-based analytical systems have leveraged breakthroughs in synthetic biology, enzyme engineering, genetic engineering, and nanotechnology. This symposium, co-sponsored by The International Association of Environmental Analytical Chemistry (IAEAC), will highlight some of the innovative research and approaches designed toward improving phage-based analytical systems for the selection, separation, and detection of bacteria. The presentations will cover emerging technologies intended for food and water analysis, focusing on pragmatism and scalability.

S16 Where the Wild Things are: Foraging for Fungi Food Safety

LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
COLLEEN SMITH: *DHHS New Hampshire, Concord, NH, USA*
LUKE LABORDE: *Penn State University, University Park, PA, USA*

Edible mushrooms, used in cuisines throughout the world, are an essential source of non-animal derived protein and offer a non-meat alternative to achieve a natural umami flavor. Mushroom varieties and versatility make them an excellent non-meat option while also stimulating culinary applications across the globe. The fermentative capabilities of mushrooms, coupled with high nutrient content, also appeal to alternative meat producers and nutrient suppliers looking for non-animal derived products (e.g., Vitamin D mushroom powder). However, there have been several edible-mushroom-associated outbreaks in the past few years, including one outbreak of Salmonella Stanley and three Listeria monocytogenes outbreaks associated with enoki mushrooms (in both the U.S. and Canada), and an outbreak from wild, inedible mushroom foraging in France leading to hundreds of illnesses and three deaths. The significant health burden of mushroom-associated outbreaks has brought mushroom food safety to the forefront for many food safety specialists, highlighting the potential biological and chemical risks of mushrooms in the food supply. As consumers become more interested in consuming various mushrooms and as processors push the envelope to develop new food products using mycoproteins, it is essential that those involved in food safety thoroughly understand mushroom production, harvest, consumption, and use. This session will provide food safety professionals with an overview of the landscape of mushrooms in the food supply. Experts will explore the production of raw and processed mushroom products, associated food safety concerns, regulatory considerations for mushrooms and mushroom-derived products through case studies of notable mushroom outbreaks, and potential problems related to the edible mushroom foraging and cottage industry. After presenting, the speakers will be open to audience member questions regarding the various aspects of mushroom food safety.

S17 Making a Big Deal over Small Things: Omics-Based Microbiological Risk Assessment

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LAWRENCE GOODRIDGE: *University of Guelph, Guelph, ON, Canada, Canada*

Biotechnology is developing with unprecedented speed. In addition, the costs of molecular analyses continues to drop to the point where new technologies are no longer limited to the realm of research and they can be applied to routine food safety control systems. As such, it is important to regularly update on advances in the field and explore how these tools can be adapted and adopted to improve food safety. MRA is well recognised as an effective tool to evaluate the risks posed by foodborne pathogens and also identify control measures to reduce their risks. The rapidly increasing availability of big data, namely from genomics, transcriptomics, proteomics and metabolomics - the omics, have the potential to not only replace, but also advance our approaches to traditional microbiological risk assessment (MRA).

This symposium will discuss the current development of omics in MRA. Questions in scope of this session are, for example: how the omics could contribute for the four components of MRA separately and holistically? What is the experience of omics used for MRA from a national, regional and international level? How could we best use this new approach to benefit the food safety globally, especially for the low-middle income countries? What and how would the new approach tackle the problems, which was difficult or impossible before?

S18 Advances in Antimicrobial Technologies and Their Translation into Industry Practices

SIROJ POKHAREL: *California Polytechnic State University, San Luis Obispo, CA, USA*
MARC POLLACK: *ZECO-Member of The Vincit Group, Chattanooga, TN, USA*
PHILLIP DEMOKRITOU: *Rutgers University, Piscataway, NJ, USA*

The use of antimicrobial ingredients and treatments is a critical component of food safety. Both safety and efficacy must be considered for the successful use of an antimicrobial. Numerous advances have been made in antimicrobial technologies, a number of which are currently being adopted by industry. For example, the development of novel antimicrobial "dry" chemistries, biological antimicrobial interventions, and equipment for its delivery are increasingly being adopted by food processing facilities. This symposium will offer an important forum for information exchange on the current novel and technological applications on the horizon for such emerging antimicrobials in the food industry. The goal of the symposium is to survey the use of these antimicrobial technologies and offer perspectives both on the development of these technologies and their translation to practice, with the aim to further facilitate this process. The speakers will include both industry members, who can offer insight as allies into the current use of antimicrobials in processing applications and academic researchers who are conducting work on developing and improving these new antimicrobial technologies. This panel will touch on recent advances in antimicrobial compounds as well as the use of antimicrobial equipment delivery in processing environments. This symposium will also feature case studies on the application of two important antimicrobial technologies – the use of bacteriophages in fresh meats: chicken and pork, and nanostructure water delivery to inactivate bacteria in crops.

S19 Parasites of Global Public Health Relevance

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

Foodborne parasitic illnesses have often been overlooked, however they have a relevant impact worldwide. Teniasis, toxoplasmosis, trichinellosis, cyclosporiasis, among others have been responsible of foodborne outbreaks, are prevalent in tropical areas and affect not only locals but also travelers, and domestic and international food trade. This symposium will present an overview of these neglected diseases, the challenges that the food industry faces and methods to reduce the risk of infection in humans. Recent studies on environmental sampling and surveillance studies will be presented.

S20 What Do Fresh-Cut Produce and Low-Moisture Food Processors Have in Common? New Considerations for Environmental Monitoring Programs

LESLIE HINTZ: *U.S. Food and Drug Administration, College Park, MD, USA*
SARAH JONES: *University of Arkansas, Fayetteville, AR, USA*
ANETT WINKLER: *Cargill, Inc., Unterschleißheim, Germany, Germany*
SURESH DE COSTA: *Lipman Family Farms, Immokalee, FL, USA*

Environmental monitoring is an essential pathogen detection and sanitation verification activity in food processing environments. Stakeholders across the supply chain are increasingly investing resources and visibility into these sampling programs in an effort to prevent potentially unsafe products from entering the marketplace. Historically, our understanding of pathogen ecology dictated unique environmental sampling approaches in various processing environments, such as Listeria monitoring in ready-to-eat product facilities and Salmonella in low-moisture food production facilities. However, new research, recent recalls, outbreaks, and evolving supply chain practices raise the question – are we looking for the right microorganisms? This short symposium brings together processors, researchers, and regulatory entities to provide clarity on designing appropriate pathogen environmental monitoring programs. A review of the ecology of Listeria and Salmonella and the external factors impacting the recovery of these pathogens in specific processing environments will kick off the discussion on the future of environmental monitoring programs in these unique environments. A representative from the regulatory sector will provide an update on new findings and learnings based on their response to recent events involving these hazards. Both fresh-cut produce and low moisture foods processors will share their experiences and insights in identifying and seeking out relevant pathogens in their environments.

S21 COVID-19 Risk Management Practices in Food Markets: What are the Impacts on Food Safety?

ELISABETTA LAMBERTINI: *GAIN - Global Alliance for Improved Nutrition, Rockville, MD, USA*
LESTER SCHONBERGER: *Virginia Tech, Blacksburg, VA, USA*
DIMA FAOUR-KLINGBEIL: *Center for Rural Development, Humboldt University, Berlin, Germany, Germany*

The COVID-19 pandemic has impacted food systems, including informal supply chains and markets that sustain a large portion of the world population. Both the pandemic itself and measures to reduce the risk of infection have shaped how food market actors perceive risk, seek or receive risk control information, handle food, and behave in the market. These changes impact food safety as well as nutrition.

Consumer and vendor behaviors are shaped by knowledge, attitudes and beliefs, motivations, and access to tools needed to implement hygienic practices. During the progression of the COVID-19 pandemic a range of messages and training efforts as well as the provision of resources have been implemented in markets (e.g., hand sanitizers, signs to facilitate distancing, instructions to not touch food), often including food safety elements. Field data show that market vendors and consumers have adopted safety measures and behaviors to mitigate the spread of the virus. The extent that new knowledge, attitudes, and behaviors have included food safety and have continued – or not – beyond the initial waves of the pandemic provides lessons on how food safety practices may be introduced and sustained in markets, and how consumers and vendors respond over time.

This symposium will report on studies carried out in several countries on the impact of COVID-19 risk management on food safety in markets, from the perspective of consumers and vendors, and reflect on key aspects relevant to food safety more in general, such as:

- How did consumers' perception and attitudes towards risk incurred at markets, including from foodborne hazards, change over time during the pandemic?
- How do practices introduced or reinforced to reduce COVID-19 in food markets impact food safety and hygiene in the markets, over time?
- What does the adoption of new practices by vendors and consumers tell us about the potential for behavior change and its sustainability in food markets?
- What aspects of market resilience are relevant to food safety management?

S22 Food Defense: Proactive Approaches to Risk Mitigation

KEVIN MORGAN: *Department of Homeland Security, Countering Weapons of Mass Destruction – Food, Agriculture, and Veterinary Defense Division, Washington, DC, USA*
KEVIN HARRIGER: *DHS CBP, Washington, DC, USA*
JEFF HARROLD: *MITRE, Beford, MA, USA*

Safe and available food is critical to our national security. However, the U.S. food supply has significant risk exposures related to public health, plant, and animal safety. New threats arise every day, and the Department of Homeland Security (DHS) Food, Agriculture, and Veterinary Defense teams are working collaboratively with other food, agriculture and veterinary sector stakeholders to identify and neutralize threats to the U.S. food supply. The complexity of these threats is growing, and DHS is rising to that challenge through research, data analytics, and the application of Artificial Intelligence. The analyses and results of these efforts guide the development of impactful policies and programs that are geared toward protecting the U.S. food supply and our citizens in a dynamic threat environment. This session will focus on some of the important developments and efforts that are occurring in this space.

S23 Evolving Familiar Tools – Recent Developments and Applications of Risk Assessment and Predictive Modeling in Government and Industry

VIJAY JUNEJA: *USDA-ARS-ERRC, Wyndmoor, PA, USA*
JOHN BASSETT: *Danone SA, Palaiseau, France, France*
YUHUAN CHEN: *Interdisciplinary Scientist, Division of Risk and Decision Analysis, Office of Analytics and Outreach, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD, USA*

Systematic approaches to food safety management using risk modeling tools continue to gain momentum for risk-based decisions in government and industry. This is well reflected in the continuing efforts to maintain and expand familiar food safety tools such as FDA-iRISK®, ComBase, and the USDA Pathogen Modeling Program. The food industry also uses predictive modeling tools and approaches to inform a range of decisions in product design, setting risk-based microbiological criteria, and establishing parameters for heat treatment and shelf life. Furthermore, risk modeling tools can be valuable to address challenges arising from supply chain disruptions, for example, by predicting changes in risk from deviations in time and temperature, changes in the initial conditions (prevalence and levels) and the growth and survivability potential of pathogens. Databases such as ComBase are valuable resources to access and compare different growth and inactivation models in various food systems. This symposium brings together three speakers from government and industry to share the latest developments—new features in FDA-iRISK, new models in ComBase, and customized tools developed in industry – some of which are available to the food safety community worldwide. The speakers will share insights on the practical applications of risk assessment and predictive modeling, drawing upon their wealth of knowledge and experiences from developing and using modeling tools for over a decade.

S24 Foodborne Disease Outbreak Update

EWEN TODD: *Ewen Todd Consulting LLC, Okemos, MI, USA*
REID SCHUSTER: *U.S. Department of Agriculture, Athens, GA, USA*
DOUG NOVEROSKE: *U.S. Department of Agriculture-FSIS, Washington, DC, USA*
MARGARET KIRCHNER: *U.S. Food and Drug Administration – CFSAN, Coordinated Outbreak Response and Evaluation Network, College Park, MD, USA*
LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
MARVIN MITCHELL, JR.: *U.S. Food and Drug Administration, College Park, MD, USA*
LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
BETH WITTRY: *CDC, Atlanta, GA, USA*
COURTNEY SMITH: *Public Health Agency of Canada, Toronto, ON, Canada, Canada*

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 an outbreak of *Salmonella* Typhimurium that sickened 31 people from 4 states from June-August in 2021 linked to a single brand of packaged salad greens. Packaged salad greens produced in a greenhouse farm were recalled. FDA visited the farm and environmental samples collected were positive for *Salmonella* Liverpool and the outbreak strain of *Salmonella* Typhimurium. An overview of several international outbreaks will be provided by Dr. Ewen Todd. Outbreaks covered in the review include a small school outbreak in Brazil, where both *E. coli* O157 NM and *Campylobacter* were isolated from children and the environment, an outbreak of *Grimontia hollisae* in a small Thailand village, and a review of *Bacillus cereus* outbreaks in France potentially associated with *B.* thuringiensis strains used as pesticides. A large outbreak of *Salmonella* Oranienburg occurred in the summer months of 2021 that sickened at least 400 people in 35 states. CDC and FDA will discuss the epidemiology, trackback, and any potential outcomes of the currently ongoing outbreak. FSIS will discuss the Agency's recent investigation of a Botulism illnesses and a suspect commercially canned soup. CDC will present on a National Environmental Assessment Reporting System (NEARS) study conducted to understand patterns of unique circumstances in the restaurant environment that precede a *C. perfringens* outbreak.

S25 Method and Validation Hurdles to Substantiate Allergen Claims

STEVE TAYLOR: *University of Nebraska, Lincoln, NE, USA*
TRACIE SHEEHAN: *Mérieux NutriSciences, Chicago, IL, USA*
STEFANO LUCCIOLI: *Food and Drug Administration, College Park, MD, USA*

Allergen claims on package can include ingredient declarations, precautionary statements, and allergen free claims. Companies that make precautionary and allergen-free claims on packages face a regulatory gray area, method challenges for testing ingredients and finished foods, and validation challenges to support that their statements are valid. There are currently very few regulations globally, validation protocols, or audit criteria for allergen free claims. This session will summarize relevant background on audits to assess facility practices and programs for allergen statements, summarize global regulations on allergen statements, and provide industry best practices on testing programs to support allergen statements.

S26 Virtual Food Safety Monitoring, Auditing and Artificial Intelligence Applications

WENDY WHITE: *Georgia Institute of Technology, Greensboro, GA, USA*
JUDI LAZARO: *AIB International, Manhattan, KS, USA*
ROB CHESTER: *Ubloquity and Supply Chain In-Sites, London, United Kingdom, United Kingdom*

The Covid-19 pandemic has commenced an irreversible paradigm shift in attitudes toward work and acceptance or acceleration of information technology (IT) solutions to increase our communication. Indeed, IT solutions have become the norm at home, work, and in our communities. Social media keeps us connected at work, virtual platforms replace travel and even interoffice meetings as equivalent to face-to-face, and home audio and video applications allow us to monitor our homes and remotely answer the door.

These IT applications are present and rapidly developing in the food industry as well as our personal lives. This symposium will explore the transition from the antiquated use of video to the present surge in IT applications for Training, Monitoring, Auditing and Real-Time Remote Management. We will address remote monitoring of large organizations and inaccessible locations (i.e., shipboard processing), food fraud and sustainable harvest, prevention of intentional adulteration, foreign supplier verification, monitoring preventative controls, and the pros and cons of remote auditing for food safety certification. The future of virtual food safety applications is now. The strategy of this symposium will be to broadly explore, (i.e., in 15-20 min. presentations) how current innovations will trend toward common implementations while highlighting benefits, challenges, risks, and philosophical conflicts.

S27 What to Expect When You're Exporting: Using FDA's Export Certification Program

ALIA BLAIS: *Canadian Food Inspection Agency, Ottawa, ON, Canada, Canada*
KATHERINE MECK: *Food and Drug Administration, College Park, MD, USA*
PENNY MARSH: *Sensient Technologies Corporation, Milwaukee, WI, USA*

As more detailed food safety regulations continue to proliferate around the world, FDA is increasingly being asked to provide the food industry with export certification in order to facilitate trade. Those exporting food to other countries have seen an increase in requests to provide evidence that their food manufacturing facilities and products are under the supervision of the exporting country's competent authority for food safety. Fortunately, FDA has established an export certification program to provide export certificates and export lists to foreign governments in order to satisfy these requirements.

This session is meant to provide IAFP participants with an overview of the FDA's export certification program for food products, which was established following the passage of FSMA, and to place the program within the context of facilitating trade. Session panel participation from food safety regulators will explain the purpose of export certification within a country's food safety system. Practical examples of how regulators and industry communicate these assurances will also be provided. Session presentations will include an overview of the FDA's export certification program from the FDA/CFSAN International Affairs Staff, an industry participant's experience with FDA's program, and a foreign regulator's perspective in using certificates as part of their food safety oversight system.

The objective of this symposium is to provide IAFP participants, particularly those that actively export food products, a better understanding of both FDA's export certification program and importing country certification requirements, examples of best practices for requesting export certification from FDA, and to answer any questions. The audience will come away from this session with more detailed knowledge of how U.S. and foreign food safety regulators work together with industry to bridge the gap between domestically focused regulatory oversight and ensuring the safety of imported food products.

S28 Data-Driven Sanitation Chemistry Selection: Does It Work Against Biofilms?

ALBERT PARKER: *MSU Center for Biofilm Engineering, Bozeman, MT, USA*
JAKE WATTS: *PSSI Food Safety Solutions, Kieler, WI, USA*
DALE GRINSTEAD: *Retired – Senior Food Safety Technology Fellow, Highlands, NC, USA*

Biofilm-forming bacteria have long been around, and they are here to stay. Acknowledging that biofilms will always be a challenge for food producers and manufacturers, how do we best remediate their existence in the production environment? One useful step in biofilm control is to opt for sanitation chemistries that are effective against biofilms, which includes those used for cleaning, disinfection, and sanitization. Expert speakers will propose ways to assess and select sanitation chemistries based on microbiological data collected from biofilm testing. First, learn about the available methods and technologies that are being used to evaluate the biofilm response to sanitation chemistries. Second, hear about a case study from industry where data from standardized ASTM testing is used to support the selection of sanitation chemistry against biofilms in food facilities. Third, gain an understanding of the data behind biofilm claims presented on labels or in company marketing materials. The combination of these topics will promote the ownership of using testing data to choose a particular sanitation chemistry over another in the drive towards biofilm control.

S29 Agricultural Water Quality for Produce: Recent Advances, Current Challenges, and Future Opportunities

SAMIR ASSAR: *Food and Drug Administration, College Park, MD, USA*
DANIEL WELLER: *Department of Statistics and Computational Biology, University of Rochester Medical Center, Rochester, NY, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*

Fresh fruits and vegetables are the foundation of a healthy diet, being an invaluable source of vitamins, minerals, and health-promoting phytochemicals. Unfortunately, fresh produce is also one of the most common vehicles for transmission of foodborne pathogens around the globe, particularly as the commodities are typically not treated with a "kill step" and are often consumed raw. Agricultural water is a critical control point for produce-growing operations since a variety of pathogenic bacteria (e.g., *E. coli* O157:H7), parasites (e.g., *Cyclospora cayetanensis*), and viruses (e.g., Norovirus) can be transmitted to fresh produce through contact with contaminated water. In 2015, the United States Food and Drug Administration (U.S. FDA) enacted the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), providing science-based federal regulations and guidance for growers to reduce the microbial risk associated with the growing, harvesting, packing, and holding of fresh produce. With the FDA announcing forthcoming regulatory changes affecting Subpart E regulations specific to water quality, there is an urgent need to provide the scientific community, Extension educational professionals, and fresh produce growers with an update on the new proposed changes to the water rule, recent research findings and current requirements affecting the production of fresh produce.

The purpose of the symposium is to provide an update on the state of agricultural water quality with an emphasis on recent changes in agricultural water quality regulations factors affecting the prevalence of pathogens in agricultural water, recent advances in agricultural water treatments (chemical treatments, ultraviolet light, etc.), and future directions for research, outreach or policy affecting agricultural water quality.

S30 Food Safety within Food Security in Africa: The Dilemma between Informal and Formal Markets

ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture Abeokuta, Abeokuta, Nigeria, Nigeria*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia, Ethiopia*
OLUWAFEMI ADEBO: *University of Johannesburg, Johannesburg, South Africa, South Africa*

At the 2019 IAFP Annual Meeting, a group of interested food safety experts from different regions in Africa held a roundtable session on "creating awareness on food safety in Africa," which aimed to provide the IAFP community with a basic awareness of the common as well as diverse food safety challenges experienced by countries and regions in Africa.

The current proposal for a short symposium will zoom in on one particular challenge, namely the dilemma existing in safety of food traded at informal and formal markets. Informal food markets are a major outlet of food products in most countries in Africa. All food products vital for human diets and livelihoods of people can be found on informal markets: animal meats, milk, eggs and fish products. The informal markets play a key role in society because they provide for employment and easy access to food at relatively inexpensive prices. In this regard they help solve some of the major social and economic challenges of countries. However, such products often escape the effective quality, health and safety regulations that characterize marketed through formal markets and may thus pose threats to food safety for public health. However, when thinking "magnitude of food safety risk," do the societal benefits weigh up to such potential food safety concerns? This is creating real dilemma regarding managing food safety risks in such settings and require initiatives towards understanding the situations. Foods from informal markets have been tested for various microorganisms including faecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Bacillus cereus* and *Shigella sonnei*.

The aim of this session is to picture the food safety situation and challenges of informal food markets in Africa as experienced by a number of researchers from different countries and to raise a discussion on what notable food risks are and how these could be considered and addressed in their local context.

S31 Using a HACCP-Mindset to Enable Enhanced Food Traceability

TEJAS BHATT: *Walmart, Rochester, NY, USA*
VIKRANT DUTTA: *bioMérieux, Inc., Hazelwood, MO, USA*
JENNIFER MCENTIRE: *International Fresh Produce Association, Washington, DC, USA*

Food traceability has been considered tangential to food protection. Recent large-scale outbreaks, such as those of romaine lettuce in the USA, have emphasized the role of traceability in outbreak investigations and recalls. For proposed traceability regulation by the U.S. Food and Drug Administration, the industry has started to consider traceability as an integral part of their food safety management system. But where and how to start enhancing traceability processes and systems? This symposium will discuss a radical methodology to enhance the traceability of food. Can we use a process that has been successfully deployed in designing the safety of the food itself, to design a more efficient traceable supply chain? Can we use the principles of HACCP to deliver a traceable food to the end consumer? A hazard for a traceability system can be defined as anything that could result in the loss of visibility of the journey of the food. For example, a transformation event, where multiple ingredients are combined to create a finished food product, be a hazard. The critical control point for this hazard is to ensure the traceability data of all the ingredients is linked to the provenance of the finished food product. The validated critical limits could be the amount of waste that could be generated through normal business operations. Monitoring of these critical limits requires the ability of an organization to measure the amount of product coming into and leaving the facility, monitoring any significant deviations from those limits. When those deviations occur, that batch of products should be marked as not traceable and be separated from the supply chain until the root cause can be identified and corrected. Could this be the way to democratize access to enhanced traceability across the food system? Come to the symposium and find out.

S32 Rapid Response Research to Support the Food Industry through COVID-19

KRISTEN GIBSON: *University of Arkansas, Fayetteville, AR, USA*
BYRON CHAVES: *University of Nebraska-Lincoln, Lincoln, NE, USA*
REZA OVISSIPOUR: *Virginia Tech, Hampton, VA, USA*
RENATA IVANEK: *Cornell University, Ithaca, NY, USA*
SAPNA DASS: *Texas A&M University, College Station, TX, USA*
ERIN SCHIRTZINGER: *Kansas State University, Manhattan, KS, USA*

The COVID-19 pandemic created huge challenges for the food industry when it first emerged in the United States given the rapid spread of the virus, particularly in environments when workers are in close proximity to each other, as well as the early lack of knowledge surrounding best practices to reduce transmission. In response, USDA-NIFA funded rapid response grants to learn more about how COVID-19 spreads and how to reduce transmission throughout the food industry, with the ultimate goal of easing the impact of the pandemic on an already stressed food system. As the pandemic evolves, the food industry continues to face new challenges. This symposium will present progress and data collected so far on six of the grants funded by USDA-NIFA, including modeling the spread of COVID-19 in food processing facilities, identifying strategies to reduce transmission of COVID-19 among individuals working in the food industry (with a particular focus on meat and poultry processing plants and food service), identifying the best sanitation strategies for retail, and creating best practices and identifying data gaps to support the food industry overall. This session will particularly focus on the extension outputs and other major takeaways so far, including lessons learned for the industry as the pandemic progresses.

S33 Global Recommendations on Risk Assessment of Allergens from the Ad Hoc Joint FAO/WHO Expert Consultation

LAUREN JACKSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
BENJAMIN REMINGTON: *Remington Consulting Group, Utrecht, NE, Netherlands, Netherlands*
JOSEPH BAUMERT: *University of Nebraska-Lincoln, Lincoln, NE, USA*

Food allergies have become a global public health priority in recent years. For packaged foods, undeclared allergens are the leading cause of product recalls in the U.S. The Codex Alimentarius Commission (CAC) first provided guidance on labeling of priority allergenic foods in 1999 recognizing 8 foods or food groups as the leading causes of food allergy globally. Subsequently, regulatory authorities in numerous countries have developed labeling regulations for packaged foods. However, global regulatory approaches are variable from one country to another. In 2020, the Food & Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) organized an ad hoc Joint Expert Consultation on Risk Assessment of Food Allergens under the auspices of the Codex Committee on Food Labeling (CCFL). Over the period from 2020 – 2021, this expert panel convened on multiple occasions to develop recommendations for consideration by CCFL and CAC on the global priority list of allergenic

foods, thresholds or reference doses, and labeling strategies using thresholds or reference doses. Simultaneously, the Codex Committee on Food Hygiene (CCFH) developed new recommended guidance on a code of practice for food allergen management. This session will summarize the recommendations made to CCFL and CCFH and the scientific basis for those recommendations along with comments on the timeline for CAC adoption of these recommendations and their impact on global regulatory considerations and harmonization.

S34 Persistence of Enteric Viruses in Low-Moisture Environments

LEE-ANN JAYKUS: *Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, USA*
SABAH BIDAWID: *Health Canada, Ottawa, ON, Canada, Canada*
ALVIN LEE: *Institute for Food Safety and Health, Bedford Park, IL, USA*

Enteric viruses including human noroviruses (NoV) and Hepatitis A virus (HAV) continue to be major causes of foodborne disease worldwide. When a food or food contact surface is contaminated by viruses from contaminated products or infected food handlers, the viruses can remain infectious for a long time and the environmental stability of viruses could lead to public health significance for low-moisture foods (LMF). A number of outbreaks have been attributed to LMFs such as dried radish, dried seaweed and sun-dried tomatoes. However, there is limited data on how viruses survive in a low-moisture environment or low water activity foods. A number of studies have demonstrated the survival of bacterial pathogens in low-moisture foods and given the environmental stability of viruses, the threat is significant because most LMFs are ready-to-eat and usually do not undergo a further processing or cook step. The objective of this symposium is to discuss the degree of viral persistence in low-moisture foods, their detection and the strategies used to inactivate viruses in these environments.

S35 Cleaning: The Perennial Overlooked Step in Sanitation and Vital Importance to Proper Environmental Surface Sanitization and Disinfection

ANGELA FRASER: *Clemson University, Clemson, SC, USA*
CHRISTOPHER LYNCH: *Stepan Company, Northfield, IL, USA*
RON MASTERS: *Stepan Company, Northfield, IL, USA*
BENJAMIN WARREN: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

Have you scrutinized your cleaners as much as you have your sanitizers and disinfectants? Cleaning is perhaps the most important step in the sanitation process. A high-quality sanitation program minimizes infection transmission and cross-contamination risks from environmental surfaces while helping prevent outbreaks such as those caused by *E. coli* or Norovirus. Unfortunately, a cleaner's performance is overlooked, and attention is given to antimicrobial products. Sanitation at any level requires both cleaning to remove food particles and organic matter, and antimicrobial action (sanitization or disinfection) to reduce or eliminate pathogenic and spoilage microorganisms. Without an adequate cleaner or cleaning step, those soils typically present on surfaces within food establishments may reduce, or totally inhibit, the efficacy of antimicrobial products. This may result in an unrecognized reassurance of sanitation by the user and a heightened risk of infection transmission. In light of this, how can we close knowledge and practice gaps around the importance of cleaning to achieve high quality sanitation? Experts from industry, academia and regulatory agencies will tackle this by addressing the following core questions. First, how do product formulation choices affect the removal of target soils in food establishments, and how is soil removal validated? Second, what is the regulatory landscape that informs the user of cleaning power requirements from their cleaners, disinfectants, and sanitizers, and how can compliance be achieved? And Third, what body of evidence exists that corroborates the survival and transmission of pathogens due to improper cleaning in food establishments, despite the use of sanitizers and disinfectants? Attendees will be empowered to critically assess, and further the conversation around, the applicability and usefulness of their sanitation products and decontamination regimes with the goal to better protect their operations, staff, and the public.

S36 The Silent Pandemic: The Emergence and Spread of Antimicrobial Resistance in Food Systems in the Middle East and North Africa (MENA) Region

NAHLA ELTAI: *Qatar University, Doha, Qatar, Qatar*
ISSMAT KASSEM: *Center for Food Safety, University of Georgia, Griffin, GA, USA*
JORGE PINTO FERREIRA: *FAO-Food and Agriculture Organization, Rome, Italy, Italy*

Antimicrobial resistance (AMR) poses a serious threat to public health worldwide. The O'Neal report famously predicted severe mortality and morbidity and catastrophic economic losses due to AMR across the globe by 2050. The rise of untreatable infections has threatened the foundation of modern medicine and can compromise food security and safety. Food systems and production can play an important role in the selection and dissemination of AMR. The CDC, FAO, and WHO have documented AMR in leading foodborne bacterial pathogens, and AMR has been dubbed a silent pandemic, a slow tsunami, and a global challenge. In addition to the public health ramifications, an outbreak with an AMR foodborne pathogen that might cause recalcitrant infections is a grave concern for the food industry. Despite a significant effort in legislation and research, AMR remains not well highlighted in many regions of the world. The latter includes the MENA region that hosts ~ 6% of the world's population and is an economic hub; a center of human movement and food trade. In The O'Neal report, Asia and Africa are predicted to be the most affected by AMR, and the MENA countries are located in these "hotspot" continents. **In this symposium, speakers from MENA countries will discuss AMR in food matrices along with challenges, potential solutions, and local and global perspectives. This symposium will shed light on a core food safety issue that has global impacts and requires immediate action.** This symposium will strengthen the global scope of IAFP while addressing the following:

- The **rise and spread of antibiotic resistance** in important food matrices in the MENA.
- **Bolstering antimicrobial stewardship** in relation to food safety and its role in mitigating resistance in the MENA.
- **The potential impact of the spread of antibiotic resistance** in food systems in the MENA on public health locally and globally.
- **Vital need for implementation of antibiotic resistance monitoring and management systems** in the MENA.

S37 Look Around, You Have All It Takes to Make Your Food Safe!

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LUCIA ANELICH: *Anelich Consulting, Pretoria, South Africa, South Africa*
HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*

There are remarkable differences between food safety management practices in developed nations and low and middle income countries (LMIC). While for the most part developed countries have robust food safety systems, the same is not always true for most LMIC. In many LMIC, food safety at its best is limited to drafted regulations that are not enforced. Consequently, most LMIC are characterized by non-existent or non-functional food safety management systems. However, it is not known whether inhabitants of LMIC do consume safe foods as reports of foodborne disease outbreaks or recalls are scarce; probably owing to the lack of adequate foodborne disease surveillance systems.

Amidst the lack of adequate food safety management systems in the majority of LMIC, many institutions in LMIC export raw, semi processed and processed foods to developed countries; suggesting that some foods from these countries do indeed meet food safety regulatory standards in the West. This also suggests that some institutions in LMIC are able to produce safe and wholesome foods despite insufficient robust national, regional and continental food safety infrastructure for the most part, to support their food safety efforts. The question arises; how are these foods grown and processed to meet acceptable food safety standards? What can the food industry learn from how food safety is practiced in LMIC? In this symposium, Food Safety Experts from LMIC will discuss how food safety standards are met in LMIC with often, minimal resources when compared with the available resources in the West. They will also share how these practices have evolved over the years and how success in the area of food safety is defined in LMIC. The speakers will also propose ways that lessons learned in LMIC can be applied to improve food safety systems in developed nations

S38 Managing Your *Salmonella* Risk: How Investing in Early Detection and Quantitation Methods Can Protect Your Poultry Business?

FRIEDA JORGENSEN: *UK Health Security Agency (UKHSA), Salisbury, United Kingdom, United Kingdom*
KAREN BEERS: *Pilgrim's Pride Corporation, Fayetteville, AR, USA*
PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece, Greece*

Salmonellosis is an important foodborne disease causing substantial medical and economic burdens worldwide. Different action plans across the food supply chain from "farm to fork" are in place to reduce the prevalence of the causative pathogen (*salmonella*). Approaches include both quantitative risk assessment and surveillance of the serovars most frequently associated with human disease. Questions addressed in this session include; How can we benefit from analyses and data interpretation? How is that supporting risk management and public health?

Investigation into the presence of *Salmonella* spp. in reformed chicken products was undertaken in response to an increase in *Salmonella* Enteritidis infections in the UK. The initial detection of *Salmonella* Enteritidis outbreak strains was achieved through the application of a real time-PCR multiplex method targeting the mostly frequently occurring serotypes in Europe. The talk from Dr. Jorgensen will illustrate the benefits of using a serotype-specific PCR method during an European wide *Salmonella* outbreak.

Quantitation of low contamination levels of *Salmonella* in poultry production is certainly an old challenge. However, semi-quantitative PCR assays were recently developed in response to USDA recommendations published in 2019. Here implementation of these types of methods in routine testing is discussed. Implementation of such technologies can support timely intervention and help control *salmonella* risks in poultry production

These two testimonials will set the scene before getting an overview on how rapid tracking and quantitation support currently developed regional road maps, established by recognized food safety authorities such as USDA and EFSA. Data analyses and progresses will be presented together with the ongoing efforts.

S39 Developments in Sample Preparation: Implications in Pathogen Detection When Difficult Matrices are Involved

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SAM NUGEN: *Cornell University, Ithaca, NY, USA*
MARTA PRADO: *International Iberian Nanotechnology Laboratory, Braga, Portugal, Portugal*
MELISSA JONES: *Department of Microbiology & Cell Sciences, University of Florida, Gainesville, FL, USA*
MASAAKI KITAJIMA: *Division of Environmental Engineering, Hokkaido University, Sapporo, Japan, Japan*
YNES ORTEGA: *University of Georgia, Griffin, GA, USA*

Detection of microbial content in foods and environmental samples becomes important to ensure the quality, value, and yield of foods. While there is no dearth of molecular techniques available for both detection and quantification of microbial content within food and environmental samples, their success is often contingent upon efficient sample preparation, especially when a complex mixture of substances is involved.

Sample preparation is a very important and often ignored step in pathogen detection and food safety. There seems to be a lack in consensus among the scientific community with regards to standardized sample preparation techniques especially when complex matrices are involved. The overall goal of this symposium is to (i) discuss challenges in sample preparation when complex matrices are involved and (ii) identify and explore developments in novel sample preparation techniques that could help improve pathogen detection.

S40 Novel Foods, Novel Challenges: Food Safety Concerns in Plant-Based and Novel Food Products

YANYAN HUANG: *ADM, Longmont, CO, USA*
LILIA M. SANTIAGO-CONNOLLY: *Kellogg Company, Battle Creek, MI, USA*
DAVID RASMUSSEN: *Kraft Heinz Corporation, Moreland Hills, OH, USA*

Booming demand for plant-based products, along with advances in novel-food technologies such as non-terrestrial animal protein via cell culture and fermentation techniques, is driving product development in our industry. At the same time, this increased focus on plant-based and novel foods, particularly alternative proteins, introduces novel challenges for food safety. Accordingly, this 90-minute session will explore four facets of food safety pertaining to novel foods:

HACCP Concerns: How do novel products affect food safety plans? What are the unique challenges using crops and "clean" inputs for illness and other hazards? In the absence of historical data, where do we get justification for plant-based food safety regarding procedures such as kill steps? Can we use the same validation data from non-plant studies?

Scope of Food Safety: Food safety programs must maintain rigorous standards for microbial threats such as *coli* that affect both plant and animal-based products. At the same time, we must also account for threats and challenges associated with specific agricultural crops (e.g., mycotoxins and allergens). How can we best develop a suitable scope?

Regulation Propagation: How might regulations increase, and what are the regulatory implications? How might current consumer and regulatory trends impact plant-based, novel, and "clean" foods? The paradigm here is Testing, Inspection, and Certification overview**.**

S41 Food Safety Aspects of Controlled Environment Agriculture Systems for Fresh Produce Production: Current Industry Practices and Future Needs

MICHELLE SMITH: *U.S. Food & Drug Administration, College Park, MD, USA*
VICTOR VERLAGE: *80 Acres Farms, Springdale, AR, USA*
KRISTEN GIBSON: *University of Arkansas, Fayetteville, AR, USA*

Food crop production in controlled environments is an increasingly important sector of U.S. and global agriculture. Controlled environment agriculture (CEA) takes advantage of technologies and automation to modify production climates, shield crops from biotic and abiotic stresses, and optimize environmental factors to maximize plant yield and quality. The ability to control environmental factors and crop inputs allows growers to extend the growing season and/or grow year-round, produce crops in extreme climates and urban settings, increase resource use efficiency (primarily of water, nutrients, labor, and energy), decrease carbon- and water-footprints, and maintain consistent food quality. Greenhouses, high-tunnels, and indoor warehouses are common CEA structures, and hydroponics, aeroponics, soilless substrate culture, and vertical farming systems are common CEA growing systems. Although CEA helps exclude pests and diseases, pathogen issues can still occur in these operations. Foodborne pathogens enter CEA in the same manner as field-grown crops: (i) through contaminated ground or municipal water, (ii) unsanitary equipment, (iii) contaminated incoming materials such as seeds or plant materials, (iv) employees and staff, and (v) insects, animals, and wind. This session aims to bring together key stakeholders including industry, researchers, and regulatory entities leading the way for the future of CEA in fresh produce production. Industry and regulatory perspectives on how CEA fits into FSMA and current best practices for food safety management will be discussed. We will then hear about the current state of the science aimed at enhancing CEA food safety through identification of current best practices as well as research gaps.

S42 Not All Acids are Created Equal

ERDOGAN CEYLAN: *Mérieux NutriSciences, Crete, IL, USA*
HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands, Netherlands*
LORALYN LEDENBACH: *Kraft Heinz, Glenview, IL, USA*

The acids have long been used as bacteriostatic and bactericidal compounds in the food industry. The effects of different acids used to control pathogenic microorganisms in foods vary with pH, concentration, acid types, and food matrices. However, the understanding of detailed interactions between these key variables in a controlled and systematic way is much needed in this FSMA era. This symposium will start with a discussion of different type of acids and their kinetics in controlling pathogenic microorganisms in foods. This will be followed by an in-depth presentation on using available mathematical/predictive modeling tools to understand and compare their efficacies of microbiocidal effects. We will conclude with a discussion of industry applications, implications, and strategies in food safety and quality control through the use of acidulants as formulation control.

S43 Cyber Attacks on the Food Industry: Virtual Threats with Real Consequences

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HENRY HEIM: *Federal Bureau of Investigation, Washington, DC, USA*
SCOTT ALGEIER: *Information Technology-Information Sharing and Analysis Center, Arlington, VA, USA*

In a digital world, cyber attacks on the food industry are a growing threat that can impede a company's ability to provide safe and quality food and endanger the health and safety of consumers. Cyber threats come in many forms, including attacks designed to steal data, ransomware that can debilitate a company, and hijacking of industrial control systems (ICS). Cyber attacks can disrupt production or supply chains, leaving product and ingredients vulnerable to deterioration or adulteration. Attacks on ICS can likewise shutdown production facilities or even result in unsafe food being unwittingly distributed and sold. Such cyber attacks on the food system are not merely theoretical but are happening at increasing frequency. Recently, high profile ransomware incidents have victimized a global meat producer, beverage companies, a supermarket chain, and a bakery company, among others. Water treatment facilities were also targeted in cities in Florida and California in the United States in which the hackers attempted to poison the water supply or disrupt water treatment operations. Identification of the vulnerabilities within global food production and water supply networks to cyber attacks is vital to the prevention of these attacks and the strengthening of cyber defense capabilities. Should hazard analysis no longer focus solely on physical, chemical, and biological hazards, but include cyber hazards as part of a robust food safety plan? Leading experts in cybersecurity and food production will examine current trends in cyber threats and discuss how cyber disruptions can become health risks. The symposium will address potential cyber vulnerabilities—including those within the ICS the food industry depends on throughout food production—and provide resources and strategies for information sharing and threat mitigation.

S44 Adjunct Antimicrobial Treatments – What are They, and How Do They Fit into a Sanitation Program?

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BENJAMIN MILLER: *The Acheson Group, Northfield, MN, USA*
SCOTT KING: *PSSI, Kieler, WI, USA*

Cleaning, sanitizing, and disinfecting are hallmark strategies that momentarily mitigate the risk of cross-contamination and/or infection. A popular questions being asked is, what about protection between traditional surface treatments? There is a strong desire among the food industry to reduce the risk presented by surfaces contaminated after or between traditional cleanings. Collectively, we refer to these as adjunct treatments because they are meant to supplement hygiene interventions. These treatments can include residual sanitizers, residual disinfectants, antimicrobial coatings, fogging, air treatments, or pesticidal devices. This is a burgeoning area of interest among the industry and an active area of research, especially since the U.S. Environmental Protection Agency released updated interim guidance for residual efficacy claims in 2021. So, how do these adjunct treatments fit into a sanitation program? Expert Speakers from government and industry will address three core topics. First, what are adjunct antimicrobials and how are these technologies regulated to protect the consumer? Second, how do we evaluate the efficacy of each type of adjunct antimicrobial treatment? Third, what are the best practices for use of these technologies in food settings? Symposium attendees will leave with a greater understanding of how these new technologies are regulated, how to evaluate the efficacy these treatments deliver to a sanitation program, and best practices for their use in a food setting.

S45 The Use of QMRA for Food Safety Interventions in Low- and Middle-Income Countries

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CLAUDIA GANSER: *University of Florida, Gainesville, FL, USA*

Within several projects in Africa, quantitative microbiological risk assessments are developed to serve a variety of goals: to estimate the risks of foodborne pathogens in specific commodities, to investigate their public health impact, and to estimate the effects of proposed interventions. A next goal is to evaluate the cost-effectiveness so as to identify the most promising interventions. Additionally, these risk assessments aim to identify main data gaps. Different food chains in different countries using different modelling techniques are investigated in different projects. Three example risk assessments will be presented and their role within the broader projects of intervening on food safety management within these food chains will be exemplified.

S46 Whole Genome Sequencing: Challenging and Defining Foodborne Pathogen Species, Risk, and Virulence

MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
DAYNA HARHAY: *U.S. Department of Agriculture – ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*
SOPHIA JOHLER: *Institute for Food Safety and Hygiene, University of Zurich, Zurich, Switzerland, Switzerland*

Whole-genome sequencing (WGS) is now the gold standard for foodborne pathogen identification, epidemiological investigations, and broader studies in microbial ecology. WGS has redefined the foodborne disease outbreak landscape through improved time-to-detection resulting in fewer cases. It has further increased the resolution of foodborne disease outbreaks by identifying smaller outbreaks that may have historically been overlooked. WGS advances have influenced detection targets, confirmation procedures, and risk assessments. WGS has and continues to redefine genus and species classifications for some foodborne pathogens, as well as provide greater insight into virulence factors. While WGS has advanced understanding of all foodborne pathogens, this technology currently has varying utility among foodborne pathogens. The case studies presented in this symposium are strategically selected to investigate the variation in use and utility of WGS as an epidemiologic and phylogenetic tool. Leading experts will present state-of-the-art case studies of *Listeria monocytogenes, Salmonella enterica,* and *Bacillus cereus* as representatives of the spectrum of advancements and limitations of WGS technology. This symposium will showcase current WGS impacts on defining or challenging current understanding of a species, its impact on regulation, insight into virulence capacity, and implications for the private sector.

S47 Consequences of Proliferating *Listeria* Species for Detection Methods

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LILIA SANTIAGO: *Kellogg's, Battle Creek, MI, USA*
LAURA BLEICHNER: *Eurofins GeneScan Technologies GmbH, Freiburg, Germany, Germany*

A combination of tremendous advances in sequencing technology and genomics that have allowed much reclassification of microbes, and continued discovery of new isolates have drastically changed the genus "*Listeria*" over recent years. It has grown from the six species listed in FDA BAM to the 26 recognized in formal taxonomy today, with the latest five added in 2021 by Carlin et al. Several attributes of the new species matter for food safety diagnostic testing including: motility is no longer diagnostic for all *Listeria;* some species could easily be misidentified, including at least one that could appear to be *L. monocytogenes* serovar 4b with molecular serotyping assays; and some may not grow above 30°C. This has important implications for reference methods such as the classic cultural methods used by e.g., FDA BAM, Health Canada and ISO, as well as the rapid methods used for most routine monitoring of food process environments. In this symposium, we will discuss the taxonomic changes and newly identified species, the significance of these changes for the food industry and the responses needed during *Listeria* test development and validation.

S48 Addressing Urban Agriculture with a One Health Approach to Food Safety Vulnerabilities and Successes

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PATRICIA MILLNER: *U.S. Department of Agriculture – ARS, Beltsville, MD, USA*
GORDON JOHNSON: *University of Delaware, Georgetown, DE, USA*

The potential environmental and societal benefits associated with urban agriculture are needed urgently to feed the world. Over the past few years, there has been unprecedented interest in the development and use of urban and peri-urban agricultural environments to grow food. The critical intersecting issues between food safety and urban agricultural environments remain under-addressed subjects. Various forms of pollution (air, water, and soil quality), as well as the varied reactions to pollutants exhibited by different crop types, may affect crop safety and quality. Traditionally, pollutants linked to urban environments have not been presented as food safety hazards during cultivation of crops (soil heath, water quality); however, the emerging infrastructure of urban food systems now must consider these pollutants as such, along with traditional foodborne hazards, to provide consumer confidence and prevent foodborne illness. A One Health approach, based on the integrated and interconnected systems of urban food production, can help identify, evaluate and address issues in urban / peri-urban agriculture related to food safety. This would also consider the unique food safety challenges of controlled environment agriculture facilities operating in novel or urban environments. Perspectives on urban agriculture in the context of the convergent triad of One Health will be explored, including: 1) the health of the environment, the people, and the animals, both domestic and wild; 2) current urban agricultural operations and their food safety priorities, challenges, and successes; 3) how food safety challenges overlap and differ between urban and controlled agricultural environments; and 4) the necessary balance between meeting standards and regulations to ensure food safety with serving under-served / under-accessed markets. Within these topics, acquiring the necessary workforce, engaging a community, and production of a microbiologically and chemically safe crop will be addressed. An output of this symposium will be a toolkit based on current and future safe urban agricultural practices that would be useful to all food safety stakeholders (industry, government and academic IAFP Members).

S49 Advances in Pedagogy, Modality, and Accessibility for Virtual Food Safety Education

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SHANNON COLEMAN: *Iowa State University, Ames, IA, USA*

New methods and approaches to food safety education often evolve slowly. However, advancements in virtual food safety education have occurred rapidly over the last two years as the year 2020 marked a cardinal shift in the proportion of trainings offered online. This change includes advantages and challenges for food safety educators, and many of these dynamics will drive future food safety educational opportunities as participants and trainers have adapted to these issues. Distance education offers several opportunities to reach broader audiences, support accessibility and accommodations, and enhance learning outcomes through the use of cutting-edge technologies and approaches. The goal of this symposium is to discuss pedagogy and experiential outcomes including knowledge transfer associated with virtual food safety education. We will first discuss pedagogical principles related to application of Bloom's taxonomy, resilient pedagogy theory, and universal design for learning as applied to food safety distance education. Secondly, we will discuss success strategies related to combination asynchronous and synchronous trainings. Finally, we will conclude the session by discussing innovative approaches for fully online food safety curriculum development.

S50 Recent Advances in Control of *Bacillus* spp. – A Pathogen of Renewed Concern

SAKSHI LAMBA: *University College Dublin, Belfield, Ireland, Ireland*
THOMAS TAYLOR: *Texas A&M University, College Station, TX, USA*
ABHINAV MISHRA: *University of Georgia, Athens, GA, USA*

In recent years, several outbreaks of foodborne illness and meat recalls linked to *Bacillus* spp. have resulted in significant public health burden. Since the pathogen is ubiquitously present in nature, it inevitably spreads through food production systems, raising quality and safety concerns to the food industry. *Bacillus spp.* have a natural tendency to adapt to environmental stresses, accomplished by altering their gene expression and physiology to form highly resistant dormant endospores. The molecular pathways for sporulation and potential biofilm development of these endospore-forming bacteria in the production environments have received recent attention. Either as spores or in biofilms, the persistence as well as dissemination in food matrices and production environments, serve as transmission vehicles for this pathogen. While the sporulation niches along the farm-to-fork continuum are intricate to identify, their control becomes a significant challenge for the meat, poultry, and bakery industry as well as the regulatory agencies. This symposium will discuss the extreme conditions encountered during preservation techniques that favor the survival and selection of specific *Bacillus* strains, their resistance profile and the interactions within physiologies at the molecular level, and potential for opportunities to design novel control strategies to enhance efficiency, performance, and sustainability in the food chain.

S51 Safety and Quality of Water Used and Reused in Fresh Produce Supply Chains

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LEON GORRIS: *Food Safety Expert, Nijmegen, Netherlands, Netherlands*
ELISABETTA LAMBERTINI: *GAIN - Global Alliance for Improved Nutrition, Rockville, MD, USA*

The use of clean water is a basic principle in fresh produce growing, handling, and processing. To achieve a consume safe product at the end of the fresh produce supply chain, it is critical to understand how the water used for their production becomes contaminated and the ways in which we can prevent and mitigate the use of unclean water. Risk assessment studies have provided useful science-based inputs into risks and need for risk reduction measures in order to protect consumers.

It is expected that water reuse will increase dramatically in the near future due to water scarcity worsening in many parts of the world. As water scarcity pressure rises, well-informed decisions as to the safety of recycled or reclaimed water (including grey water, produced water, return flows, and recycled wastewater) used in fresh produce production is also necessary when promoting water reuse. To date, many sources of reusable water have not been widely explored and adequately assessed for their health risks at the detail required to ensure food safety and protect consumer health.

Various types of hazards are potentially introduced to fresh produce through water reuse; hence, these need to be thoroughly identified and evaluated for food safety risks. Where necessary, risks have to be reduced to acceptable levels through adequate treatment/technical intervention. Ultimately, criteria or thresholds to hazards/indicators may need to be established for day-to-day monitoring of an operation (re-)using water, as well as for regulatory purposes such as public health protection. Question is whether such approaches can be easily adopted and implemented in Low- and Middle- Income Countries, where water scarcity frequently occurs.

This symposium will discuss food safety and quality aspects of water use and re-use in the context of fresh produce supply chains. Questions in scope of the session are, for instance: which potential reusable water sources can be used in a fit-for-purpose approach to alleviate water scarcity problems? What is the experience of water (re-)use for different commodities and in different countries? How can we best use testing and threshold values to monitor water quality and how can we develop tailored values to different water (re-)use conditions?

S52 Data Trusts for Food Protection

BRENDAN RING: *Creme Global, Grand Canal Quay, Dublin, Ireland, Ireland*
TOM MADRECKI: *Consumer Brands Association, Arlington, VA, USA*
JENNIFER VAN DE LIGT: *ToxStrategies, Inc., Saint Paul, MN, USA*

Data has been referred to as the new oil of the 21st Century, given its resource value and potential to drive the economy. Like oil, refinement of and access to data are critical to realize its full potential. The FDA's New Era of Smarter Food Safety, an initiative to leverage technology and other tools and approaches to create a safer, more digital, traceable food system exemplifies the power of data sharing in the food community. Food companies and regulatory bodies around the globe share a common goal to deliver safe food to consumers. Both are also becoming increasingly aware of the value of sharing data, yet only few examples exist of data being shared across the food industry. Data sharing between companies (private), between companies and government regulators (public-private), and between regulating agencies (public) shows great promise for improving traceability, adopting and scaling artificial intelligence (AI) applications, and delivering safer food for all. However, there are significant obstacles to sharing data including privacy, trade secrets, and the potential for regulatory action. Data trusts, in which an independent organization serves as the fiduciary for the data and governs proper use of data, are a potential solution to these problems. In this symposium, speakers will describe what data trusts are, discuss data trust frameworks for secure and anonymous data gathering, provide examples of existing data trusts for food safety and protection, and illustrate the use of big data to predict critical supply chain infrastructure threats.

S53 Surrounded on All Sides: A Dive into the Unseen Microbiomes of Residential and Industrial Built Environments and Food Safety Implications

CHRISTINA K. CARSTENS: *University of Texas, School of Public Health, Houston, TX, USA*
MATT HENDERSON: *Land O'Frost, Inc., Munster, IN, USA*
BRANDON KOCUREK: *U.S. Food and Drug Administration, Laurel, MD, USA*

Next generation sequencing has become more prevalent in the research areas of ecology, food safety, and public health and has opened a whole new world of exploration into microbial communities. Microbiome research is not limited to the evaluation of microbial populations in food matrices, and it extends into the environments in which we live and work and produce and prepare our foods. The microbiome of the built environment, or the human-constructed environment, has the potential to influence food safety and public health outcomes at multiple stages along the farm-to-fork continuum. In this symposium, presentations from academia and industry will discuss the microbiomes of the domestic kitchen environment and the defined zones in the food production environment, respectively, and how sanitation and consumer food safety behaviors effect these communities. In addition, research from MetagenomeTrakr, a pilot program from the U.S. Food and Drug Administration with the goal of rapid foodborne pathogen detection from built environment microbiomes, will also be presented. How microbiome research will functionally improve testing and pathogen detection methods in the built environment to improve food safety will be discussed.

S54 Increasing Access to and Cultivating Diversity within Food Safety Spaces

REBECCA BRIGHTWELL: *University of Georgia, Athens, GA, USA*
YVONNE MASTERS: *John B. Sanfilippo & Son, Inc., Chicago, IL, USA*
MANAN SHARMA: *USDA ARS Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*

As a whole, the food and agriculture workforce represents the rich diversity of the United States. However, the diversity is not represented uniformly throughout all levels of industry, which has led to gaps in access and appropriate resources throughout the system. Food safety professionals must rise to meet various needs within the consumer base and their workforces alike, and work towards creating a fully representative system. This is especially important as food preparation methods change with cultural diversity, the medically vulnerable population increases, and the unique food safety needs of other historically underserved groups are recognized.

In 2020, the formation of IAFP's Diversity, Equity, and Inclusion (DEI) Council dedicated resources to expand the response to the plea for a more diverse space for food safety. The efforts of this council should enhance our association by fostering a safe, inclusive space for members; however, there remains a gap in equipping food safety professionals to bring DEI into our work outside of IAFP.

This symposia strives to facilitate a conversation around the significance of barriers associated with increasing access to food safety spaces, intersecting aspects of food safety and DEI. The first two presentations will cover basic topics, including cultural sensitivity and relevance in communication, creating and implementing culturally-aware materials and communications relevant to underserved populations, and accommodations in traditional and virtual food safety education spaces. The final presentations will highlight implementation of these topics in the workplace while incorporating relevant data, lessons learned, and specific action steps. Attending food safety professionals will leave with the knowledge to begin or continue evaluating workplace endeavors for equitizing access among diverse populations and the tools to implement accessible changes. With the sponsorship of IAFP's Webinar Committee, additional webinar sessions will follow the 2022 Annual Meeting for members to attend throughout the year. These sessions will provide more detailed topical discussions aimed at furthering effective implementation discussed in the symposium in real food safety settings.

S55 New Advances in *Alicyclobacillus* Detection, Differentiation, and Control

ABIGAIL B. SNYDER: *Cornell University, Ithaca, NY, USA*
ADAM JOELSSON: *Invisible Sentinel, Philadelphia, PA, USA*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*

Alicyclobacillus spoilage represents a significant threat to product quality for manufacturers of fruit juices and other beverages. Some estimates suggest that Alicyclobacillus spoilage has been an issue for the majority of juice manufacturers, resulting in the need for time consuming laboratory analyses, rejection of contaminated ingredients, and potential consumer dissatisfaction. Yet, our limited understanding of the mechanism of Alicyclobacillus spoilage has hampered our ability to effectively detect and differentiate spoilage causing species from those species unlikely to cause product spoilage. This reduces the ability of the food industry to make highly refined product disposition decisions and set meaningful microbial specifications for raw materials. Spoilage has primarily been associated with the production of the off-aroma guaiacol, although other halophenolic compounds have also been identified. Recent genomic and biochemical analyses have improved our ability to predict which strains are guaiacol producers. The translation of these findings into usable tools for the industry can enhance quality control programs and shorten the time required for analytical tests. This symposium will start with a discussion of recent advancements in our understanding of the Alicyclobacillus genus and distribution of genes associated with guaiacol biosynthesis therein. This will be followed by an in-depth presentation on recent innovations in analytical technologies for detection of Alicyclobacillus spp. and the use of genetic tools to predict guaiacol production. We will conclude with a discussion of industry applications, implications, and strategies in quality management enabled through the use of analytical testing.

S56 Infusing Cannabis Edibles with the Time-Tested Science of Food Safety

KATHY KNUTSON: *EAS Consulting Group, a Certified Group Company, Green Bay, WI, USA*
JOEL CHAPPELLE: *Attorney, Food Industry Counsel LLC, Milwaukee, WI, USA*
DARWIN MILLARD: *Final Bell Corp., Bowmanville, Ontario, Canada, Canada*

This session is intended to give insight into the growing trend of Cannabis being used as an ingredient in various foodstuffs. Contrary to many other ingredients, parts of the Cannabis plant are federally illegal and pose unique challenges to incorporation into food. Additionally, many of those creating Cannabis-Infused edibles have little experience in the food safety measures that are necessary to keep these ingestible items safe. We will discuss the Cannabis industry in general, highlight some regulations for food production, discuss best practices for the manufacture of Cannabis-infused foods and close with testing and labeling of these products.

S57 What Environmental Surveillance and Water Quality Can Tell Us about Antibiotic-Resistant Bacteria in Pre-Harvest Environments

JONATHAN FRYE: *USDA ARS Bacterial Epidemiology & Antimicrobial Resistance Research, Athens, GA, USA*
NIKKI SHARIAT: *University of Georgia, Athens, GA, USA*
ANDREA OTTESEN: *U.S. Food and Drug Administration, Center for Veterinary Medicine, Office of Research, Laurel, MD, USA*

Antibiotic-resistant (AMR) foodborne pathogens associated with surface water are an under-characterized threat to food animals, produce, and human health. Surface water serves as a catchment for runoff from natural, human, and agricultural activity which can lead to its contamination with AMR bacteria; conversely, surface water can also transmit AMR bacteria to food animals, produce, and humans. As water becomes a more critical resource, ensuring its suitability and quality for natural, agricultural, and human use has a direct impact on meat, fruit, and vegetable food safety. As water becomes a more limited agricultural resource, and as more and more sources are potentially enlisted in irrigating fruits and vegetables, more attention needs to be paid to the potential pathogens introduced to foods that humans consume. Attempting to understand factors that influence the presence of AMR pathogens in irrigation water as it is increasingly sourced from mixed-used (residential and agricultural) watersheds for farms of all sizes is essential to designing mitigation strategies for AMR in agricultural water. To that end, exploring strategies to sample, analyze and quantify pathogens and antibiotic resistance in the pre-harvest produce growing environments are critical. Presenters in this session will discuss methods to recover enteric pathogens and describe AMR genes and pathogens recovered from water. This symposium will describe how enrichment bias during recovery of pathogens may limit our understanding of specific AMR bacteria and genes. Speakers will also address how the resistome of specific watersheds can impact food safety. This information will aid in the development of informative environmental surveillance programs that will yield appropriate data targets for interventions to reduce the threat of waterborne AMR bacteria on food safety. The session will provide academic, government, and industry professionals up-to-date information on this emerging trend.

S58 Gluten in Fermented or Hydrolyzed Foods – Regulatory, Consumer, and Analytical Perspectives

CAROL D' LIMA: *U.S. Food and Drug Administration, College Park, MD, USA*
AMY KELLER: *Gluten Free Watchdog LLC, Marysville, OH, USA*
TRICIA THOMPSON: *Gluten Free Watchdog, LLC, Manchester, MA, USA*
RAKHI PANDA: *U.S. Food and Drug Administration, College Park, MD, USA*

Celiac disease affects approximately 1 in 141 individuals in the United States, requiring adherence to a strict gluten-free diet. In an effort to ensure consumers are provided with truthful and accurate information, in 2013, the FDA issued a regulatory definition of the food labeling term "gluten-free" and uniform conditions for its use. Gluten-free was defined as less than 20 ppm (mg/kg) gluten. The 2013 gluten-free rule also addressed the uncertainty in interpreting the results of current gluten test methods for fermented or hydrolyzed foods in terms of intact gluten. Detection of gluten in fermented or hydrolyzed foods such as cheese, yogurt, vinegar, sauerkraut, pickles, green olives, beer, wine, or hydrolyzed plant proteins is uniquely challenging due to the complexity of the fermentation process and high variability in protein/peptide profiles, which may vary based on numerous parameters used during fermentation or hydrolysis. Currently there are no scientifically valid analytical methods for accurately quantifying the gluten protein content in fermented or hydrolyzed foods. The presence of gluten in fermented or hydrolyzed foods that are labeled as "gluten-free" could pose significant health challenge for individuals with celiac disease. Therefore, in 2020, the FDA issued a new regulation for fermented, hydrolyzed, and distilled foods that uses alternative means to verify compliance with the provisions of the 2013 gluten-free food labeling final rule. This symposium will present an overview of the new regulation on the use of gluten-free labeling for fermented, hydrolyzed, and distilled foods. The challenges faced by consumers in maintaining a gluten-free diet due to the presence of gluten in "gluten-free" labeled fermented or hydrolyzed foods will also be discussed. The session will close by discussing the shortcomings of current gluten detection methods for accurate quantification of gluten in fermented or hydrolyzed foods, and recent progresses in this direction.

S59 Computer Modeling – The Next Step in the Dairy Industry Evolution

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SARAH ENGSTROM: *Grande Custom Ingredients Group, Fond du Lac, WI, USA*
MANON RACICOT: *Canadian Food Inspection Agency, St-Hyacinthe, QC, Canada, Canada*

As the world moves toward digitization and automation, an exponential increase in the pool of generated data has occurred across all aspects of society, including the food industry. These data can be leveraged to produce added value through the development of computer models able to assist in future decision-making. The key focus of this symposium will be to introduce the benefits of these computer models to the dairy industry.

This symposium will cover computer models that were developed in recent years for application in the dairy industry. Four expert speakers from the dairy industry, academia and government will each present on the development process and use of computer modeling tools in their specific sectors, with specific focus on:

- Spoilage prediction in fluid milk,
- Thermalization processes for safer raw milk cheeses,
- Data-driven food safety risk assessment in dairy processing, and
- Facility-specific COVID-19 mitigation strategies for dairy establishments.

Through these presentations, the symposium attendees will be introduced to a diverse set of different computer modeling tools that will cover food safety and quality applications in cheese, fluid milk, and yogurt. Further, we believe this symposium can open the door to future innovations under the common term "Digital Dairy".

The target audience of this symposium is members of the academia, regulatory and industry sectors who are interested and/or involved in data-driven quality and risk management.

S60 Life at the Extremes: Fungal Spoilage in Low Water Activity, High Acid, and Thermally Processed Foods and Beverages

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EMILIA RICO: *BCN Labs, Rockford, TN, USA*
MARGARITA GOMEZ: *Retired, Long Beach, CA, USA*

Fungal spoilage in processed foods remains a challenge. Physiological features of yeasts and molds contribute to their tolerance to thermal processing, acidity, desiccation, and oxygen and nutrient limitations. These features variably include growth form, cell wall structure, cytoplasmic composition, cell membrane-bound proteins, and secretion of secondary metabolites. Collectively, these mechanisms contribute to the ability of fungi to disperse, survive, and propagate in highly restrictive food environments. The diversity of fungal growth and survival mechanisms has resulted in organisms adapted to nearly all food environments; although, only a small subset of fungi are particularly suited for spoilage of a given product. The relationship between the individual physiology and metabolic capabilities of a yeast or mold and the product's specific physicochemical attributes and processing history determines spoilage potential. Improved understanding of these relationships contributes to more targeted and effective control strategies. The goal of this symposium is to highlight research that facilitates recognition of the specific spoilage organism in a given product system and identification of the most practical mitigation methods for industry. We will start with a discussion of how the extremotolerance of different fungi impacts the likelihood that a given species will cause spoilage in particular products. Next, we will discuss how to recover and identify spoilage fungi present in different and diverse food systems. Finally, we will conclude with a discussion from industry highlighting inactivation of heat resistant molds assessing findings from other fields – in this case, spacecraft decontamination of bacterial spores.

S61 Mixed Methods Approaches to Investigating Microbial Produce Safety Hazards and Mitigation in Hydroponic and Aquaponic Operations

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MENGYI DONG: *University of Illinois At Urbana-Champaign, Urbana, IL, USA*
SANJA ILIC: *The Ohio State University, Columbus, OH, USA*

Research addressing microbial hazards in hydroponic and aquaponic crop production systems is in the beginning stages, in part due to the relatively recent growth of these operations in major commercial markets. For this reason, there is a lack of evidence-based produce safety guidance for growers and for regulatory inspectors. Speakers will address the intersection of research and practice by explaining the rationale behind their mixed methods research approaches and sharing practicable insights from their results.

First, an assessment of hydroponic grower food safety needs was conducted along with an investigation of microbial loads on hydroponic and soil-grown leafy greens obtained at retail. Results of the microbial assessment showed no significant difference in microbial loads on hydroponic and soil-grown greens. The grower needs assessment highlighted the need for hydroponic specific food safety training for growers.

Secondly, two commercial hydroponic and aquaponic farming systems were investigated utilizing a food safety practice survey and NGS microbial community analysis. This approach allowed for characterization of bacterial transmission routes within each system, detected spoilage microorganisms and potential pathogens, and yielded system-specific suggestions for improving produce safety and system health.

Lastly, researchers tracked the survival of *Listeria monocytogenes* and *Salmonella* Typhimurium in experimental hydroponic leafy greens systems using the nutrient film technique (NFT). The persistence of *Listeria* and *Salmonella* in the nutrient solution and plant rooting media, along with detection on leaves, provided evidence for the ability of this pathogen to disperse in an NFT system and potentially contaminate produce. On-farm sanitation strategies for pathogen control were also evaluated and results were incorporated into a publication for hydroponic grower training.

Mixed research approaches can help us to understand the on-farm context for produce safety decisions while providing quantitative evidence to inform guidance for growers. We hope to provide a starting point to inspire future projects and collaboration around the topic of produce safety in hydroponic and aquaponic operations.

S62 Mitigating the Risk of *Salmonella* in Food Products

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J. EMILIO ESTEBAN: *U.S. Department of Agriculture – Food Safety Inspection Service, Washington, DC, USA*
NIKKI SHARIAT: *University of Georgia, Athens, GA, USA*
ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*

The landscape for mitigating the risk of pathogens in food is constantly evolving for the industry. Risk is an inherent part of business and companies cannot predict every challenge but deploying mitigation strategies can prove to be beneficial. While HACCP plans have been employed, and companies have worked diligently to reduce outbreaks of *Salmonella*, they still happen. We can do better. We have to.

The intention of this Symposia is to highlight the continued threat that Salmonella poses to the food supply chain and to share a few emerging strategies for mitigation.

In this session, we will discuss the FDA and USDA perspectives on *Salmonella* and the associated risk and then discuss a novel CRISPr application for *Salmonella* serotyping. Finally, we will close with a look at how Cargill reduces *Salmonella* risk.

S63 Precision Genomics: A Toolbox for the New Era of Food Safety

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XIANGYU (SEAN-U) DENG: *University of Georgia Center for Food Safety, Marietta, GA, USA*
ABIGAIL B. SNYDER: *Cornell University, Ithaca, NY, USA*

Whole genome sequencing has been utilized for the past 10 years to characterize antimicrobial resistance, virulence genes, and serovars. The NCBI Pathogen Detection browser contains over 390,000 *Salmonella* isolates, over 190,000 *E. coli* isolates, and roughly 46,000 *Listeria monocytogenes* isolates. Researchers have shown that AMR genotypes correlate strongly with AMR phenotypes and this data has been extremely important to public health. Recently, NCBI's Pathogen Detection Browser has implemented AMRFinder Plus which also detects genes that can lead to tolerance of biocides, heavy metals, heat, and acids. To gain even more insights from this data, we need to focus on Precision Genomics and utilizing functional genomic studies to further enhance our knowledge of these pathogens.

In this session we are going to present different viewpoints and examples of the use of Precision Genomics to further utilize the vast library of sequencing data. Virulence gene profiling of *Salmonella enterica* can aid in risk assessment and determination of severity illness due to select serovars similarly to what is currently being performed with Shiga-toxin producing *Escherichia coli.* Further, complete metadata along with the sequence data allows for future genomic source prediction from newly sequenced strains using machine-learning approaches. The presence of biocides and heavy metals tolerance genes and the further phenotypic analysis (MIC/MBC) of bacteria isolated from food-relevant environments can shed light on strain history and selection dynamics. The mechanisms responsible for survival and persistence in specific environmental conditions and stresses can be further elucidated using transcriptomics, epigenetics, and dense transposon mutant libraries.

S64 The Regulation of Food Ingredients in Diverse Global Markets

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LUCA BUCCHINI: *Hylobates Consulting, Rome, Italy, Italy*

Regulation of ingredients added to food is a critical component of food safety and presents a valuable opportunity to address safety concerns before products reach consumers. However, the path towards regulating an ingredient for use in food can be complex and depends on the proposed food categories and use level, the intended technical effect, the production process for the ingredient, and other relevant safety data and information. For example, before an ingredient may be lawfully added to products marketed in the United States, the ingredient must be 1) an approved food or color additive, 2) determined generally recognized as safe (GRAS) by qualified experts for the intended conditions of use, 3) prior sanctioned for that use by the Food and Drug Administration (FDA) or the United States Department of Agriculture (USDA), or 4) fall into another exempt category. Advances in food processing technologies - including the use of antimicrobial ingredients, microbial fermentation processes, and ingredients derived through biotechnologies – and the increasing diversity of the global food system present regulatory bodies and food safety professionals alike with novel challenges in evaluating the safe use of food ingredients. This symposium will serve as a primer for food safety professionals on the process of food additive regulation, focusing on the regulation of food and color additives in global markets including the United States, Europe, China, and Asia. Speakers will explore the role of food ingredient regulation and other pre-market review programs in promoting a safe food supply. Speakers will also cover the regulatory pathways for ingredients added to food and highlight new regulatory responses to emerging technologies in food production.

S65 Lessons Learned from Produce Safety Rule Trainings to International Audiences in Latin America

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ANA MARISA CORDERO: *IICA, San Jose, Costa Rica, Costa Rica*
SERGIO NIETO-MONTENEGRO: *Food Safety Consulting & Training Solutions, LLC, El Paso, TX, USA*
CLARE NARROD: *Joint Institute for Food Safety and Applied Nutrition/ University of Maryland, College Park, MD, USA*

The Food Safety Modernization Act (FSMA) of 2011 resulted in a number of new rules and the development of associated training material with each rule to ensure the safety of food consumed in the U.S. FSMA also for the first time mandated the U.S. Food and Drug Administration (FDA) to provide capacity building abroad to aid exporters to comply with the new requirements. FDA provided funding through several cooperative agreements to deliver training on the Produce Safety Rule (PSR) through train-the-trainer and grower training programs in Latin America as well as conduct monitoring and evaluation research to capture the impact of such efforts. The purpose of this symposium is to share what has been learned as these educational efforts took place so we can share with others involved in rolling out training to international audiences so they can do it more smoothly in the future. Rolling out such training in Latin America has proved challenging on some grounds. In the symposium we will discuss lessons learned from FDA funded delivery of the train-the-trainer and grower trainings in Latin America by U.S and Latin American trainers. This includes findings from the monitoring and evaluation of courses and the needs and challenges for mentoring potential lead trainers. Findings from a needs assessment that took place in 2020 resulting in the development of different types of supplemental training material for growers ranging from owners, supervisors, and workers in different formats (on-line, telephone apps, infographic, and mimes, etc.) will be discussed. Lastly, the behavioral experiments associated with evaluating the effectiveness of the supplemental material to the current PSR grower training and various training platforms will be discussed.

S66 To Biofilm, or Not to Biofilm: *Listeria monocytogenes*' Emerging Existential Dilemma

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LISBETH TRUELSTRUP HANSEN: *National Food Institute, Technical University of Denmark, Lyngby, Denmark, Denmark*
MAGDALENA OLSZEWSKA: *Center for Food Safety, University of Georgia / Department of Industrial and Food Microbiology, University of Warmia and Mazury, Olsztyn, PL/ Griffin, GA, USA*

Considering that *Listeria monocytogenes* (Lm) can form a biofilm on a food-contact surface, it could become a consistent contamination source. It has been reported that the same Lm pulsotypes have been isolated from a food processing plant multiple times throughout the year. Previously, multiple studies have observed significant differences in gene expression between sessile and planktonic Lm cells. In contrast, newer studies uncover novel routes by which biofilm formation by Lm can be impacted, for instance, crucial roles for SigB and wall rhamnosylation. Also, which genes are involved in Lm biofilm formation under conditions resembling the food processing environment, like lower temperatures or dynamic conditions.

Moreover, studies on whether or not high biofilm-forming capacity is related to the persistence and prevalence of specific genotypes in food processing environments have yet to be completed. To generate structural data to understand better the complex behavioral and survival strategies of Lm biofilms as well. A vast majority of published data comparing biofilm formation by Lm are based on the global quantitative crystal violet assay, which does not give access to structural information. Using a high-throughput confocal-imaging approach, deciphering the architecture of Lm biofilms is feasible, shedding some light on Lm biofilm traits, including its extracellular matrix. While exopolysaccharides have been described as the extracellular matrix's primary component in many bacterial biofilms, their presence and contribution in Lm biofilm await further investigation. Indeed, as observed in some model biofilm-forming bacteria, a thick and slimy extracellular polymeric substance is not present in Lm. However, recent reports have begun to recognize the significant role of exopolysaccharides in this pathogen. One of them is cell surface-bound which protects cells against disinfectants and desiccation, indicating its potential significance for listerial persistence in the environment and food safety.

This symposium will address the question on Lm capability to form biofilms with relevance not only to different commodities at the pre-harvest, processing and retail environment.

S67 Transmissible Locus of Stress Tolerance (tLST) in Bacteria, a Potential Threat to Food Safety and Public Health

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MICHAEL GÄNZLE: *University of Alberta, Edmonton, AB, Canada, Canada*
UTE RÖMLING: *Department of Microbiology, Tumor and Cell Biology Biomedicum C8 Karolinska Institutet, Stockholm, Sweden, Sweden*
JOSEPH BOSILEVAC: *U.S. Department of Agriculture – ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*

A genomic island found in various species of proteobacteria can confer heat resistance. It was termed as the locus of heat resistance in *Escherichia coli*, but as the transmissible locus of quality control in *Pseudomonas aeruginosa*. It has been renamed to the transmissible locus of stress tolerance (tLST) recently, due to its involvement in tolerance to multiple antimicrobial interventions (e.g., elevated temperature, pressure and chlorine stresses) and its transmissibility. tLST may contribute to the persistence of pathogenic bacteria in food production and clinical settings. The *D60°C* (the time used to inactivate 90% of population at 60°C) of tLST negative *E. coli* is generally < 2min, however, *D60°C* of tLST positive strains can be from ~2 min to even >70 min. Human activities including thermal interventions in food production chain, chlorination of municipal waste water and the disinfection practices in hospitals may select for tLST positive bacterial strains. tLST harboring *E. coli* have been isolated from clinical samples, livestock, meat processing environments, and found to be predominant in raw milk cheese (36.3%) and wastewater (59%) isolates. Whether or not tLST confers tolerance to other stresses, for instance, antibiotics is unknown. In addition, the original host of tLST and the selective pressure for the spread and maintenance of tLST still remains elusive. Recent genomic and functional analyses may shed light on our understanding of the evolution of tLST, which may lead to its potential control.

The objective of this session is to call for scientists in this area and to discuss the recent findings regarding tLST, with a focus on the mechanisms of tLST conferring stress resistance, the implications of tLST to food safety and public health, the potential anthropogenic and natural selective pressures for the maintenance of tLST and the potential measures that can help to reduce the spread of tLST.

S68 Foodborne Pathogens and Vulnerable Populations: Protecting and Educating the Immunocompromised

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YAOHUA (BETTY) FENG: *Purdue University, West Lafayette, IN, USA*
RENEE BOYER: *Virginia Tech, Blacksburg, VA, USA*
SANJA ILIC: *The Ohio State University, Columbus, OH, USA*

Foodborne illnesses are more likely to occur and are of greater severity among the immunocompromised. Immunocompromised individuals make up almost 20% of the population in the UK and the USA. Numerous factors can lead to increased susceptibility and illness by the major foodborne pathogens. Hence, novel methods should be explored to prevent foodborne disease among this contingent. Some of these include HACCP-based initiatives as well as population-specific education. The aged (≥65 years of age) make up a segment of society that are at greater risk for foodborne illnesses. Due to a decreased immune response as the body ages, the elderly are more likely to succumb to gastroenteritis, which, for the aged, can be fatal. Furthermore, they may also more frequently suffer chronic illness, increasing their susceptibility. As the average lifespan increases, so will the proportion of elderly who are more at risk. Thus, food safety associated with senior care facilities are key. Studies have also indicated that the aged may succumb to illness at a lower infectious dose. Other immunocompromised also include expectant mothers and their unborn. In some cases, the mother may be asymptomatic to foodborne pathogens yet the unborn may suffer severe consequences. Specialized instruction can enhance knowledge of food safety choices and food handling practices for expectant mothers. Of special concern are the non-English speaking who may need more targeted instruction. As the western world becomes more heterogeneous, the need for multilingual instruction in the area of food safety for expectant mothers is critical. The final presentation will discuss food intake, food safety attitudes, knowledge, and behaviors among cancer patients receiving treatment, with a special focus on socioeconomic factors, such as food insecurity, and reliance on food assistance programs. The opportunities for novel food safety interventions for this population will be highlighted. This symposium will address the above-mentioned topics, providing an overview of protecting and educating vulnerable populations to foodborne pathogens.

S69 Spoiled Seafood? Advancements in Detecting Decomposition

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PATTI ROSS: *U.S. Food and Drug Administration, Collage Park, MD, USA*
PAUL SARNOSKI: *University of Florida, Gainsville, FL, USA*
RANDY SELF: *U.S. Food and Drug Administration, Bothel, WA, USA*

A primary reason for seafood refusal at importation globally, is evidence of decomposition. Decomposition is assessed by inspection programs that apply human sensory and/or chemical analyses (histamine, indole) and/or review of temperature monitoring records (HACCP). Sensory programs use highly trained and standardized human analysts; however, concerns over the subjectivity of this program have been raised. Additionally, there are concerns over the applicable of current chemical indices for low temperature decomposition. Although sensory-driven predictive models using chemical analyses have been developed, the main challenge is the unique chemical profile associated with the decomposition of different seafood species. Recent advancements in using instruments for the analysis of sensory attributes and microbial communities have been somewhat successful in increasing our knowledge of the decomposition process. This session will discuss the sensory analysis process applied to seafood and new technologies that have been developed to augment the sensory analysis of seafood decomposition.

S70 Mind the Gap: The Role of the Frontline Voice in Food Safety Culture Improvement

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EMMA SAMUEL: *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom, United Kingdom*
NIC SHARMAN: *Nic Sharman Consultancy, Didcot, United Kingdom, United Kingdom*
SOPHIE TONGYU WU: *University of Central Lancashire, Preston, United Kingdom, United Kingdom*

Effective recognition systems are key in cultivating and maintaining a positive food safety culture. Consequently, an alignment of management and food handler food safety priorities is necessary to ensure that misalignment does not result in underperforming control systems, stifled cooperation between hierarchies, ineffective communication and under-utilization of the workforce competence, as shown in an intercontinental study done in Zambia, Tanzania, Greece and China. Therefore, to identify where misalignment in accordance with food safety culture characteristics may exist, evaluating hand hygiene behaviour (essential to support food safety) in food manufacturing and processing environments in Wales (UK) may be a useful approach to indicate strengths and weaknesses within the prevailing culture that facilitate or hinder such working relationships. Adopting

a triangulated research approach, underpinned by the Global Food Safety Initiative's Food Safety Culture Dimensional Framework, ensures that management have greater insight into the day-to-day challenges faced by production staff and that the frontline voices are recognised and heard when managing the change process. Reflecting on observations from food safety audits conducted around the world, audits can offer a lens into a business's culture based on observations and talking to the frontline. Although audits are a snapshot, when assessing companies' approach to managing food safety, it shows their strengths and weaknesses with how the frontline staff and management team deliver food safety on an everyday basis. Indeed, businesses of different sizes approach their food safety culture journeys differently. Regardless of the structure for communications, frontline's voices can make a significant impact in a company's culture. Using a novel real-time culture feedback program in food businesses in the United Kingdom, frontline's opinions, attitudes, perceptions, and self-reported behaviors are collected, which serve the basis for deriving incremental weekly action. This incremental 'nudging' is critical to establish internal rhythm for consistently communicating food safety and ultimately achieving continuous improvement. While transitional changes are also identified as necessary in some participating companies, the key is to maintain consistent frontline engagement, and the utilization of real-time feedback technology to capture workforce opinions has been shown to positively promote general discussions around safety.

Special Session

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

ABDOULIE JALLOW: *Food Safety & Quality Authority of the Gambia, Serre Kunda, KMC, Gambia, Gambia*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia, Ethiopia*
ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture Abeokuta, Abeokuta, Nigeria, Nigeria*
BARBARA KOWALCYK: *The Ohio State University, Center for Foodborne Illness Research and Prevention, Translational Data Analytics Institute, Columbus, OH, USA*
LUCIA ANELICH: *Anelich Consulting, Pretoria, South Africa, South Africa*

Note: this is not a symposium or round table, but an interactive – "walking around" type event

The African continent is enormously large and includes 55 countries that are very diverse in social, cultural, and economic aspects. For the last 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.

There has been an effort made by the proposers and others to increase the general awareness within IAFP about the many and diverse food safety challenges across Africa that professionals in government, industry and academia meet day-by-day. Our next aim is to support further interactions between food professionals in IAFP that may help address such challenges.

We note that the African Union is increasing its attention to food safety, building a dedicated food safety strategy and driving for more regulatory harmonization, amongst other strategic objectives. There is a need and opportunity to more effectively address food safety challenges across the region/continent, but the scale of this is one that would benefit greatly from international collaboration of food safety professionals.

Such collaborations have been going on for many decades between countries, international Organizations as well as local and foreign academics and scientists abroad and have made progress. Yet, more needs to be done and more can be done.

For both the food safety professionals from Africa and members of the IAFP community, there is thus ample opportunity to join forces and help tackle food safety challenges by for instance sharing knowledge, establishing new, tailored insights/solutions and developing food safety capabilities.

But how do you know what the options are? What are the challenges you could engage on? Who are your possible contacts in Africa or elsewhere in the IAFP community? What are options for support/funding? A key answer to this is: you have to get connected!

For that reason, an interactive session ("market") is proposed as part of the IAFP Annual Meeting. This market would be an opportunity for food safety food professionals working in various different public and private sector roles to connect with others, share their ideas on challenge areas or solutions to collaborate on, offer their scientific/technical expertise or practical experience in developing joint projects and mobilizing funding.

Roundtable Abstracts

RT1 How Relevant is Finished Product Testing for Pathogens to Public Health Outcomes?

HEATHER CARLETON: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ROY BETTS: *Science Fellow, Campden BRI, Chipping Campden, United Kingdom, United Kingdom*
MARTIN DUPLESSIS: *Food Directorate, Health Canada, Ottawa, ON, Canada, Canada*
BENJAMIN WARREN: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*

FDA's final rule "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" (the CGMP & PC rule) requires a facility that has identified hazards requiring preventive controls to verify that the preventive controls are consistently implemented and are effectively and significantly minimizing or preventing the hazard. Facilities also need to show the product has not been recontaminated after the preventive control. Verification activities can include physical measurements of the critical limits, product design measurements, environmental and/or product testing for indicator organisms or pathogens, which depend on the preventive control(s) and risks. However, many producers are asked by their customers to test for a multitude of pathogens in their finished product and their environment, to satisfy requirements for lot acceptance, regardless of whether the tests are relevant or even validated for the matrix in question. As a result, excessive testing diverts resources that could be focused on the real defined risks as well as causes products to be rejected to then be reworked or thrown away/wasted when they would not cause a public health issue. In addition, consumers may lose confidence in the food industry when recalls are announced that had no public health implications. This roundtable will discuss criteria for choosing organisms for testing finished product and/or the environment, debate if testing finished product vs. using HACCP monitoring, verification, and validation data are better indicators for lot acceptance, how human behavior fits into public health relevance, what should be recalled when no imminent public health risk has been identified, and whether zero tolerance should be the focus when testing.

RT2 Flour and Shiga Toxin-Producing *Escherichia coli* (STEC): What Can be Done to Prevent Outbreaks?

YAOHUA FENG: *Purdue University, West Lafayette, IN, USA*
KELLY STEVENS: *General Mills, Minneapolis, MN, USA*
STEPHANIE NGUYEN: *Conagra Brands, Omaha, NE, USA*
KALIRAMESH SILIVERU: *Kansas State University, Manhattan, KS, USA*
LINDA J. HARRIS: *Department of Food Science, University of California, Davis, Davis, CA, USA*
APARNA TATAVARTHY: *U.S. Food and Drug Administration, College Park, MD, USA*

Approximately 95% of the flour produced in the US goes to food manufacturers, processors, and institutions where a pathogen reduction step in the form of baking, frying, or cooking is applied commercially. The remaining 5% of the flour that enters retail markets (e.g., all-purpose flour, baking or cake mixes, seasoning blends, raw dough products) relies on the consumer to use safe handling practices in the home to prevent cross-contamination and to reduce pathogenic microorganisms by adequately heating or baking products made with flour. However, recent research has shown that consumers have misperceptions surrounding the safety of handling and consuming raw flour and may not be following instructions appropriately. Several recent outbreaks of Shiga toxin-producing E. coli (STEC) gastroenteritis in the US have been associated with consumption of raw wheat flour-based products prepared in the home. These outbreaks, especially the ones suspected to be associated with consuming raw batter prepared from flour and baking mixes, heightened the need to apply risk-based preventive controls to identify sources of contamination and pathogen reduction strategies.

Research to identify a pathogen reduction preventive control for retail flour continues as current interventions such as heat treatments may alter flour functionality and quality. Applying mitigation strategies and preventive controls earlier in the wheat milling process (e.g., tempering) may be necessary. However, a multipronged approach including various steps before and after milling coupled with effective consumer education and outreach may be necessary to prevent future outbreaks. Panelists in this roundtable include researchers that will highlight potential process preventive controls and consumer perceptions and potential educational strategies. Members from the flour industry will speak to the existing prevention strategies and challenges during an outbreak. The U.S. Food and Drug Administration will provide a regulatory perspective on the current thinking on prevention strategies for this commodity.

RT3 COVID-19: What Have We Learned to Make Our Food Systems More Resilient in the Future?

JORGE PINTO FERREIRA: *FAO-Food and Agriculture Organization, Rome, Italy, Italy*
PETER BEN-EMBAREK: *WHO, Geneva, Switzerland, Switzerland*
SUCHART CHAVEN: *PepsiCo, Valhalla, NY, USA*
JEFFREY FARBER: *Food Safety Consultant, Toronto, ON, Canada, Canada*
LUCIA ANELICH: *Anelich Consulting, Pretoria, South Africa, South Africa*

The globalization of food sourcing and trade, the extensive movement of people and intensive food production practices are challenging the resilience and sustainability of food systems and value chains around the world. While COVID-19 is not a foodborne illness, the perception in many parts of the world was very different early on in the pandemic. International intergovernmental organizations such as FAO and WHO, supported by governments and science organizations, have made efforts to clarify the scientific insights and drive for risk-based management.

The pandemic illustrates the challenges for stakeholders to establish and communicate sound science- and risk- based information in a timely and understandable manner when confronted with a rapidly emerging issue. Additional challenges for stakeholders included organizational impacts of COVID-19 and the need to rapidly adapt and develop new ways of working to e.g. be able to discharge governance responsibilities and keep food supply chains operational.

Although the pandemic is still having a significant impact on society, it is important to start compiling and sharing the learnings from within government, industry and academics. These should be used to better manage consumer safety risks and potential disruptions of food supply chains within and across borders when faced with the next crisis, whether due to another influenza pandemic or a serious international foodborne outbreak.

This roundtable session is an initiative of the International Commission on Microbiological Specification of Foods. It brings together food professionals from around the globe representing several stakeholder groups that will share learnings from their perspectives and will discuss how these learnings are or can be used to make food safety management more resilient going forward and better control foodborne illnesses.

RT4 The Intersection of Adjacent and Nearby Land Use and Produce Safety

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NATALIE KROUT-GREENBERG: *California Department of Food and Agriculture, Sacramento, CA, USA*
ASHLEY EISENBEISER: *Food Marketing Institute, Arlington, VA, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
REBECCA BELL: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
KAREN KILLINGER: *U.S. Food and Drug Administration, College Park, MD, USA*

Recent multi-state outbreaks associated with leafy greens, peaches, and onions have highlighted that certain types of adjacent and nearby land use can impact the safety of fresh produce. The FDA's investigational findings from these outbreaks suggest that potential contributing factors include the proximity of cattle and poultry operations and of other animal activity to produce fields and agricultural water sources and systems. FDA, other federal and state agencies, industry and other groups are addressing this produce safety concern through a variety of efforts. Outreach and educational efforts are underway to increase awareness about this potential produce safety concern. These efforts include emphasizing Produce Safety Rule requirements and good agricultural practices that relate to addressing potential hazards associated with adjacent and nearby land use practices. Research is also underway to better understand the relationship between adjacent and nearby land use and the safety of nearby produce. This roundtable will provide an opportunity for participants to learn about the various initiatives underway to address this produce safety concern, and engage in discussion with thought-leaders on how to continue making progress in this challenging area.

RT5 Practical Approaches to Enhance Food Safety Culture: Shared Learnings from a Dairy Industry-wide Program

BRAD SUHLING: *Prairie Farms Dairy, St. Louis, MO, USA*
JONATHAN FISCHER: *HP Hood LLC, Wilmington, MA, USA*
KAREN MCCARTY: *Agropur, Inc., Le Sueur, MN, USA*
JEREMY TRAVIS: *Hilmar Cheese & Ingredients, Hilmar, CA, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*

The U.S. Dairy Industry frequently works together pre-competitively to identify, share, and adopt Best Practices. In 2020 we launched a Food Safety Culture initiative across companies with a culture maturity survey, anonymous industry-wide benchmarking dashboard, and the sharing of Culture implementation best practices. As of late 2021 over 50 U.S. dairy plants have completed assessments, a dashboard has been shared, and we've met to share observations and discuss best practices. In many ways, Dairy represents the entire food industry, but we'll also share some of the differences when compared to a global database across Food. The panel will include the Culture champion from several dairy processors in addition to Lone Jespersen who is guiding the efforts with us.

RT6 What Do We Know and Still Not Know about Pathogen Control in Low-Moisture Foods?

BECKY DOUGLAS: *Tree Top, Inc., Selah, WA, USA*
RICO SUHALIM: *PepsiCo, Plano, TX, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
JUMING TANG: *Washington State University, Pullman, WA, USA*
MERYL SILVERMAN: *USDA/FSIS, Washington, DC, USA*
BRADLEY MARKS: *Michigan State University, East Lansing, MI, USA*

Foodborne disease outbreaks and recalls associated with a wide range of low-moisture foods have occurred over the past few decades, including in almonds, spices, dried fruits and meats, baby formula, crackers and other snacks. These outbreaks have raised concerns among scientists in the food industry, regulatory agencies, and the wider scientific community as they pose new food safety challenges in food supply chains. One concern is that bacterial pathogens, in particular *Salmonella* and *Listeria monocytogenes,* show much higher resistance to thermal treatments in low-moisture foods than when they are present in high-moisture foods. Lack of knowledge of the impacts of key parameters on pathogen thermal resistance have hindered industry efforts to assess, develop, and validate thermal treatments for pathogen control in low moisture foods.

Over the past ten years, the USDA's National Institute of Food and Agriculture (NIFA) has supported major research projects on this subject. Food companies have also devoted much effort to develop and validate intervention strategies for specific low=moisture foods.

This roundtable session brings together six experts from academia, the food industry and regulatory agencies to share their views around the question of what we know and do not know related to pathogen control in low-moisture foods. The session will also serve as a forum to gather expert input from the audience and identify knowledge gaps to help inform future research and development efforts. In the session, each panelist will give a 5-min talk on established knowledge based on sound experimentation/research or many years of effective industry practices, and identify the missing knowledge and data gaps that have hindered industry practices to ensure food safety in low-moisture foods. The rest of the session will allow active dialog between the audience and panelists.

RT7 Recent State and Local Outbreak Investigations

MUGDHA GOLWALKAR: *Tennessee Department of Health, Nashville, TN, USA*
BEVIN DURANT FIDLER: *Pennsylvania Department of Agriculture, Harrisburg, PA, USA*
CHANDRA KANWAT: *South Carolina Department of Health & Environmental Control, Columbia, SC, USA*
KELSEY HOLLOMAN: *Virginia Department of Health, Richmond, VA, USA*
RANDY J. TREADWELL: *Association of Food & Drug Officials, York, PA, USA*

Each year state and local food regulatory agencies investigate thousands of potential foodborne outbreaks. This session will highlight the efforts of state and local food regulatory agencies in the investigation of foodborne illnesses including the U.S. Food and Drug Administration-funded Rapid Response Teams (RRT). The session will focus on the use of techniques such as environmental assessments, environmental sampling, and whole-genome sequencing to solve outbreaks at the state and local levels. The session will also discuss lessons learned and contributing factors identified during the investigations.

RT8 Hold the Phone! The Role of Celebrity Chefs and Influencers in Food Safety Messaging

CHEETIE KUMAR: *Garland, Raleigh, NC, USA*
BRITANNY SAUNIER: *Partnership for Food Safety Education, Arlington, VA, USA*
ELLEN EVANS: *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom, United Kingdom*
KATIEROSE MCCULLOUGH: *North American Meat Institute, Washington, DC, USA*
NICOLE ARNOLD: *East Carolina University, Greenville, NC, USA*
AARON LAVALLEE: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*

As more consumers turn to social media while cooking at home for recipes and cooking tips, celebrity chefs and food "influencers" have an opportunity to share food safety information through a wide array of methods – from recipes shared on Facebook and Instagram to short informational videos on TikTok. In many cases, these individuals miss the opportunity to communicate effectively about food safety, sometimes going as far as sharing incorrect or misleading information. In other public health fields, data shows that consumers are more likely to follow health guidance when it comes from a source that they trust, but it is not fully understood whether this plays out with celebrity chefs/food influencers and consumer food safety behavior.

In this roundtable, panelists will discuss current trends among celebrity chefs and food influencers as it relates to food safety. This session will also include a discussion of engaging with food influencers about incorrect food safety information "in the wild." Panelists will share what they see the role of celebrity chefs/influencers to be in consumer food safety in the coming years, as well as strategies currently under exploration for supporting these audiences in providing food safety information.

RT9 Can We Rely on Third Party Auditors to Assess Whether a Supplier's Microbial and/or Chemical Test Methods are the Right Fit for the Food Commodity?

SANDRA JOHNSON: *SGS North America, Oklahoma City, OK, USA*
APRIL BISHOP: *TreeHouse Foods, Oak Brook, IL, USA*
HOWARD POPOOLA: *The Kroger Co., Cincinnati, OH, USA*
JEFFREY LUCAS: *Mérieux NutriSciences, Luling, TX, USA*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*
MOLLY MILLS: *Kerry, Beloit, WI, USA*

A certificate of analysis (COA) is generally required for every shipment of micro-sensitive or high-risk ingredients prior to unloading of the material into the manufacturing facility. The COA would either accompany the shipment or submitted electronically prior to delivery. Plant personnel responsible for authorizing receipt of goods, however, cannot be expected to have the training and understanding of whether the pathogen test method and the compositing scheme, or the chemical test method used are appropriate. This issue can be compounded by the fact that the COAs may not declare the actual test method used. Receiving personnel often have been trained to look for a test result indicating "negative" or "absent" for a pathogen test, or the maximum value of other microbiological or chemical test parameters stipulated on company specifications. Should the responsibility of reviewing the adequacy of test methods and sample collection technique fall on GFSI auditors who are expected to have the skill and knowledge to evaluate the suitability of microbiological and chemical test methods for every identified hazard? Or is it a shared responsibility between the customer and supplier during the ingredient approval process? The speakers will share the challenges, opportunities and measures they have implemented as we strive to comply with the requirements of the Food Safety Modernization Act. This roundtable symposium will attempt identify potential training needs that can help augment the knowledge and capability of GFSI auditors, corporate internal auditors and plant quality personnel.

RT10 Back to Front and Front to Back: How to Manage out Toxins and Naturally Occurring Hazards throughout the Supply Chain

ERIK WESTBLOM: *Provision Analytics, Calgary, AB, Canada, Canada*
HOSAHALLI S. RAMASWAMY: *McGill University, Ste-Anne-de-Bellevue, QC, Canada, Canada*
KANTHA SHELKE: *Corvus Blue LLC/Johns Hopkins University, Chicago, IL, USA*
ANGELA ANANDAPPA: *Alliance for Advancing Sanitation, Glenview, IL, USA*

Natural toxins are of growing concern in agricultural products, especially those grown in climate sensitive regions and in novel products as they are introduced to more markets. As the new age of digital traceability transforms data availability, transparency related to natural toxins and naturally and regionally relevant hazards is an important factor to consider both for risk assessment and for the implementation of effective control strategies. Currently there is little mechanistic knowledge about how richer, more layered, risk-oriented food safety data can be used to make confident risk-based decisions that are both pragmatic and relevant to agricultural products and processed foods. This symposium will open with a historical perspective of new product development technologies and processing methods, followed by a discussion of naturally occurring toxins that are increasing in interest and relevance. A presentation of extraction methods for heavy metal toxin removal and the opportunities for applying existing methods to related products will then be followed by a look into a traceability-based view into yet un-mined data that can be used to work backwards to predict naturally occurring hazards.

RT11 Mission Impossible? Bringing Equivalency to Virtual Audits and Inspections

SHAWN STEVENS: *Food Industry Counsel, LLC, Random Lake, WI, USA*
ANDREW CLARKE: *Loblaw Companies Ltd, Brampton, ON, Canada, Canada*
NICK SCIRE: *FDA, HHS, Washington, DC, USA*
MANDY SEDLAK: *Ecolab, Naperville, IL, USA*
DAN SOLIS: *FDA, HHS, Los Angeles, CA, USA*
PHILLIP PIERCE: *NSF, Key West, FL, USA*

Virtual auditing and inspection has always taken a back-seat to onsite evaluations as food safety practitioners have been reluctant to assign equivalency between these two formats. This drastically changed in 2019 when the need for a remote alternative became urgent for third-party auditing companies, customers, and governmental regulators. Plato famously wrote, "our need will be the real creator," which over the years, has evolved into the proverb, "necessity is the mother of invention." This urgent need has escalated innovation in the processes, tools, and Information Communication Technologies (ICT) needed to bridge the gap between virtual and onsite evaluations.

Even though we're seeing an end to pandemic restrictions on travel and visitor access, the demand for virtual inspections and audits won't be returning to pre-2019 levels. Advances in processes and technology continues to enhance these remote evaluations. But the question remains: will virtual evaluations ever meet the standards of their onsite counterparts? This diverse roundtable is comprised of experts from many sectors to give unique opinions, perspectives, and case studies from their experiences with virtual inspections and audits. The audience will gain a better understanding of the challenges and considerations of pursuing this format and what needs to happen to gain true equivalency.

The roundtable panelists include:

1. A state/local governmental regulator will discuss their agency's virtual inspection pilot.
2. A federal governmental regulator will discuss their agency's plans to expand virtual inspections for both foreign and domestic facilities.
3. A third-party auditor will help us understand the challenges and wins they've faced when conducting remote audits, and the audit processes used to enhance these visits.
4. An IT expert will explore the technology, both visual tools and Information Communication Technologies, that is currently being utilized by remote auditors/inspectors.
5. A food lawyer to discuss some of the potential implications to remote sharing of confidential food safety data and other legal considerations for remote inspections and audits.

RT12 How Much S.M.A.R.T.E.R. Have Agricultural Water Quality Metrics Become?

DON SCHAFFNER: *Distinguished Professor and Extension Specialist in Food Science, Rutgers University, New Brunswick, NJ, USA*
ELIZABETH BIHN: *Cornell University, Produce Safety Alliance, Geneva, NY, USA*
NATALIE KROUT-GREENBERG: *California Department of Food and Agriculture, Sacramento, CA, USA*
GREG KOMAR: *California Leafy Greens Marketing Agreement, Salinas, CA, USA*
EMILY GRIEP: *International Fresh Produce Association, Washington, DC, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*

The S.M.A.R.T. criteria, a system commonly used in management, are Specific, Measurable, Achievable, Relevant, and Time-bound. Additional letters E and R are used to represent Evaluated and Reviewed.

In this roundtable discussion, key stakeholders will describe contemporary agricultural water quality management for produce safety in the United States. Key elements of the produce safety landscape include Good Agricultural Practices (GAPs) guidance, third-party audit systems, and industry/buyer requirements such as the Leafy Greens Marketing Agreement and Tomato GAPs metrics. In addition, the US Food and Drug Administration has released a proposed revision to the FSMA Produce Safety Rule Subpart E: Agricultural Water for further rule making. This proposed revision moves away from quantitative test results and toward a broader systems-based approach for risk evaluation to support decisions about appropriate use of agricultural water.

The panelists in this session represent organizations/programs that stay current with best practices for produce safety risk management by evolving over time in response to 1) lessons learned from outbreaks (e.g., retrospective root-cause analysis), 2) available sampling data and 3) research-driven data interpretation and principles development.

Water quality program evolution is driven by a goal to better guide practical water use decisions to achieve risk reduction. Water quality considerations include not only typical fecal-indicator bacteria data but also other observations and conditions that detect or indicate risk of foodborne pathogen presence in water.

The Round Table discussion will provide a platform to view and discuss this essential process of agricultural water quality program evolution through the lens of the S.M.A.R.T.E.R. criteria.

RT13 Identity Matters: Building a More Inclusive Workplace for Women in Food Safety

LAURA GUTIERREZ BECERRA: *Amazon, Seattle, WA, USA*
LISA ROBINSON: *Ecolab Inc., Eagan, MN, USA*
CATHERINE CUTTER: *Penn State University, University Park, PA, USA*
MINDY BRASHEARS: *Texas Tech University, Washington, DC, USA*
YVONNE MASTERS: *John B. Sanfilippo & Son, Inc., Chicago, IL, USA*
MELODY GE: *StarKist Co., Reston, VA, USA*

Background: A 2020 survey conducted by Women in Food Safety revealed that: Self-learning and motivation are two leading drivers for career success; Work/life demands and feeling of a glass ceiling are identified as the main career barrier among women; 52.7% of all participants find a lack of connection with a company-experienced employee as the main obstacle when entering the job market; and 90% of participants felt stuck at least once in their position throughout their career or job. Ensuring a workplace is inclusive of all membership is a priority of many employers. There is a need to hear the voices of women within food safety to assist with these inclusivity efforts.

Objective: The objective of this session is to listen to the experiences of a diversity of women in food safety careers and learn how to make the work environment more inclusive. This roundtable will include women from diverse identities sharing their workplace experiences and providing advice to fellow women and employers. There will be time within the roundtable for attendees with other identities to share action steps toward inclusion. This roundtable is intended for all IAFP attendees. The audience will leave this roundtable with multiple action items to immediately put into practice within their workplace to be more inclusive toward women as well as their own career development

RT14 Strengthening Food Safety Education and Research across Programs and Departments in the Universities

ELNA BUYS: *Department of Consumer and Food Sciences, University of Pretoria, Pretoria, South Africa, South Africa*
KEITH WARRINER: *University of Guelph, Guelph, ON, Canada, Canada*
WILLIAM HALLMAN: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
MICHELLE R. EMBRY: *Health and Environmental Sciences Institute (HESI), Washington, DC, DC, USA*
NADIYA BOYKO: *Uzhhorod National University, Uzhhorod, Ukraine, Ukraine*
ADEWALE OBADINA: *Federal University of Agriculture, Abeokuta, Abeokuta, Ogun State, Nigeria, Nigeria*

Food safety is a multidisciplinary program that must be taught in various disciplines and programs such as food science, nutrition, public health, pharmacy, crop science, animal science, chemistry, engineering, law, policy, and medicine. As an example, to reduce exposure to harmful levels of a

hazard requires research on understanding how its prevalence changes in food as it moves from farm to table. Multidisciplinary efforts are needed to understand how to reduce risk in terms of its production, detection, prevention, control and post-exposure management. That information then needs to be used in scientific analyses that support regulatory decisions to mitigate such risks. Then supply chain actors need to be taught how to implement such measures. Hence the training needed to reduce such risk may not be adequately covered in one discipline or University program. However, food safety topics often are only taught in programs where there is integrated course content or when the lecturer has an interest in the topic. If food safety topics are not captured in traditional course content, then their inclusion in teaching and research is the sole discretion of the lecturers. Currently, only a few universities worldwide offer standalone food safety programs. It is unclear whether students outside of those programs are receiving adequate training and are involved in research that really prepares them to become food safety professionals. The purpose of this roundtable is to discuss the need and how to enhance food safety education and increase teaching and research collaboration among various university departments/programs, where food safety is not offered as a stand-alone program, so that their graduates are well informed on the subject. The panelists from different academic disciplines, industry, private sector and non-profit organizations will discuss several successful collaborations and positive outcomes of such efforts as well as challenges and lessons learned.

RT15 Life after Graduate School and Beyond Academia

CHIP MANUEL: *GOJO Industries, Inc., Akron, OH, USA*
AJITA SUNDARRAM: *Mission Barns, San Francisco , CA, USA*
SUZY HAMMONS: *USDA-FSIS, Washington, DC, USA*
DAVID BUCKLEY: *Diversey, Charlotte, NC, USA*

Students and post-grad researchers often find themselves in a conundrum; on one hand, academia appears to be the next logical step based on their research and education, yet the academic life does not always feel like a solid fit. Graduate training, generally speaking, does not provide a lot of exposure to career opportunities outside of academia. Food safety career paths in industry and government remain vague to students because these options are not frequently discussed during school and relatively few students are able to obtain industry experience. By allowing new, rising, and seasoned industry and government leaders the opportunity to share their knowledge and experience, this roundtable aims to bridge the gap between academia and the food industry and shed light on how to obtain a career in these sectors. A mixed panel of industry and government leaders will provide the necessary tools and expertise to not only sustain a successful career, but to also provide young scientists with a fresh perspective and insight on what working in industry and government entails. Topics for discussion will include, but are not limited to; (a) finding the right company or fit; (b) transitioning from graduate school to industry or government; and (c) expectations of a new hire given one's education and experience post-graduation (skills written on CV that can be transferred into real-life industry skills). The purpose of this roundtable is to make the food industry/government workforce more transparent and to introduce graduate students to employment opportunities in these sectors. This roundtable was highly successful in 2016 and graduate students today would benefit from an encore.

RT16 Public-Private Data Sharing: A New Opportunity for Risk-Based Decision Making in Food Safety

KELLY STEVENS: *General Mills, Minneapolis, MN, USA*
SEAN LEIGHTON: *Cargill, Inc., Wayzata, MN, USA*
STEVE BREWER: *University of Lincoln, Lincoln, United Kingdom, United Kingdom*
MINDY BRASHEARS: *Texas Tech University, Washington, DC, USA*
ROBERTA WAGNER: *Consumer Brands Association, Arlington, VA, USA*
BARBARA KOWALCYK: *The Ohio State University, Center for Foodborne Illness Research and Prevention, Translational Data Analytics Institute, Columbus, OH, USA*

In this roundtable, food safety leaders with private sector, academic, and government experience, along with a data governance expert will discuss the potential value and impact of data sharing for improving food safety, and acknowledge the challenges.

Each segment of each value chain – from growers to processors to distribution to retail – has a trove of data that are relevant for food safety. Within each organization, these data are used to make go/no-go decisions. These data have great potential to provide larger value. If these data were integrated and managed as a resource, they could be used by many researchers to assess trends, identify factors that increase food safety risk, and test the real-world impact of interventions. With the advent of enormous computing power and powerful analytical tools, integrating and learning from large amounts of data is do-able. To supplement scientific studies that collect primary data, this observational data would greatly increase our shared understanding of opportunities for reducing risk.

However, there are many barriers to sharing data including data governance concerns related to access and usage. In addition, work must be done to develop a structure for overseeing the data and to develop standards and processes for integrating the data into one data set. Finally, data quality must be managed as well. No one organization currently has the expertise or capacity to take on this role, so collaborations must be established to accomplish this. There are examples from other sectors, such as environmental science and the automotive industry, that can be leveraged.

This roundtable will discuss the challenges and opportunities for public-private data sharing to increase food safety, and identify elements of a path forward.

RT17 Acidified Foods: Addressing Challenges in Product Classification Beyond Food Safety. What Role Do Water, Syrups, and Other Low-Water Activity Ingredients Play?

MICHAEL MIGNOGNA: *U.S. Food and Drug Administration, Washington D.C, DC, USA*
LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
ASHWINI WAGH: *The Clorox Service Company, Pleasanton, CA, USA*
LAURE PUJOL: *NOVOLYZE, Daix, France, France*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*
DERRICK BAUTISTA: *Del Monte Foods, Inc., Walnut Creek, CA, USA*

In 2010, FDA issued a "Draft Guidance for Industry: Acidified Foods". In 2015, FDA withdrew the draft guidance. At present, there is no draft guidance on acidified foods. The withdrawal of the guidance has prompted reflections from the Food and Beverage (F&B) sector, academia, Food Process Authorities, inspectors, and regulatory bodies on interpreting acidified foods criteria: pre-acidified ingredients, low-acid powders, water, other liquids, the definition of a small amount of low-acid ingredient, and the definition of a significant difference from the pH of the predominant acid or acid foods.

Water, syrups, salt, sugar, spices and other ingredients are critical to the makeup of most dressings, condiments, sauces and beverages. The pH of water is 7–9 depending on the county and it has no buffering capacity. FDA considers spices, salt, sugar, preservatives as low acid ingredients. If these powdered low acid ingredients are dissolved in water the solution's pH tends to be similar to the pH of the water. Once an acid is added to the solution, the solution's pH drops below 4.6 almost immediately. Where is the food safety risk in these product categories—acidified beverages, dressings and condiments? The number of outbreaks associated with these product categories is low to nil. However, if regulatory agencies are considering powders to pose the same risk as large particulates, the F&B sector is seeing a shift in paradigm.

Since the risk posed by these product categories is low, should they be subjected to a 5-log reduction? Or should a risk assessment be conducted and then the product be challenged with the appropriate inoculum level (2–3 logs). How does it impact the F&B sector?

How are the F&B sector and the regulatory bodies embracing this changed opinion of "water being considered low acid and other powdered low moisture dry ingredients being considered low acid"?

RT18 Application of New Technologies for Improved Food Safety

JOSEPH HOLT: *OSI Group, Aurora, IL, USA*
JAMES YUAN: *PepsiCo, Purchase, NY, USA*
CINDY JIANG: *McDonald's Corporation, Woodridge, IL, USA*
TIMOTHY JACKSON: *U.S. Food and Drug Administration, CFSAN, College Park, MD, USA*
DERRICK BAUTISTA: *Del Monte Foods, Inc., Walnut Creek, CA, USA*
MARK MOORMAN: *Food and Drug Administration, College Park, MD, USA*

The announcement of FDA's "New Era of Smarter Food Safety" initiative in 2020 brought heightened visibility to new technologies and approaches to create a safer food supply, including those to enhance traceability, improve predictive analytics, respond more rapidly to outbreaks, and reduce contamination of food. However, with more questions than answers on how the food industry can implement these technologies within their facilities and infrastructures, there exists an opportunity to help close the gap between what's achievable with these technologies and the current state. In this roundtable, a panel of regulatory and industry scientists will discuss how recent innovations in predictive analytics; new tools for risk assessment and environmental monitoring, including how to handle tracking and mapping data; and virtual monitoring technology for use in auditing and inspections can be used to improve food safety and sustainability. In addition, panelists will discuss whether the industry is using these technologies to their fullest extent, and limitations to implementing these technologies in the food industry.

RT19 Moving Closer to Zero – Challenges and Opportunities for Reducing Children's Exposures to Toxic Elements from Foods

EVE STOODY: *Director, Nutrition Guidance and Analysis Division, Center for Nutrition Policy and Promotion, Food and Nutrition Service, U.S. Department of Agriculture, Alexandria, VA, USA*
DE ANN DAVIS: *Senior Vice President Science, Western Growers Association, Pacific Grove, CA, USA*
TOM NELTNER: *Senior Director, Safer Chemicals, Environmental Defense Fund, Washington, DC, USA*
CHERYL CALLEN: *Senior Director of Regulatory Affairs, Gerber/Nestle Infant Nutrition, Arlington, VA, USA*
PAMELA STARKE-REED: *Deputy Administrator, Nutrition, Food Safety/Quality, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD, USA*
CONRAD CHOINIERE: *Director, Office of Analytics and Outreach, Food and Drug Administration, U.S. Department of Health and Human Services, College Park, MD, USA*

Mitigating the presence of lead, arsenic, cadmium, and mercury in the food supply is complex and multifaceted. These contaminants are naturally occurring in the environment or present due to man-made activities and are taken up by crops. These exposures can impact child development, so reducing levels in foods consumed by babies and young children presents an important public health opportunity, yet can pose challenges for government, industry, and consumers.

In April 2021, FDA announced *Closer to Zero* (*C2Z*), an action plan to reduce exposures to these contaminants from foods commonly consumed by babies and young children to the lowest extent feasible. *C2Z* implements a science-based, iterative approach for achieving continual improvements over time. The effort will draw upon a wide range of scientific disciplines and gather data and input from a wide range of stakeholder perspectives as it develops reference levels of exposure to the contaminants from foods, proposes action levels for foods, and assesses achievability for meeting action levels and feasibility for making reductions in the levels of contaminants. Beyond food safety and toxicology, the *C2Z* approach also considers nutritional needs during development, including those of the Dietary Guidelines for Americans, to better understand the role of nutrition for modulating adverse health effects from children's exposure to toxic elements.

This roundtable brings together experts from industry, consumer advocacy, and government to discuss the various aspects of the *C2Z* initiative in order to highlight the challenges and opportunities for reducing exposures among the very young. The panel members will present brief remarks to spark discussion and the audience will be invited to pose questions for an important dialogue on this complex, multifaceted issue.

RT20 Rapid Methods and Automation in Food Microbiology: 40 Years of Developments, Promises, and Disappointments

WILLIAM SHAW: *USDA Food Safety & Inspection Service, Washington, DC, USA*
THOMAS HAMMACK: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
JOSEPH MEYER: *Kerry, Waunakee, WI, USA*
ROY BETTS: *Science Fellow, Campden BRI, Chipping Campden, United Kingdom, United Kingdom*
STAN BAILEY: *Senior Director Scientific Affairs, bioMérieux, Athens, GA, USA*
PURNENDU VASAVADA: *UW- River Falls and PCV & Associates, LLC., River Falls, WI, USA*

Rapid methods and automation in food microbiology have been an attractive and favorite target of research and development in food microbiology. The food industry's necessary focus on food safety and quality has long driven the need for effective testing of raw materials, ingredients, and in-process and finished products to detect, identify and characterize microbiological and chemical hazards. The overarching reason is the promise and prospect that better methods will result in safer foods for all consumers. Over the past 40 years, microbiologists have endeavored to develop new and "better" methods for enumeration and detection of organisms of concern in food. But what does "better" mean? Faster? More automated? More sensitive or specific? Cheaper? More objective?

Since 1980s, a plethora of methods based on a wide range of techniques have been introduced. Some methods offer results that are different from those gain in conventional tests. While some of these methods have been successfully adopted by the industry and regulatory agencies, a vast majority of these methods have fallen by the wayside. A few have stood the test of time and become widely used and accepted by both industry users and regulatory authorities as definitive and trusted test methods. Also, more recently, "culture independent" methods have been introduced as an answer to industry needs. Will these new development succeed where others have failed?

This roundtable is designed to develop answers to some of these questions and try to look at the future of rapid methods and automation in food microbiology. Panelists from the academia, industry, regulators and method developers will present their unique perspectives based on their own extensive knowledge of test method development, evaluation, validation and use. This interactive session will offer opportunity for audience participation and provide comments on microbiological testing in the food industry.

RT21 Watching GRAS Grow: Understanding What It Means to be GRAS in the U.S.

TONY PAVEL: *Perfect Day, Berkley, CA, USA*
KATIE OVERBEY: *U.S. Food and Drug Administration, Rockville, MD, USA*
CLAIRE KRUGER: *Spherix Consulting Group, Rockville, MD, USA*
SYLVESTER MOSLEY: *Coca Cola, Atlanta, GA, USA*
ALEX EAPEN: *Cargill, Inc., Wayzata, MN, USA*
STEVEN HERMANSKY: *Conagra Brands, Omaha, NE, USA*

Manufacturers of food ingredients and the finished products containing the ingredients, have a legal responsibility under the Federal Food, Drug, and Cosmetic Act to ensure that all ingredients are safe under their intended conditions of use before entering the market. In the United States, the FDA's Generally Recognized As Safe (GRAS) Notification Program is a popular regulatory path. A GRAS conclusion for a given ingredient is tied to the safety of the ingredient under the conditions of its intended use. One of the components of a GRAS safety evaluation is to estimate the dietary exposure for the ingredient of interest. This is done by incorporating the intended use levels and the foods in which the ingredient will be used. In addition, a cumulative dietary exposure for the ingredient, taking into account other sources (e.g., other regulated uses, background), is estimated as appropriate. The safety of the ingredient is evaluated in the context of the identity of the ingredient, manufacturing process, intended use, dietary exposure, and publicly available safety information. A clearer understanding of GRAS is important for both the producer and the consumer of the ingredient, to drive supply chains, and increase confidence in the overall safety of the U.S. food supply. The objectives of this session is to provide an overview of what it means for a food ingredient to be GRAS, discuss steps that ingredient manufacturers can take to ensure their ingredients are GRAS for their intended uses, illustrate challenges when determining whether an ingredient is GRAS, and highlight ways that finished product manufacturers can determine whether a food ingredient meets the legal definition of GRAS.

RT22 Understanding and Overcoming Challenges in Helping Underrepresented Minority Audiences Meet the FSMA PSR 112.22(c) Training Requirements

RICARDO ORELLANA: *Produce Safety Alliance, Amherst, MA, USA*
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The Food Safety Modernization Act (FSMA) outlines the federal regulation governing the production, harvest, and packing of fresh fruits and vegetables. The Produce Safety Rule (PSR) has a specific training requirement outlined in Section 112.22(c) that "At least one supervisor or responsible party for your farm must have successfully completed food safety training at least equivalent to that received under standardized curriculum recognized as adequate by the Food and Drug Administration." The Produce Safety Alliance (PSA) developed and implemented a grower training course to meet this requirement and provide growers with relevant knowledge to comply with the PSR. PSA Trainers have delivered trainings to over 67,000 individuals, but many growers have not been reached. The PSR training is only required for covered farms. However, all growers would benefit from understanding regulations that govern their industry and learning about practices to reduce microbial risks on their crops.

While extension and industry educators have increasingly recognized the importance of promoting diversity and inclusiveness in produce safety training, the results of these efforts have been highly variable across the wide-range of communities found in the fresh produce industry. It seems that there are underrepresented minority growers who have not had the opportunity to attend a PSA training. Therefore, this roundtable will discuss novel approaches used to reach underrepresented communities to help them meet the FSMA-PSR training requirement. Our discussion will focus on empirical results and practical recommendations related to the utilization of appropriate communication channels to reach these communities and how to build effective working relationships based on cultural awareness and understanding. Because of the great diversity in linguistic, cultural, and socio-economic realities found in underrepresented communities in the industry, it is important for educators to continually improve our understanding of the needs of these communities to know how to better serve them.

Technical Abstracts

T1-01 Data-Driven Discovery of Novel Polymer Coatings for Biofilm Reduction

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Introduction: Polymer coatings that are inherently resistant to bacterial attachment have demonstrated potential in reducing biofilm formation in food processing environment. Optimization of their design and synthesis, however, is often time- and resource-intensive.
Purpose: Here we introduce a high-throughput, machine-learning (ML) workflow for virtual screening and optimization of biofilm-resistant polymer coatings.
Methods: We first constructed a polymer-biofilm library based on the experimental antibiofilm performances of 2240 unique copolymers against *Pseudomonas aeruginosa* PA01 (PA) and 1121 computed molecular features. Then, we trained a kernelized supported vector regressor (SVR) using the latent space features generated using autoencoder and optimized the model hyperparameters by minimizing root mean squared errors (RMSE). As a proof-of concept, we targeted at the virtual screening of amphiphilic copolymers, which resulted in a list of comonomer pairings with superior predicted biofilm resistance. For experimental validation of model predictions, we synthesized one of the top-ranked amphiphilic coatings, poly(2-Hydroxyethyl methacrylate-*co*- 2,2,3,4,4,4-Hexafluorobutyl acrylate) (or pHEMA-HFBA), with various hydrophilic-to-hydrophobic ratios, using an all-dry, solvent-free coating technology.
Results: The polymer library used in this work encompasses > 4 times more unique copolymers than the prior record (497), which improved the generality of the learned 'patterns' and hence the utility of models for screening unseen copolymers. The SVR model achieved a training RMSE of 0.3599 and a testing RMSE of 0.3950, corresponding to less than 6% of the average predicted biofilm amount. Furthermore, the model has shown promise in identifying the optimal hydrophilic compositions given amphiphilic coating chemistry. Specifically, the model successfully predicted the minima of biofilm formation on the pHEMA-HFBA coatings to occur at ~70vol.%HEMA (hydrophilic monomer), agreeing with the biofilm assay results.
Significance: The ML workflow reported herein has shown encouraging potentials in virtual screening and optimization of novel antibiofilm coatings for food processing environment. In the future, multi-objective optimization considering cytotoxicity and acid/base resistance of the coating chemistry must be included in the workflow for successful translation into industry practices.

T1-02 Predicting Food Spoilage Using Rapid and Non-Invasive Near Infrared Spectroscopy in Synchronicity with Machine Learning Models

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❖ ***Developing Scientist Entrant***

Introduction: Rapid prediction of spoilage status of perishable foods can reduce food waste and loss while assisting in decision making for the food value chain actors. Near infrared spectroscopy, an easy-to-use and rapid technology is used to detect food composition and quality analysis.
Purpose: The objective of this study is to use near infrared spectroscopy in combination with machine learning algorithms to predict the microbial spoilage of raw chicken as a model for perishable food
Methods: Commercially deboned boneless skinless chicken breast fillets were purchased from a local processor and placed at refrigerated temperature for 0, 2, 4, 6, and 8 days. These broilers deboned fillets were analyzed using a portable near infrared spectroscopy device (350-2500 nm). The reflectance of each wavelength was gathered at 6 different positions on each fillet (2151 data points x 6 positions). Microbial (aerobic plate count) sampling on deboned fillets was also performed and used as a reference to train the supervised learning algorithm [K-Nearest Neighbor (K-NN) and Back Propagation Neural Network(BPNN)] for spoilage classification into baseline microbial count (up to 3.0 log CFU), propagation 1 (3.0-4.9 log CFU), propagation 2 (5.0-7.0 log CFU) and spoiled (> 7.0 log CFU).
Results: The BPNN and K-NN models were able to detect the difference in reflectance value for microbial growth in fillets stored at different storage periods with varying levels of accuracy. Models were able to predict the four spoilage phases with a 73.30-100% accuracy rate. Classification accuracy was 90.00-100 % for baseline counts, 87.5-93.8 % for propagation 1, 73.30-100% for propagation 2 and 100 % for spoiled.
Significance: Near infrared spectroscopy in combination with machine learning algorithms can be used by food value chain actors for rapid shelf-life prediction and take actions accordingly to reduce food waste and loss.

T1-03 Prediction of Population Behavior of *Listeria monocytogenes* in Food Using Machine Learning and Combase Database

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Introduction: In predictive microbiology, statistical models are employed to predict bacterial population behavior in food using environmental factors such as temperature, pH, and water activity. As the amount and complexity of data increase, handling all data with high-dimensional variables becomes a difficult task.
Purpose: We propose a data mining approach to predict bacterial behavior using a database of microbial responses to food environments. *Listeria monocytogenes*, which is one of pathogens, population growth and inactivation data under 1,007 environmental conditions, including five food categories (beef, culture medium, pork, seafood, and vegetables) and temperatures ranging from 0 to 25 °C, were obtained from the ComBase database (www.combase.cc).
Methods: We used eXtreme gradient boosting tree, a machine learning algorithm, to predict bacterial population behavior from eight explanatory variables: 'time', 'temperature', 'pH', 'water activity', 'initial cell counts', 'whether the viable count is initial cell number', and two types of categories regarding food.
Results: The root mean square error of the observed and predicted values was approximately 1.0 log CFU regardless of food category, and this suggests the possibility of predicting viable bacterial counts in various foods.
Significance: The data mining approach examined here will enable the prediction of bacterial population behavior in food by identifying hidden patterns within a large amount of data.

T1-04 Foodborne Salmonellosis Outbreak Severity Prediction Based on Genetic and Meteorological Trends Using Machine Learning

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Introduction: Several studies have shown a correlation between outbreaks of *Salmonella enterica* and meteorological trends, especially related to temperature and precipitation. Additionally, current outbreak-related studies are performed on data pooled by *Salmonella* species without taking into account its intra-species and genetic heterogeneity.

Purpose: The purpose of this study was to analyze and quantify the effect of differential gene expression and a suite of meteorological factors on salmonellosis outbreak severity (typified by case numbers) using a combination of machine learning and count modeling methods.

Methods: *Salmonella* outbreak data and corresponding meteorological data were obtained from the National Outbreak Reporting System and National Climatic Data Center databases. *Salmonella* whole-genome sequences obtained from the Pathogen Detection database were employed in a pan-genome creation. Elastic Net regularization was used to identify significant genes, and a multi-variable Poisson regression was developed to fit the individual and mixed-effects data. Best-fit models were identified using pseudo R^2 values and the significance level was set at 0.05.

Results: The best-fit Elastic Net model ($\alpha = 0.5000$; $\lambda = 2.18399$) identified 53 significant gene features. The final multi-variable Poisson regression model ($x^2 = 5748.22$; pseudo $R^2 = 0.6688$; probability > $x^2 = 0.0000$) identified 127 significant predictor terms ($P < 0.10$), comprising 45 gene-only predictors, average temperature, average precipitation, and snow cover, and 79 gene-meteorological interaction terms. The significant genes ranged in functionality from cellular signaling and transport, virulence, metabolism, and stress response, and included gene variables not considered as significant by the baseline model.

Significance: The results of this study indicate the need to co-evaluate genomic data with environmental data to develop a more holistic model to predict disease outcome severity, which could extend to re-evaluating the risk to human health.

T1-05 Using *E. coli* Population to Predict Foodborne Pathogens in Pastured Poultry Farms

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* is the most widely used indicator of fecal contamination in food, and its elevated counts on poultry carcasses have been routinely linked with inadequate or unhygienic handling, processing, or storage.

Purpose: The purpose of this study was to investigate the association between *E. coli* and foodborne pathogens such as *Campylobacter*, *Salmonella*, and *Listeria* in pastured poultry farms, as well as in related processing plants.

Methods: A total of 1,690 samples were collected from 11 pastured poultry farms in the Southeastern United States from 2014 to 2017. Five different sample types: (i) feces, (ii) soil, (iii) whole carcass rinse during processing (WCR-P), (iv) whole carcass rinse of final product after chilling and storage (WCR-F), and (v) ceca were measured for *E. coli* population and the presence of *Campylobacter*, *Salmonella*, and *Listeria*. A logistic regression model for pathogen presence was developed for each sample type. These logistic regression models were used to predict the probability of each pathogen's presence as a function of *E. coli* concentration.

Results: The increase of *E. coli* population significantly increased the predicted probability of *Salmonella* presence in soil and WCR-P samples ($p = 0.0011$ and $p = 0.0157$ respectively). A similar trend was found for detecting *Listeria* presence in soil, WCR-P, and WCR-F samples. For *Campylobacter*, the initial prevalence in feces and ceca was high and a decreasing trend of detecting *Campylobacter* was observed as *E. coli* concentration increased. In soil and WCR-P models, the probability of *Campylobacter* presence significantly increased as the *E. coli* population increased.

Significance: These models provide a practical and effective way of evaluating the relationship between *E. coli* and foodborne pathogens and enable the prediction of foodborne pathogen presence based on *E. coli* prevalence. These results can be used in the risk assessment of pastured poultry products.

T1-06 Changes in *Salmonella O*ccurrence Since the Introduction of Performance Standards for Chicken Parts

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Introduction: In 2016, the Food Safety and Inspection Service (FSIS) implemented *Salmonella* performance standards for chicken parts to reduce *Salmonella* contamination on chicken parts samples (i.e. legs, breasts or wings) by 30%. This study presents a review of changes in *Salmonella* contamination before and after implementation of the standards to determine if the predicted 30% reduction was achieved.

Purpose: The study quantifies the reduction in the occurrence of *Salmonella*-contaminated chicken parts samples following implementation of the standard in 2016. Changes in other factors that may affect the risk of exposure, illness, and severity are also addressed.

Methods: FSIS developed a modeling framework using changes in the parameters of fitted beta-binomial distributions to estimate the effect of performance standards on the chicken parts industry on an annual basis. Additional insights into the timing and magnitude of these changes were modeled using penalized b-spline regression models. Insight into changes in other risk factors for salmonellosis (e.g., seasonal exposure, distribution of serotypes, and resistance to medically important antibiotics) were assessed using a combination of Fourier series models and compositional data analysis techniques. We analyzed 50,237 samples collected by FSIS in parts producing establishments and 33,739 samples collected at retail by the Food and Drug Administration.

Results: The reduction in *Salmonella*-contaminated chicken parts samples was greater than 75% ($p < 0.05$). Chicken parts samples collected at retail demonstrates significant reductions of a similar magnitude. Seasonal variation in *Salmonella* contamination has changed dramatically the early 2000s, with a peak in December and nadir in June for the FSIS samples ($p < 2.2e\text{-}16$). The peak and nadir for the FDA retail samples occurred one month later. *Salmonella* Infantis is rapidly replacing Kentucky and Enteritidis as the dominant serotype in chicken parts.

Significance: Evaluation of pathogen reduction performance standards is vitally important to understand their effectiveness and identify areas of improvements for future efforts to reduce *Salmonella* illnesses attributable to poultry.

T1-07 Quantitative Microbial Risk Assessment (QMRA) of Salmonellosis from Chicken and Pork Salad Consumption in Cambodia

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Introduction: *Salmonella* is an important foodborne bacterial pathogen that causes high risk to human health globally.
Purpose: This study aimed to estimate the risk of Cambodian consumers of acquiring salmonellosis after consuming contaminated chicken and pork salad using a quantitative microbial risk assessment.
Methods: Chicken meat and pork samples (n=204 each) were collected from traditional markets in 25 provinces for *Salmonella* analyses. Practices of cooking chicken and pork salad from 93 Cambodian households were surveyed and used to design an experiment to assess *Salmonella* cross-contamination from raw meat to ready-to-eat salad. Data on *Salmonella* contamination, salad consumption and dose response (alpha=0.00853 and beta=3.14) were modelled using Monte Carlo simulations with @Risk at 10,000 iterations.
Results: The prevalence of *Salmonella* in chicken meat and pork were 42.6% (87/204) and 45.1% (92/204) respectively. The average concentration of *Salmonella* in chicken meat was 10.6 MPN/g, and in pork 11.1 MPN/g. Half of the interviewed households processed and cooked meat for salad directly after purchasing. The QMRA model showed that the annual risk of salmonellosis estimated from consuming chicken salad, pork salad and mixtures of chicken and pork salads were 11.2% (90%CI 0.0 – 35.1), 4.0% (90%CI 0.0 – 21.3), and 14.5% (90%CI 0.0 – 33.5), respectively. The factors with the highest influence on the estimate were cross-contamination while preparing salad, followed by the prevalence of *Salmonella* on chicken meat and pork at the market. A wide confidence interval in the estimated incidence was mainly due to the variability in the degree of reduction in bacteria concentration by cooking, and salad consumption pattern.
Significance: The risk of salmonellosis due to chicken and pork salad consumption appears to be high. Control measures may include improving the safety of retailed chicken and pork at markets and improving hygiene practices and equipment during salad preparation at household.

T1-08 Quantitative Risk Assessment of *Salmonella* in Ground Beef and the Resulting Impact on Public Health

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❖ ***Developing Scientist Entrant***

Introduction: Rates of *Salmonella* infections have not declined over the last 15 years in the US. The continued emergence of highly virulent strains of *Salmonella* in beef cattle populations poses challenges to controlling *Salmonella* in beef products. Enumeration values have been proposed as being more useful than presence or absence for implementing risk-based mitigation strategies to prevent *Salmonella* in ground beef.
Purpose: The purpose of this study was to develop a quantitative microbial risk assessment (QMRA) model for ground beef to assess the impact on public health of removing lots highly contaminated with *Salmonella*.
Methods: QMRA models were developed using FDA-iRISK to account for various consumption scenarios including parameterized thawing methods, cross-contamination coefficients, consumption setting, and pathogen reduction after cooking. *Salmonella* was characterized by enumeration (MPN/g) and different dose-response models were used for virulence profiles. Relative disability-adjusted life year (DALY) values were used to differentiate pan-susceptible from antimicrobial resistant *Salmonella* infections. The effect of removing highly contaminated lots was based on threshold levels of 1 and 10 MPN/g. All enumerated 25g or 325g co-enriched ground beef samples (n=1060) submitted to FSIS from 2010-2020 were included in this analysis.
Results: Total illnesses and mean risk of illness were reduced when highly contaminated lots were assumed to be diverted from raw product sales. When considering all consumption setting scenarios, removing lots contaminated with >10 MPN/g resulted in a 13.5% reduction of illnesses and removing lots contaminated with >1 MPN/g resulted in a 36.2% reduction of illnesses.
Significance: This risk assessment framework identifies risk management strategies for reducing the public health burden of *Salmonella* in ground beef products.

T1-09 Improving Dairy Powder Sampling Plans for Detecting Pathogens through Simulation Analysis

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is an important food safety hazard in dairy powder products because it can survive low water activity and infect at low dose. However, *Salmonella* in dairy powder products is hard to detect from the low prevalence and contamination level. With existing regulatory discretion in sampling, academic literature and industry members identified the lack of scientific reasoning behind current sampling plans and the need for comprehensive, science-based guidance.
Purpose: We aim to adapt a previously developed sampling tool for optimizing dairy powder sampling and benchmark industry-relevant sampling plans for detecting *Salmonella* contamination.
Methods: We visited a large milk powder producer in Michigan for initial process assessment and model parameterization. For modeling, powder production was mapped to a two-dimensional (2D) space with production time as X-axis and production rate as Y-axis by adapting a previously developed 2D simulation model for sampling produce in the field. Hazard introduction was simulated with a defined prevalence (area) and level (CFU/g). Grab and autosampling plans were created with equivalent total sample masses and the model iterated to determine the probability of hazard detection.
Results: We developed a process diagram describing product sampling points, hazard introduction scenarios, and industry-relevant sampling plans. The simulations of this process had of five modules: hazard simulation, sampling simulation, assay simulation, decision-making simulation, and iteration. Initial adaptation of the model to dairy powder sampling with 100 iterations confirmed the general trend in sampling; taking more, smaller, randomized samples led to a higher probability of detection for clustered rare contamination.
Significance: Initial analysis confirms that autosamplers are likely more effective than grab sampling methods when a highly clustered hazard exists for diary powders. Future work using a web-based app will benchmark the industry sampling plan. The model will help industry stakeholders design safer and science-based optimum sampling plans to manage specific hazards by entering parameters describing their hazard scenarios and sampling plans of interest.

T1-10 Evaluating Product Testing Combined with Other Strategies for Reducing Risks from Pre-Harvest Contamination of *E. coli* O157:H7 on Generic Leafy-Green Produce Using a Farm-to-Facility Simulation

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❖ ***Developing Scientist Entrant***

Introduction: Current food safety testing approaches have proven, at times, to be unable to detect a contamination event, leading to foodborne illness. The marginal gain of food safety sampling compared to risk reduction strategies is unknown, making it difficult to assess the real power of sampling plans.
Purpose: This model focuses on the marginal gain of product testing at different stages combined with other risk reduction techniques. This will assist stakeholders in making decisions for when to apply testing to reduce risk.
Methods: A simulation was built in Python to represent *Escherichia coli 0157:H7* dynamics in the farm-to-facility process for leafy greens. Pre-harvest contamination of 100,000 CFUs was spread (i) randomly, (ii) a 10% cluster or (iii) a 1% cluster in a 100,000 lb. field. Interventions such as washing, holding, pre-cooling, and sanitization were modeled to represent two baseline scenarios, (i) No-Intervention and (ii) All-Interventions. Sampling was performed at pre-harvest, harvest, receiving, finished product, and end consumer. A total of 42 scenarios were generated to evaluate the relative efficacy at reducing consumer exposure for 7 sampling plans across 2 baseline systems, under 3 different contamination spreads.
Results: The All-Intervention system showed to reduce consumer exposure by 3.42, 2.84, and 2.68 compared the No-Intervention system for the random, 10%, 1% contamination spreads respectively. For the No-Intervention system sampling before harvest, and finished product sampling showed to be the more effective sampling strategies to reduce consumer exposure, reducing exposure by 28%-71%, and 28%-56% respectively. For the All-Intervention system sampling showed limited efficacy and power due to low levels of contamination at sampling points. Sensitivity analysis (PRCC) showed that in-field die-off (0.53), pre-wash (-0.47), and the chlorinate wash (-0.38) are the most important factors for the reduction of consumer exposure, sampling plans showed to have limited effect (-0.2).
Significance: The model shows that when GAPs and GMPs are in place, product testing has limited detection power and effect on the final consumer exposure.

T1-11 Quantifying Free Chlorine Inactivation Efficacy of *Escherichia coli* O157:H7 during Produce Wash

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Introduction: Free chlorine is the major disinfectant used in industrial fresh produce wash cycles. Reduction of free chlorine concentration due to organic load levels can significantly reduce the efficacy of antimicrobial properties of free chlorine. We present a comprehensive mathematical model quantifying the reduction of the inactivation coefficient of *E. coli O157:H7* during produce wash.
Purpose: Quantify efficacy of free chlorine during produce wash cycles.
Methods: Our wash system consists of a tank containing 20L of tap water maintained at 4°C at a pH of 6.5 and an initial free chlorine level of 230 µM. Batches of green lettuce were introduced to the tank at a rate of 8.33 g/s, and inoculated red leaf lettuce with *E. coli* concentration levels of 5-log MPN/g were introduced to the tank at a rate of 1.67 g/s. The average time of exposure of lettuce to the wash water was 30 s. Free chlorine concentrations, organic load as measured by chemical oxygen demand, and pathogen levels in the wash water and on the uninoculated lettuce were collected at intervals of 2 minutes. After 10 min, the addition of produce to the wash water was paused for 5 min to replenish free chlorine levels to 500 µM. Measurements were repeated two more times for a total experimental time of 40 min. Experiments were independently repeated three times.
Results: The inactivation coefficient of *E. coli O157:H7* was 1.17 ± 0.05 L/mg/s when no organic matter was present in water. The inactivation coefficient dropped by over 70% when chemical oxygen demand levels was above 600 mg/L.
Significance: There is a possibility of cross-contamination of fresh produce via the wash water if free chlorine levels are not properly maintained during commercial wash cycles, while excess levels of free chlorine can lead to formation of harmful disinfectant by-products. We aim to optimize levels of free chlorine to prevent cross-contamination, while reducing the formation of disinfectant by-products via our model.

T1-12 Insights into the Relevance of *Bacillus cytotoxicus* as a Foodborne Pathogen

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Introduction: *Bacillus cytotoxicus* belongs to the *Bacillus cereus* group that also comprises the foodborne pathogen *Bacillus cereus sensu stricto*, *Bacillus anthracis* causing anthrax, as well as the biopesticide *Bacillus thuringiensis*. The first *B. cytotoxicus* was isolated in the context of a severe food poisoning outbreak leading to fatal cases of diarrheal disease. Subsequent characterization of the outbreak strain led to the conclusion that this *Bacillus* strain was highly cytotoxic and eventually resulted in the description of a novel species, whose name reflects the observed toxicity: *B. cytotoxicus*. However, only a few isolates of this species have been characterized with regard to their cytotoxic potential and the role of *B. cytotoxicus* as a causative agent of food poisoning remains largely unclear.
Purpose: The aim of this study was to gain further insights into the toxicity of *B. cytotoxicus* to allow for conclusions on the relevance of the organism as a foodborne pathogen.
Methods: Nineteen *B. cytotoxicus* isolates were obtained from mashed potato powders and characterized by toxin gene profiling for *nhe, cytK,* and *ces,* as well as by Vero cell cytotoxicity assays.
Results: All isolates harbored the *cytK1* (cytotoxin K1) gene and species-specific variants of the *nhe* (non-hemolytic enterotoxin) gene. The isolates exhibited low or no toxicity towards Vero cells. A high prevalence (95%) of *B. cytotoxicus* in mashed potato powder was observed. Isolates from mashed potato powder harbored *cytK1* and *nhe* genes but exhibited no or low cytotoxicity in a Vero cell assay.
Significance: Our findings indicate that while *B. cytotoxicus* are highly prevalent in mashed potato powder, their cytotoxic potential is likely lower than initially assumed.

T2-01 Using Whole Genome Sequencing Data to Inform Food Safety Actions

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Introduction: The Food Safety and Inspection Service (FSIS) analyzes whole-genome sequencing (WGS) data from ready-to-eat (RTE) meat and poultry establishments to inform food safety actions.
Purpose: This study describes the results from the WGS analysis FSIS has performed from July 2020 to September 2021 in RTE establishments and describes how the agency has used this data to inform Public Health Risk Evaluations (PHREs) and Food Safety Assessments (FSAs).
Methods: FSIS analyzes RTE product, food contact, and non-food contact samples WGS data from *Listeria monocytogenes* (*Lm)* positive isolates. The Agency uploads the sequences from the isolates to the National Center for Biotechnology Information (NCBI) database. FSIS then analyzes the data to determine if the isolates are part of a cluster containing clinical isolates uploaded within the last two years. The Agency also considers whether there are multiple isolates from other sources in the same NCBI cluster to determine if and how the information should be used to inform the PHREs and or FSAs performed by FSIS.
Results: During the study time period, 76 sampling events occurred where one or more FSIS *Lm* isolates were sequenced. Of these, five isolates were part of a cluster with one or more human illness isolates, and WGS analyses for two of the FSIS *Lm* isolates supported investigations. In one analysis, an isolate from a RTE product at one establishment was found to be closely related to a RTE product from another establishment. Investigations identified

product commonalty, and clinical isolates were later identified, leading to an outbreak investigation and an Agency recommendation to recall the product.

Significance: FSIS utilizes the results from WGS analyses to inform PHREs/FSAs and to identify information for outbreak investigations and potential recalls. Analyzing this information helps FSIS protect public health by identifying adulterated product and removing it from the marketplace.

T2-02 Detection of Diverse *Salmonella* serovars in Various Food Matrices Using Quasimetagenomics

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Introduction: Previous studies investigating quasimetagenomics (direct sequencing of microbial enrichments) established its promise at reducing time to results, however, more work is needed to adapt this promising methodology to broader implementation such as routine food testing.

Purpose: This study sought to advance the understanding of quasimetagenomic approaches by investigating culture-independent molecular serotyping for *Salmonella enterica* in previously untested serovars, food matrices, and mixed culture.

Methods: Four different *Salmonella* strains representing serotypes Montevideo, Heidelberg, Infantis, and Enteritidis were investigated. Monoserotype inoculations were carried out on lettuce, pet food, and raw chicken, with one mixed-serotype inoculation on lettuce, inoculated products were incubated overnight in RV broth. Enrichments were then subject to immunomagnetic separation, followed by multiple displacement amplification and then DNA sequencing on the MinION sequencer from Oxford Nanopore Technologies. The resulting sequencing reads were filtered for *Salmonella*-specific reads using Kraken, assembled using Raven, followed by *in silico* serotype determination using both SISTR and SeqSero2, in addition to SNP analysis using the CFSAN SNP-pipeline.

Results: Successful *in silico* serotype predictions were achieved from *Salmonella* inoculated on all three food matrices. Predictions were achieved with as little as 30 minutes of sequencing in lettuce and pet food, but required 90 minutes of sequencing in raw chicken. Sequencing obtained from quasimetagenomics was indistinguishable from whole genome sequencing of inoculated strains, with no SNP differences in trials using serovars Enteritidis and Montevideo. Attempts to detect two different inoculated serovars from the same sample were unsuccessful, with only one of the two serovars being detected.

Significance: Quasimetagenomics can generate accurate serotype predictions within 24-48 h of receipt of sample, without the need for culture isolation. While our study achieved correct serotype calls with even less sequencing time than previously reported, the ability to correctly distinguish multiple serotypes in complex samples still needs refinement for successful implementation.

T2-03 Targeted High Throughput Quasimetagenomic Sequencing Using Hybridization Capture for Detection of *Salmonella* in an Outbreak Investigation

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Introduction: In 2020, the FDA investigated an outbreak of *Salmonella* Enteritidis infections linked to the consumption of peaches. Shotgun metagenomic profiling was performed on outbreak implicated sample enrichments along with hybridization capture to identify *Salmonella* isolates.

Purpose: Our objective was to identify *Salmonella* serovars directly from culture enrichments using shotgun metagenomic profiling with hybridization capture.

Methods: *Salmonella* was isolated from outbreak implicated peach leaves (n=3) and fruits (n=2) using FDA BAM method with an addition of sonication step in sample preparation. Two random sub-samples (1 leaves, 1 fruits) were spiked with a GFP-labeled *Salmonella enterica* Gaminara strain to ensure method validity. Shotgun metagenomic sequencing was performed on both 24hr pre-enrichments (H24) and selective enrichments (H48) along with hybridization capture. The baits design was based on SeqSero O and H antigen database and contained ~5000 baits (80 nt long), targeting over 373 genes. Results were analyzed using a custom-built k-mer bacterial database and using alignment-based tools such as BLAST and BURST against custom built databases.

Results: Proportional abundance of *Salmonella* ranged from 1 to 5% in H24 pre-enrichments and 60 to 80% in H48 enrichments. Only *S.* Gaminara (control strain) and *S.* Alachua were identified through culture methods. BLAST and BURST alignment against database of ~2600 *Salmonella* genomes identified multiple serotypes on all enrichments. *Salmonella enterica* Alachua was predominant with maximum hits and *Salmonella Enteritidis* was detected at very low levels on peach fruit TT enrichments. SeqSero predicted *S.*Alachua, and *S.* Gaminara in RV and TT enrichments of fruits, and mixed serotypes in peach leaf enrichments.

Significance: Using custom target capture baits may be a useful tool for serovar detection directly from enrichments. Improving the selectivity of *Salmonella* enrichment from foods and coordinating with sequencing approaches facilitates expedited source attribution.

T2-04 Predictive Analytics within Food Safety: Source Attribution of *Salmonella* Using Whole-Genome Sequence Data and Random Forest

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Introduction: *Salmonella* continues to be a vexing public health problem; incidence rates have not declined in two decades

Purpose: Investigate likely food sources of human salmonellosis by developing a random forest model trained on whole-genome sequenced *Salmonella* isolates with known food source and apply it to sequenced human isolates to estimate their likely source.

Methods: Allele profiles for 18,379 isolates from NCBI were determined based on a whole genome multi locus sequence typing (wgMLST) approach that included 22,457 loci. Isolation sources were binned into 15 food categories (most isolates were from chicken (N=5,741, 31%), vegetables (N=2,313, 13%), and turkey (N=2,281, 12%)). We assessed random forest performance in predicting the likely food source by varying measures of feature importance and class weighting. We evaluated performance using accuracy and kappa for out-of-bag (OOB) estimates and for a test dataset from a stratified 75% train-25% test (TT) split. The optimal model was used to predict the isolation source of 7,240 isolates from patients in FoodNet sites during 2014-2018; any isolate with <50% certainty was categorized as having an "unknown" source.

Results: The optimal model used the 6,360 most informative loci by permutation importance with inverse class weighting (overall accuracy: 81% OOB, 75% TT; kappa: 78% OOB, 70% TT). Forty-eight percent (N=3,467) of isolates were categorized as having an unknown source. Among the remaining 52% (N=3,773), chicken was predicted the most common source (N=1,789, 47%), followed by vegetables (N=943, 25%), and pork (N=348, 9%). Chicken was also predicted the most common source of illness caused by serotypes Enteritidis (N=1,215/1,427, 85%), Typhimurium (N=185/308, 60%), Infantis (N=31/53, 58%), and Heidelberg (N=48/87, 55%).

Significance: These results show the promise of predictive analytics to inform food safety and outbreak investigations because they can help identify food categories of interest and complement attribution estimates based solely on outbreak data.

T2-05 Identifying Sub-Populations in *Salmonella* Serovars from Genomic Virulence Markers

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Introduction: Current *Salmonella enterica* serovar classification does not discriminate between isolates with dissimilar evolutionary histories, obfuscating our understanding of serovar-level human health risks.
Purpose: To identify sub-populations within serovars isolated from humans, cattle, and beef using virulence gene carriage in genome assemblies and differences in clinical presentation.
Methods: A curated set of 12,337 *S. enterica* genomes, comprising 37 commonly isolated serovars, was compiled from human Salmonella isolates with clinical case information (CDC-FoodNet), *Salmonella* isolates from beef/cecum at slaughter (USDA-FSIS), and retail beef samples (FDA). Putative virulence factors were predicted from each genome to establish isolate-based virulence gene catalogues. Pairwise similarity between each gene catalogue (isolate relatedness) was estimated using unsupervised Random Forest. Isolates from the resultant pairwise similarity matrix were clustered using Ward's method and split into 37 clusters (serovar number). Cluster consistency was estimated via non-parametric bootstrap. Sub-populations in serovars were defined as serovar presence in ≥ 2 clusters, each cluster population representing ≥ 0.20 of the total serovar population. Difference in virulence between serovar sub-populations, measured by the proportion of extraintestinal infections (PrEI) from 6,052 isolates with clinical case information, was tested using relative risks (RR) (posterior confidence of RR>1, PrRR>1).
Results: 10 of the 37 serovars analyzed had potential genomic virulence sub-populations: Muenchen, 1,4,[5],12:b:-; 1,4,[5],12:i:-; Infantis; Montevideo; Oranienburg; Kentucky; Saintpaul; and Typhimurium, with PrEI ranging from 0.071 (0.009 – 0.190) to 0.423 (0.263 – 0.593). Significant differences in PrEI were only found for sub-populations of Infantis (RR: 2.0, 1.3 – 3.2, PrRR >1: 99.9%) and Typhimurium (RR: 2.0, 1.2 – 3.3, PrRR > 1: 99.7%). In both Typhimurium and Infantis sub-populations, bovine and beef strains were resident along with human clinical strains.
Significance: Serovars sub-populations may present different virulence profiles. Differentiating sub-populations linked to more severe clinical presentations could improve the assessment of risks and control of *Salmonellae* in foods.

T2-06 Diversity and Phylogeny of Selected Nontyphoidal *Salmonella* Serovars Associated with Meat and Poultry

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❖ ***Developing Scientist Entrant***

Introduction: Several nontyphoidal *Salmonella* (NTS) serovars with clinical significance have been shown to be polyphyletic and present virulence differences between clades within a given serovar.
Purpose: Analyze the evolutionary history of NTS serovars associated with meat (*S.* Montevideo) and poultry (*S.* Kentucky and *S.* Enteritidis), and assess the association of clades within these serovars with human salmonellosis.
Methods: For each serovar, a phylogeny was reconstructed using representative isolates of all NCBI Pathogen Detection SNP clusters and singletons identified. Distinct phylogenetic groups were defined and subdivided into two nested levels of subgroups. Based on epidemiology evidence, subgroups were classified as human-associated (HA), non-human-associated (NHA), or neutral by calculating odds ratios; one-sided Fisher's exact tests (with FDR correction) was used to determine statistical significance.
Results: All three serovars were determined to be polyphyletic, with *S.* Montevideo (total isolates: 7,618), *S.* Enteritidis (total isolates: 66,210), and *S.* Kentucky (total isolates: 10,050) comprising three (designated Montevideo A-C), five (Enteritidis A-E), and two (Kentucky A-B) phylogenetic groups, respectively. For both *S.* Montevideo and *S.* Enteritidis, one phylogenetic group contains the majority of isolates (Montevideo A: 7,062 isolates; Enteritidis A: 66,118 isolates) and contains both HA and NHA clades. For *S.* Kentucky, while Kentucky A (8,314 isolates) chiefly contains US isolates that were rarely associated with human salmonellosis, Kentucky B (1,734 isolates) is featured by a large, HA SNP cluster mainly consisting of UK isolates. Interestingly, Montevideo C contains only two isolates, which clustered with *S.* Mbandaka with few SNP distances, likely representing a recent serovar conversion.
Significance: The polyphyly of the serovars and the differential human virulence across clades within a serovar suggest a shift of NTS control strategies from serovar-classification-based to risk-based approaches to maximize public health impacts. These findings will guide future comparative genomics and phenotypic experimentations to identify genomic features associated with enhanced or reduced human virulence.

T2-07 Contribution of Plasmid Diversity to the Genomic Plasticity of *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: Plasmids play an indispensable role in the evolution of bacteria. The emergence and spread of plasmid-borne genetic determinants encoding virulence and/or antimicrobial resistance in foodborne pathogens pose a significant threat to food safety, particularly in *Salmonella enterica* which is a leading cause of foodborne bacterial disease outbreaks.
Purpose: This study assessed plasmid diversity and their dissemination dynamics in *S. enterica.*
Methods: 2855 *S. enterica* genomes contained in the *Salmonella* Food Systomics (SalFos) database were screened for the presence of plasmids. Genetic determinants encoding virulence and antimicrobial resistance were assessed. Plasmids were mapped against single nucleotide polymorphism (SNP)-based phylogeny to infer their distribution and dissemination among *S. enterica* serotypes and genetic clusters.
Results: Overall, 766 plasmids from 57 bacterial host species were detected in 63% (n = 1810/2855) of the *S. enterica* genomes studied. Additionally, 71 different rep types were identified, including 24 Inc and nine Col groups. Plasmid content analysis identified 35 different virulence determinants in 259 plasmids from 564 *S. enterica* genomes. Also, 229 plasmids carried a total of 94 genes encoding resistance to 12 classes of antibiotics. Interestingly, 80% (184/229) of these plasmids were predicted to be mobilizable. Co-occurrence of genes encoding resistance to multiple antimicrobials, including biocide (*sugE*); arsenic (*arsAD*-operon); tellurite (*tehA*) and antibiotics; aminoglycosides*(aph(3")-Ib*), fosfomycin *(fosA),* tetracycline *(tetA),* among others were associated with the mobilizable IncFIB plasmid type, and prevalent in *S. enterica* subsp. *enterica* serovar *infantis.* Mercury resistance determinant (*merB*) borne on mobilizable plasmids with IncC and lncH12A rep types, co-occurring with genes encoding resistance to four classes of antibiotics were identified in multiple *S. enterica* serotypes and genetic clusters.
Significance: This data suggests that plasmid surveillance would contribute to understanding the emergence and spread of plasmid-borne genetic determinants encoding for virulence and resistance to antimicrobial compounds in *S. enterica*.

T2-08 QAC Efflux Genes are Common in *Listeria monocytogenes* Isolates from U.S. Food Processing Environments and are Variably Associated with Clonal Complex, Isolation Source, and Persistence

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Introduction: QAC efflux genes increase the minimum inhibitory concentration of *Listeria monocytogenes (Lm)* to benzalkonium chloride, but the prevalence of these genes and their contribution to persistence is variably reported.
Purpose: To assess patterns in *Lm* ecology and QAC efflux gene distribution by leveraging a large-scale collection of isolates.

Methods: We analyzed WGS data for 4,969 *Lm* isolates collected from U.S. food facilities. A custom BlastP search screened for QAC efflux genes *bcrABC*, *qacH*, *emrC*, and *emrE*. Single Nucleotide Polymorphisms (SNPs) were calculated within groups of *Lm* using the CFSAN SNP Pipeline v2.2.1 for facilities with >20 isolates collected across at least four years. Logistic regression was performed using the generalized linear model (glm) in the emmeans package v1.6.2-1 with the Tukey method of p-value adjustment for multiple comparisons.

Results: Cassette *bcrABC* was present in 46% of all isolates. Isolates from mixed facilities had the highest prevalence of QAC efflux genes (75%) followed by seafood facilities (67%, $p < 0.001$). Groups of isolates collected in different years with a mean SNP distance ≤ 20 were identified in each of the nine individually analyzed facilities, which we defined as evidence of persistence. Changes in the prevalence of QAC efflux genes were generally linked with changes in the prevalence of specific clonal complexes. For example, in seafood facility D, as the prevalence of CC321 strains rose from 25% in 2011 to 76% in 2017 so did the prevalence of QAC efflux gene. This confounds assessments on the role of QAC efflux genes as clonal complexes possess many genetic differences that may contribute to persistence. Among persistent isolates, differing patterns of QAC efflux gene gain and loss were observed.

Significance: Leveraging large-scale, open-source DNA sequence databases reveals patterns in QAC efflux gene distribution across *Lm* in the U.S.

T2-09 The Association between Pastured Poultry-Related Microbiomes and *Campylobacter* Presence

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter* is a commensal microorganism in the gut of poultry and is mainly present in the ceca, feces, and colon. Bird feces has been identified as a major source of flock transmission of *Campylobacter* at the farm level.

Purpose: The purpose of this study was to investigate the association between microbial communities and *Campylobacter* presence in pastured poultry farms.

Methods: Feces (n=543) and soil (n=542) samples were collected from 11 pastured poultry farms in the Southeastern United States from 2014 to 2017. Bacterial communities of these samples were characterized using Illumina 16S rRNA gene amplicon sequencing. Meanwhile, *Campylobacter* spp. were tested for the presence or absence in the samples. Feces and soil samples were taken three times, start, middle, and end, throughout a flock's lifecycle. Models were developed by pasture times separately for feces and soil samples. Linear Discriminant Analysis Effect Size (LEfSe) and random forest were performed to select the differential features and predictive features between *Campylobacter* present and absent groups, respectively.

Results: The LEfSe identified *Bacteria* (phylum), *Firmicutes* (phylum), *Clostridia* (class), and *Clostridiales* (order) as features that differentiate *Campylobacter* negative group from *Campylobacter* positive group in feces samples at the beginning of pasture. In soil samples of early pasture time, *Proteobacteria* (phylum) was selected as the differential feature. In prediction of *Campylobacter* presence in feces samples at end of the pasture time, genus *Caloramator* was identified as the top feature using random forest. For soil sample, genus *Limnohabitans* was the most important feature in predicting *Campylobacter* presence at start and end of the pasture times.

Significance: These models provide an alternative way of differentiating and predicting *Campylobacter* presence in pastured poultry farms. The results showed the potential of integrating microbial community data in predictive microbiology and risk assessment models.

T2-10 Genomic Characterization of Five Plasmids Harbored by Two Environmentally Persistent *Cronobacter sakazakii* Strains Recovered from Powdered Infant Formula Manufacturing Facilities

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Introduction: *Cronobacter sakazakii* is a foodborne pathogen that causes serious disease in neonates, infants, and adults.

Purpose: This study describes the complete genome sequences of two environmentally persistent *C. sakazakii* (H322 and GK1025B) strains isolated from powdered infant formula (PIF) manufacturing settings. In addition, genomic characterization of five plasmids, pH322_1, pH322_2, pGK1025B_1, pGK1025B_2, and pGK1025B_3 is described.

Methods: Using PacBio single-molecule real-time (SMRT®) sequencing technology, whole genome sequence (WGS) assemblies of *C. sakazakii* H322 (ST 83, clonal complex [CC] 83) and GK1025B (ST64, CC64) were generated. Plasmids, also sequenced, were aligned with phylogenetically related episomes to determine, and identify conserved and missing genomic regions.

Results: A truncated ~13-kbp type 6 secretion system (T6SS) gene cluster was identified on virulence plasmids pH322_2 and pGK1025B_2, and a deletion (6 kbp) was identified on plasmid pH322_2, which included genes encoding a tyrosine-type integrase, a hypothetical protein, and a phospholipase D. Within the T6SS, an arsenic resistance operon was also identified, which is common among plasmids pSP291_1 and pESA3. In addition, an intact *Salmonella* SSU5 prophage gene cluster was mapped to plasmids pH322_1 and pGK1025B_1 which were shown to be phylogenetically related to *C. sakazakii* phage-plasmids pCS1, pCsa767a, pCsaC757b, and pCsaC105731a. Plasmid pGK1025B_3 was identified as a novel conjugative *Cronobacter* plasmid. Furthermore, WGS analysis identified a ~16.4-kbp type 4 secretion system gene cluster harbored on plasmid pGK1025B_3, which contained a phospholipase D gene, a well-known virulence factor.

Significance: These data provide high resolution information on *C. sakazakii* genomes and emphasizes the need for furthering surveillance studies to link genotype to phenotype of strains from previous investigations. These results provide baseline data necessary for future in-depth investigations of *C. sakazakii* that colonize PIF manufacturing facility settings and contribute to a better understanding of this pathogen's survival and persistence within various "built environments" like PIF manufacturing facilities.

T2-11 Validation of the Single-Use Glove Microbiome Shotgun WGS Metagenomic Analysis

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Introduction: Until the current multi-year project, previous studies on single-use gloves (SUGs) employed in food & healthcare environments have suffered from technical limitations preventing adequate microbial hazard identification. Eagle Protect PBC w/ partners have developed an effective methodology enabling 16S & shotgun sequencing of SUG isolates using the NCBI BLAST nt sequence analysis platform.

Purpose: Having overcome multiple technical challenges reported previously, robust bioinformatic validation of WGS sequence read findings is an important final step in data analysis.

Methods: Isolates from pooled rinsate (inside &/or outside) of 26 (25 nitrile & 1 vinyl) SUG brands consisting of positive MPN enrichment broth samples had previously been subjected to 16S/ITS1 amplicon & Shotgun sequencing. Being reported here is BLAST-based validation that was employed in tandem w/ an ensemble concordance between BLAST, Sourmash 3.5.0, MetaPhlAn 3 (default parameters & database). BLAST annotations were quality-filtered using a read-level implementation of the Bazinet et al. (2018) protocol designed for bioforensic casework. This included a prefiltering of annotations against a set of negative controls, initial quality-filtering using protocol-specific cutoffs for e-value and bit score ranges, and the establishment of bit score thresholds (b-s) for non-target taxa. With non-target taxa chosen for each target taxon amongst pathogens, fecal indicators or specific taxonomic groups, the completed validation parameters were selected and used for benchmarking.

Results: Approximately 6.4% of 25 million reads were qualified prior to b-s filtering. Including identification of human specific fecal indicators (~50% / samples), 16 enterotoxigenic strains of *Bacillus cereus* & *B. anthracis* were validated along with the presence of *Listeria monocytogenes*, *Clostridoides difficile*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* & *Streptococcus pneumoniae.*

Significance: Frank & opportunistic pathogens identified on gloves in this study may represent public health issues for glove users, food or healthcare entities that rely on these hand surrogates for safe operation.

T2-12 Tracking the Source of *Bacillus Thuringiensis* in Spinach and Tomato

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❖ ***Developing Scientist Entrant***

Introduction: *Bacillus thuringiensis* (Bt), a member of *B. cereus* group, can be naturally found on plants as the inherent microbiota, but also can be used as a biopesticide and persist on crops, and be introduced as such to the food supply-chain and the final produce at sale.

Purpose: This study aims to track the source of Bt on spinach, from fresh to frozen spinach with or without prior treatment of Bt biopesticides, and on tomato from the retail level.

Methods: Fresh spinach from the field, washed and frozen spinach samples were obtained from 3 processing companies. Tomato samples were bought in the local supermarkets in Ghent. Samples were analysed at Ghent University and typical presumptive *B. cereus* colonies were counted from MYP agar. Isolates were checked by the production of parasporal crystals under phase-contrast microscopy for the initial identification of Bt. Selected isolates were further analysed by WGS at Aarhus University or the TaqMan qPCR method by screening the *cry* genes (*cry1Aa/Ab*, *cry2Aa* and *cry1D*) developed at WUR to differentiate Bt biopesticide strains from the other *B. cereus* group strains.

Results: Higher occurrence and counts of presumptive *B. cereus* with more Bt populations were found in spinach samples prior sprayed with Bt biopesticides than samples without use of Bt biopesticides. Eight out of 12 Bt isolates (identified by microscopy) from spinach samples treated with Bt biopesticides were highly likely to be the same strain as the respective 2 used Bt biopesticides strains, both by WGS and TaqMan analysis. Besides, 57 out of 109 tomatoes (52%) were positive for presumptive *B. cereus*, and 97% (206/213) isolates from these samples were identified as Bt by microscopy. Twelve of 13 of these Bt isolates were identified as the Bt biopesticide strains by TaqMan analysis.

Significance: Bt found in spinach products and tomatoes are more likely from Bt biopesticide commercial products.

T3-01 Efficacy of Acetic Acid Dissolved in Oil with Water-in-Oil Emulsions Against Desiccated Cells of *Salmonella* Enteritidis and *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Desiccated bacteria possess cross-tolerance against environmental stresses and thereby persist in dry food-processing environments such as peanut butter and chocolate facilities.

Purpose: The objective of this study was to develop oil-based antimicrobial delivery systems for dry cleaning and sanitation of food processing equipment.

Methods: A contact time of 30 min at 22 °C was used as treatment, against *Salmonella enterica* serovar Enteritidis and *Listeria monocytogenes* inoculated onto stainless-steel coupons to 7 log CFU and desiccated at a constant relative humidity (RH). Bacterial survival was determined using plate count method with TSA, and using the Most Probable Number (MPN) method with buffered TSB. Paired and unpaired *t*-tests were performed at a significance level of 95%.

Results: Treatment with 200 mM acetic acid dissolved in oil (the acidified oil) reduced desiccated cells of *S.* Enteritidis (75% RH) by 0.69 log. The cells desiccated at 33% RH exhibited significantly greater resistance, which were reduced by the acidified-oil treatment by 0.05 log ($P < 0.05$). However, when 0.3-9% v/v water was added to the acidified oil in the form of water-in-oil (W/O) emulsions (the acidified W/O emulsions), the desiccated *S*. Enteritidis was reduced to below limit of detection (0.48 log, i.e., a reduction of > 6.52 log MPN/coupon) after treatment, regardless of the desiccation level. The acidified oil and W/O emulsions were also effective against desiccated cells of *L. monocytogenes*. Furthermore, the antimicrobial activity of acetic acid was found contingent on the prevailing osmotic gradient. With a fixed water concentration, lowering the water activity (a_w) of the acidified W/O emulsions (by using glycerol) led to reduced efficacies.

Significance: This study demonstrated that W/O emulsions with low levels of water (≥ 0.3% v/v) were a viable delivery system for acetic acid to sanitize stainless-steel surfaces, and the antimicrobial activity of acetic acid was found dependent on osmotic stress.

T3-02 AI-Enabled Biosensing for Rapid Identification of Pathogens in Food and Agricultural Water

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❖ ***Developing Scientist Entrant***

Introduction: Food and agricultural processes are water-intensive, and thus managing microbial water quality is essential for food safety, food security, and public health. However, timely detection of pathogenic bacteria is interfered with by complex and noisy environmental background matrices and requires a well-trained workforce.

Purpose: This study aimed to develop an artificial intelligence (AI)-biosensing platform for rapid and automated pathogen identification in food and agricultural water.

Methods: We present an AI model to detect diffusive microscopic patterns of T7 bacteriophage-induced cell lysis specific to *Escherichia coli*. The model was trained with a deep learning algorithm on augmented datasets using *E. coli* monoculture (10–10^3 CFU/mL) and selected non-*E. coli* bacteria (*Listeria innocua*, *Bacillus subtilis*, *Pseudomonas fluorescens*), followed by fine-tuning on a mixed culture of these four species. The model inference was performed on lysed *E. coli* in unseen real-world samples with a gradient of food and environmental background noise, including coconut water, spinach wash water, and irrigation water. Samples without phage addition were used as controls. The model performance was examined based on the observed-predicted plot for *E. coli* counts with 95% confidence prediction intervals, its regression slope and evaluation metrics (R^2, RMSE), and overall prediction accuracy.

Results: The results show that our AI-biosensing platform could accurately detect and quantify *E. coli* in food and agricultural water with a reduced assay time of 5.5 h (< a single food processing shift) and a detection limit of 10 CFU/mL. Moreover, the model trained solely on lab-cultured bacteria achieved prediction on the unseen real-world water samples with 80–100% accuracy in 403 images, depending on the sources of background noise (i.e., food debris, organic matter, and natural microbiota).

Significance: Overall, this study demonstrates the potential of the AI-biosensing platform to automate and accelerate microbial water quality monitoring during food and agricultural processing.

T3-03 Characterization and Detection of Finfish Parvalbumin

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❖ ***Developing Scientist Entrant***

Introduction: As the major allergen in finfish, parvalbumin is responsible for more than 90% of finfish allergies. To reduce the occurrence of finfish allergy, on the one hand, food allergen characterization could assist in protein modifications to decrease allergenicity. On the other hand, it is critical to develop assays for allergen residues quantification.

Purpose: This project aims to (1) characterize finfish parvalbumin's physical composition and immunochemical properties and (2) develop and characterize single-stranded (ss) DNA aptamers against finfish proteins.

Methods: First, parvalbumin from four finfish species (cod, salmon, mullet, and tilapia) was purified using column chromatography. Effect of chelators, reducing chemical and oxidizing agent on antibody-antigen interaction was studied using immunoblot. Second, ssDNA aptamers were developed against cod parvalbumin using microplate systematic evolution of ligands by exponential enrichment (SELEX). Their target analyte, selectivity, structure, and affinity were analyzed.

Results: First, parvalbumin was successfully purified from four finfish species with more than 97% purity based on gel electrophoresis analysis. The yield of parvalbumin from cod, salmon, mullet, and tilapia was 1.1, 0.4, 3.4, and 1.2 mg/g of muscle (wet basis), respectively. Native parvalbumin contained both monomers and oligomers. In the presence of reducing agent (dithiothreitol) and detergent (sodium dodecyl sulfate), parvalbumin oligomers dissociated into monomers. As for the immunochemical properties, immunoreactivity of four finfish parvalbumin decreased when its calcium was chelated. Second, a total of 20 aptamer candidates were obtained. All aptamers had a hairpin structure. It is noted that at different temperatures, the same candidate sequence may have different structures. The developed aptamers showed reactions with several finfish proteins.

Significance: The purified parvalbumin has the potential to be used in the development of capturing agents such as antibodies and aptamers, which can be further utilized in the assay development for the detection of fish allergen. It also points out the possibility of reducing parvalbumin antigenicity by calcium chelation.

T3-04 Combined Effect of Conjugated Linoleic Acid over Converting *Lactobacillus casei* and Berry Phenolic Extracts Against Colonization of *Campylobacter* in Chicken

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❖ ***Developing Scientist Entrant***

Introduction: In previous studies, we have observed that either probiotic *Lactobacillus casei* with *mcra* (myosin-cross-reactive-antigen) over-expressed (LC^{+mcra}) or berry phenolic extracts (BPEs) individually can reduce the colonization of *Campylobacter* in chicken model and combination of them can do the same in *ex-vivo* condition.

Purpose: In this study, we aimed to evaluate the combined effect of LC^{+mcra} and BPEs in reducing the colonization of *Campylobacter* and in gut microbiome modulation using a day-old chicken model.

Methods: A day-old chicks (160) were fed with either LC^{+mcra}, BPEs, combination of LC^{+mcra} and BPEs or antibiotic growth promoters to evaluate *Campylobacter* colonization trend on gut of birds. A Kanamycin resistant *C. jejuni* strain (CJ-KM) was used as reference pathogenic strain for oral challenge. Culture based techniques were used for *Campylobacter* enumeration and 16S metagenomics approach was used to compare microbiome profiles. ANOVA was used to determine differences in treatments/control groups.

Results: Colonization of CJ-KM as well as the natural colonization of *Campylobacter* was reduced by the combined effect of LC^{+mcra} and BPEs significantly ($p<0.05$) at all time points. Combined treatments reduced the colonization of both CJ-KM and *Campylobacter* by >1.0 log CFU/g in different intestinal portions of the chicken gut at all time points (day-14, 21, and 28). Similar pattern of reduction was observed in antibiotic-fed group of chicken. At phylum level, there was notable change; there was significant lower percentage of Proteobacteria in the combined treatment group compared to control group ($p<0.05$). At genus level *Lactobacillus* was significantly higher (1.7-fold) and *Campylobacter* was significantly lower (6.3-fold) in the combined treatment group compared to control group ($p<0.05$).

Significance: Colonization of *Campylobacter* in poultry gut can be controlled by combination of LC^{+mcra} and BPEs without observed detrimental effect on normal gut flora. Therefore, LC^{+mcra} with BPEs could be an alternative to improve safety of poultry products and reduce campylobacteriosis.

T3-05 Norovirus Detection in Fresh Produce, Water, and Hand Rinses: Potential for Environmental Transmission during Production?

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❖ ***Developing Scientist Entrant***

Introduction: Norovirus is a leading cause of produce-associated foodborne disease outbreaks in the United States, however, the contribution of production practices to produce contamination is unclear.

Purpose: To investigate the presence of amplifiable human norovirus RNA in produce and environmental samples taken from agricultural production, and the relationships between paired agricultural samples.

Methods: Composite samples (185) were collected from 10 farms in Nuevo Leon, Mexico, including 82 produce rinses (cantaloupe [77], jalapeño [50], tomato [58]), 49 hand rinses, 27 irrigation, and 27 source waters. Of these, 88 represented produce paired with pre-harvest agricultural water and 97 represented produce paired with post-harvest hand-rinse samples. Samples were concentrated by ultracentrifugation and polyethylene glycol precipitation followed by detection of norovirus (GI, GII) RNA by RT-qPCR. Presumptively positive samples (cycle threshold <40) were sequence-confirmed. Logistic regression models were used to assess the association between viral RNA detection on matched samples.

Results: Among all samples, 75 (40.5%) were presumptively-positive for the presence of human norovirus RNA, with a higher proportion representing GII (36.9%, 66/179) than GI (9.7%, 18/185); 5% (9/185) were positive for both GI/GII. Among these presumptive-positives, 30% (25/84) were sequence-confirmed (sequence quality was variable): (8% [2/25] GI.1; 32% [8/25] GII.6; 60% [15/25] GII.2). The predominant genotype in water and on hands was GII.2, whereas for produce it was GII.6. The highest percentage of sequence-confirmed norovirus RNA-positive samples was in irrigation water (26%, 7/27), which was significantly more than hands (8%, 4/49, p=0.04), source water (15%, 4/27), and produce (11%, 9/82). Across sample types, the only significant association was the frequency of GII RNA in irrigation water (p=0.04), relative to hands.

Significance: Detection of human norovirus RNA in agriculture samples suggests that fresh produce production practices may play a role in contamination events. However, norovirus infectivity data are needed to fully understand the public health significance of these findings.

T3-06 Comparison of Genetic Information on Stress-Resistant and -Sensitive *Listeria monocytogenes* Isolated from Foods, Humans, and Animal-Related Sources

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes,* is an opportunistic foodborne pathogen that can survive under severe environmental conditions (low pH, high salinity, and low temperature). To reduce a potential outbreak caused by *L. monocytogenes*, identification of essential genes associated with survival under harsh growth conditions is needed.

Purpose: The purpose of this study is to compare genetic characteristics between stress-resistant and -sensitive *L. monocytogenes* to identify gene compositions related to virulence, antibiotic resistance, and survival.

Methods: A total of 65 (36 stress-resistant and 29 stress-sensitive) *L. monocytogenes* were selected via stress-resistance test under pH (3), salinity (4%), and temperature (1 °C). Whole-genome sequencing was conducted using an Illumina MiSeq according to the FDA protocol. Genetic characteristics of stress-resistant and -sensitive *L. monocytogenes* strains were analyzed in pan-genome, functionality, virulence, and antibiotic resistance genes. Identified unique genes present in stress-resistant *L. monocytogenes* were confirmed using a PCR assay.

Results: The number of unique genes in stress-resistant *L. monocytogenes* showed 466 while stress-sensitive group possessed 35 unique genes. Unique genes of stress-resistant group included *PadR*, *OsmC*, and *CsbD*, and 29 stress-resistant *L. monocytogenes* had *FadR*. The unique genes of stress-resistant *L. monocytogenes* exhibited several functionalities, such as replication, recombination & repair (17.9 %), and translation, ribosomal structure & biogenesis (3.5 %) than the stress-sensitive group, 6.6 % and 1.7%, respectively. Stress-sensitive *L. monocytogenes* possessed *lin* and *mprF* genes (antibiotic resistant genes) more than in the stress-resistant *L. monocytogenes*. In the analyses of virulence genes, stress-resistant group possessed more *lisK* genes than stress-sensitive group.

Significance: This study will contribute to understand genetic relationship between stress-resistant and -sensitive *L. monocytogenes* isolated from diverse sources.

T3-07 *Salmonella* Surveillance in United States Broiler Production, 2016–2021

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❖ ***Developing Scientist Entrant***

Introduction: Despite significant reduction of *Salmonella* incidence during processing, poultry remains a considerable source of foodborne salmonellosis. Therefore, there is a need to understand the dynamics of different *Salmonella* serovars in pre- and post-harvest poultry.

Purpose: Prior to this study, some poultry companies reported to us that some serovars they find during live production do not align with those found during processing. We initiated this study to determine whether this pattern occurred more broadly across national and regional *Salmonella* monitoring data.

Methods: *Salmonella* data from the United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) monitoring of processed poultry products and regional flock monitoring was analyzed from 2016-2020. CRISPR-SeroSeq was performed on breeder samples collected between 2020-2021 to investigate the incidence of multi-serovar populations in breeder flocks.

Results: The overall *Salmonella* incidence in broiler carcasses and intact parts (parts) has decreased from 8.86% (1,344/15,162) to 6.31% (1,422/22,535). The average incidence in parts was 11.14% (4,737/42,511) compared to 4.51% (2,145/47,538) in carcasses. Regional differences include higher proportions of serovars Infantis and Typhimurium found in processors in the Atlantic regions and higher relative amounts of serovar Schwarzengrund in the Southeast. For Georgia, the largest broiler producing state, USDA-FSIS data was compared to *Salmonella* monitoring data from breeder flocks over the same period, revealing that serovar Kentucky was the major serovar in breeders (67.91%; 104/134), but not at processing, suggesting that it is more effectively removed during interventions. Our population analyses completed with CRISPR-SeroSeq showed that 32% (43/134) of samples contain multiple serovars, with up to 11 serovars found.

Significance: Using serovar population analyses by CRISPR-SeroSeq revealed that many pre-harvest samples contain multiple serovars, which explains the serovar diversity seen during processing. Although this study is focused on broiler production, the findings here are broadly applicable to other industries where *Salmonella* is a concern. Additionally, this study highlights the importance of comprehensive surveillance monitoring in food production systems to identify and control pathogens.

T3-08 Capture and Concentration of Human Noroviruses in Foods and Environmental Samples by Engineered Bacterial Strains

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Introduction: Due to the low levels of virus present in food and environmental samples, its low infectious dose, and the lack of a feasible culture enrichment step for routine testing in foods, it is important to specifically concentrate human norovirus prior to detection. Existing concentration methods have several limitations, such as higher cost, lack of scalability, and generally low capture efficiency (<30%).

Purpose: The purpose of the current study was to evaluate the use of nonpathogenic *E. coli* mutants engineered to express norovirus-specific peptides on their surface for concentration of human norovirus (GII.4) prior to detection.

Methods: E. *coli* strains were engineered to present norovirus-specific peptides by cloning them to the C-terminus of the ice nucleation protein after introduction of a serine-glycine spacer (SGGGGSGGGGSGGGGS). Mutants were grown, induced with 0.5mM IPTG overnight and diluted to 10^6 cfu/ml, then suspended with norovirus GII.4 containing diluted patient stool sample. Following incubation for one hour, capture efficiency was determined by suspension assay-RT-qPCR by calculating removal of input virus from suspension.

Results: Capture efficiencies ranged from 66% (SD ±4.0) to 81% (SD ±0.24) among the 7 different engineered *E. coli* strains and *E. cloacae,* a native-binding norovirus bacterium. The highest capture efficiencies observed with the engineered *E. coli* strains, 81.34 % (SD ±0.2) and 76.55% (SD ±1.0) were higher than that observed with *E. cloacae* (76%, SD ±3.0), as well as the no-peptide scaffold control. Using engineered *E. coli* had the added benefit of being inducible, potentially making the mutants less prone to media/growth conditions as has been reported for *E. cloacae*

Significance: We were able to demonstrate the potential of engineered *E. coli* strains expressing norovirus specific peptides for concentration of noroviruses prior to detection. Owing to the ease of deployment, cost-effectiveness, and potential to be scaled up for handling larger volumes, we envision this method can be easily adopted for concentration of norovirus from foods, patient, and environmental samples.

T3-09 Detection of the Viable but Non-Culturable State (VBNC) of *Listeria monocytogenes* and *Listeria innocua* induced by Biocide Stress Using Raman Microspectroscopy

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria innocua* is a Gram-positive ubiquitous bacteria, non-pathogenic, widely distributed in a range of environments (vegetation, water, soil). *L. innocua* is frequently found in the food-processing environments with other species, such as *Listeria monocytogenes*. *L. monocytogenes* is an important foodborne pathogen and the etiological agent of human listeriosis, a rare but frequently fatal disease. *L. monocytogenes* can also persist in the industrial environment and (re)contaminate food, which has already been evidenced in several epidemics. The bacterial persistence may be due to their resistance to cleaning and disinfection procedures, that may induce a viable but non-culturable (VBNC) state of bacteria. Bacteria in the VBNC state have very low metabolic activity and are not detected by standard culture-based methods. These bacteria retain the ability to recover. Raman microspectroscopy coupled with deuterium isotopic probing (Raman-DIP) offers metabolic labelling. DIP appears to be a powerful technique for the in-situ study of bacterial metabolism, through vibrational spectroscopies.

Purpose: We evaluated the capacities of the Raman-DIP to discriminate between different states of viability (VC, VBNC, dead) found in *L. innocua* after biocide (P3-topactive DES) treatment.

Methods: We treated the bacteria with biocides used in the seafood industrial sector and we analysed their impact on bacterial viability by qPCR, PMA-qPCR and plate count agar (total, viable and VC population respectively) and by Raman-DIP microspectroscopy. We selected different concentrations of biocide to ensure a VC/VBNC/dead population.

Results: Dead bacteria can't be analyzed using Raman-DIP. For viable bacteria, the Raman-DIP results showed that the VC population metabolised the isotope and showed measurable labelling, with a high C-D peak in the Raman spectra (2150cm^{-1}), which was not the case for the VBNC state, where no labelling was observed without C-D peak on the bacteria.

Significance: We were able to discriminate each viability status of *L. innocua* and *Listeria monocytogenes* after a biocide stress, and use this technique to measure the viability status after application of other stresses.

T3-10 Characterization of Very High Pressure (550 MPa) Resistant Bacterial Spores

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❖ ***Developing Scientist Entrant***

Introduction: Resistant bacterial spores pose a food safety risk and often demand intensive food processing for inactivation. As a gentle alternative, a high pressure (HP) based strategy is being investigated. It relies on germinating spores by HP to make them susceptible to a second, mild inactivation treatment. For moderate (100-300 MPa) and very (vHP, 400-800 MPa) HP germination, a sub-population of HP germination deficient, so-called HP superdormant (HPSD), spores prevents the implementation of the germination-inactivation strategy.

Purpose: To implement the germination-inactivation strategy, it is essential to characterize HPSD spores, e.g. their susceptibility to a second vHP treatment, and investigate possible causes for vHP superdormancy like a genetic inheritance.

Methods: *Bacillus subtilis* PS533 spores were treated at 550 MPa, 60 °C for 40-60 s (ACES buffer pH=7.0). Then, HPSD spores were quantified using flow cytometry (FCM), isolated by buoyant density centrifugation and subjected, parallelly to an untreated control, to an identical second HP treatment to characterize the permanence of vHP superdormancy. To test genetic inheritance of vHP superdormancy, isolated HPSD spores and an untreated control were resporulated, HP treated again as above and analyzed by FCM.

Results: While HPSD spores did not germinate in the first pressure treatment, > 99 % of HPSD spores germinated after exposure to an identical second HP treatment (n=3) indicating that vHP superdormancy might be transient or that the first HP treatment made them more susceptible to vHP. Due to similar germination capacities of resporulated HPSD spores and their control (e.g. 50 s HP: 3±1 % and 4±2 %, n=4), inheritance of a genetic change can be excluded as main cause for vHP superdormancy.

Significance: For the first time, vHP superdormant spores were isolated and characterized. This study advances the understanding of germination at industrial pressure levels and the development of a mild spore inactivation strategy.

T3-11 Development of a Strain-Specific *Shigella* Isolation Method from Model Food Commodity Using Genomically Predicted Antimicrobial-Resistance Traits

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❖ ***Developing Scientist Entrant***

Introduction: Isolation of *Shigella* from shigellosis-outbreak-associated foods is often problematic due to the lack of effective selective enrichment media.

Purpose: To investigate whether strain-specific selective enrichment media, developed based on antimicrobial resistance (AMR) predicted from *Shigella* whole-genome sequence (WGS) data, can improve recovery of *Shigella* from implicated foods.

Methods: AMR profiles of *Shigella* strains associated with historical foodborne outbreaks were predicted based on draft genomes and validated using broth microdilution in *Shigella* Broth (SB) and mTSB. Approximately 3, 30 and 300 CFU of a target outbreak strain was inoculated into 25-g portions of carrots in triplicate and recovered using the Canadian reference method (MFLP-25) and two strain-specific enrichment media developed based on the AMR of the target *Shigella* strain (SB with 50 µg/mL streptomycin (SB-S), mTSB with 25 µg/mL trimethoprim (mTSB-T)). Efficiencies of enrichment media was assessed by: (1) determining proportion of colonies on differential plates recovered from post-enrichment broths (93 colonies per sample) and (2) determining relative fraction of the *Shigella* marker gene (*ipaH*) to total bacterial loads (16s rDNA) by quantitative PCR (qPCR).

Results: *Shigella* AMR profiles could be accurately predicted based on WGS; however, resistance to some of the antibiotics (e.g. trimethoprim) was reduced in SB. *Shigella* was not isolated from SB or SB-S at any inoculation level, but recovered from all of the mTSB-T enrichments (37.5-100% of colonies). Assessment of samples by qPCR was consistent with microbiological analyses. The relative proportion of the *Shigella* marker was highest in mTSB-T (1:1.57 to 1:5.76), followed by SB-S (1:41 to 1:391), with lowest proportions in SB without antibiotics (1:1170 to 1:49300).

Significance: Improved *Shigella* recovery was achieved in this study with the addition of custom selective agents during cultural enrichments. Genomically-informed custom selective enrichment of *Shigella* could be an important tool for supporting future foodborne shigellosis outbreak investigations.

T3-12 Genotypic and Phenotypic Characterization of *Salmonella enterica* and *Listeria monocytogenes* Recovered from Alternative Irrigation Water on the Eastern Shore of Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Alternative water sources for the irrigation of field crops are gaining interest due to the pressures that water scarcity places on traditional irrigation sources. Investigating characteristics of bacterial pathogens recovered from water is required to appropriately identify public health hazards.

Purpose: This study investigated the genetic diversity and antimicrobial resistance patterns of *Salmonella enterica* and *Listeria monocytogenes* isolates recovered from six alternative irrigation water sources on Maryland's Eastern Shore using whole genome sequencing (WGS) and minimum inhibitory concentration (MIC) tests.

Methods: Eighty-two *S. enterica* and 94 *L. monocytogenes* PCR-confirmed isolates from irrigation water were sequenced on the MiSeq platform using 500 cycles of paired-end reads and analyzed for identification, subtyping, virulence marker detection, and antimicrobial resistance predictions. For phenotypic resistance patterns, MICs were determined using the Sensititre micro-broth dilution method for Gram-negative (*S. enterica*) or Gram-positive (*L. monocytogenes*) bacteria.

Results: Eighteen *S. enterica* serotypes were found, with *S.* Infantis (26%) being the most prevalent. Seven *L. monocytogenes* serotypes were identified, with 4b being the most prevalent (40%). Thirty-five percent of the *S. enterica* isolates showed resistance to one antimicrobial and 29% exhibited multidrug resistance (MDR) (resistance to two or more antimicrobial classes). Ninety-seven percent of the *L. monocytogenes* isolates were resistant to one drug, and 42% exhibited MDR. WGS predicted resistance genotypes of all *S. enterica* isolates to the *aac(6')-Iaa* gene and 99% of *L. monocytogenes* isolates to the *fosX* gene. All *S. enterica* isolates carried the *invA*, *sopE2*, *ssaQ*, *ssaR*, *mgtC*, and *prgH* virulence genes. All *L. monocytogenes* isolates carried the *hly*, *inlA*, *inlB*, *prfA*, and *mpl* virulence genes.

Significance: A diverse array of *Salmonella* serovars, including those causing human illness, were recovered from alternate irrigation water sources. These findings, along with the identification of multi-drug resistant *L. monocytogenes* isolates, highlight the need for mitigation strategies for irrigation water intended for produce crops.

T4-01 Sustainable Packaging for Meat Products: Fermentation Can Help!

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Introduction: Oxygen, together with light, is the most important factor affecting the color stability of sliced meat products packed under modified atmosphere. With the increasing use of L.E.D at retail and the use of (more) sustainable packages (monolayer and/or packaging more permeable to oxygen) avoiding greying and oxidation of meat products during their whole shelf life is a challenge.

Purpose: The purpose was to evaluate if the addition of a specific *Lactococcus lactis ssp. lactis strain,* mutated within the gene sequence coding for the lactate dehydrogenase, could consume enough oxygen from meat packages to delay oxidation and greying of products.

The alternative reaction used by this strain for recycling NAD+ is catalyzed by a NADH oxidase. NADH is oxidized to NAD+ by the concomitant consumption of stoechiometric amounts of oxygen.

Methods: Cooked products (poultry ham, cooked ham, mortadella) were sprayed with *Lactococcus lactis ssp. lactis strain* (concentration around 6.5 Log cfu/g) during slicing, MAP packed and stored between 4 and 7°C during their shelf life. Samples were first stored in darkness to mimic storage at meat producers and then exposed to the light to mimic storage at retail.

Residual oxygen, pH, red color intensity and sensory assessment (hedonic test) were carried out over the shelf life of the meat products.

Results: *Lactococcus lactis ssp. lactis strain* significantly reduced the residual oxygen level in MAP packages to reach a level significantly lower than in the control. The a-value was more stable and no greying was measured. From a taste point of view, no negative impact of the culture was noticed by the panelists.

Significance: With increasing demand for more sustainable packaging and reduced food waste, the application of the *Lactococcus lactis ssp. lactis* strain is a natural solution to improve the quality of meat products.

T4-02 Characterization and Isolation of Lactic Acid Bacteria Probiotic Candidates from Fermented Meats

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Introduction: With increasing public awareness of a healthier lifestyle, there is growing market demand for functional foods/supplements which contain probiotics. Probiotics lactic acid bacteria (LAB) are defined as live microorganisms which confer health benefits to the host when administered in adequate amounts.

Purpose: The purpose of this study was to screen for novel probiotic candidate(s), as probiotic features and health benefits conferred, which are known to be strain-specific.

Methods: The biodiversity of 106 LAB isolates sourced from fermented meats was investigated using 16S rRNA and GTG_5 PCR fingerprinting. Antagonistic activity and production of organic acids were investigated against 22 distinct strains. Antibiotic susceptibility, hydrophobicity, auto-aggregation capacity, ability to withstand simulated gastric juice (0.3% pepsin), bile salt (0.3%), phenol (0.2 & 0.5%) and NaCl (1.5 to 3.5%) were assessed to select for probiotic candidates. Presence of bacteriocin-associated gene clusters and probiotic gene markers were analyzed. Two-way ANOVA verified with Tukey's multiple comparison tests was performed; a p-value of < 0.05 was considered statistically significant.

Results: *Lactiplantibacillus plantarum* was the most dominant species in the fermented meats. *L. plantarum* strains were found as the strongest lactate producer (106 mM to 158 mM). *L. plantarum* strains effectively inhibited all bacteria and fungal indicator strains tested. *L. coryniformis* subsp. *torquens* and *L. plantarum* displayed significantly greater hydrophobicity (31.2 to 42.2%) compared to *L. curvatus*, *L. sakei* and *P. acidilactici* (4.9 to 18.1%). *L. plantarum* strains showed highest auto-aggregation levels (63.6 to 69.0% at t = 24 h), highest tolerance to 0.3% bile salts, simulated gastric juice, phenol, and salt compared to strains of other species. Overall, *L. plantarum* 41G was identified as the most robust probiotic candidate possessing probiotic gene markers (*bsh*, *fbp*, *mub*) and also a bacteriocin producer.

Significance: Novel proposed probiotic candidate *L. plantarum* 41G was successfully isolated, characterized and potentially could be further developed into functional foods.

T4-03 Prevalence and Antibiotic Resistance of *Salmonella* in Organic and Non-Organic Chickens

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* infections related to chicken products have been intensely increasing worldwide and becoming a universal public health crisis.

Purpose: This study aimed to determine the prevalence of *Salmonella* in organic and non-organic chickens and investigate antimicrobial resistance

profiles.

Methods: Whole broiler carcasses organic (n=20) and non-organic (n=20) were obtained monthly for one year (n=480) from a retail store on the Eastern Shore of Maryland. Each carcass was mixed with 500 mL of buffered peptone water and incubated at 37 ºC for 24 h. *Salmonella* isolation and identification were conducted by following the whole carcass enrichment method recommended by USDA-FSIS. Two hundred thirteen confirmed *Salmonella* isolates (organic n=76; non-organic n=137**)** were serotyped and tested for antibiotic susceptibility using standard methods.

Results: Forty-nine percent of the carcasses were positive for *Salmonella.* Organic and non-organic positivity rates were 37.1% and 61.8%, respectively. There was no significant difference in the prevalence of *Salmonella* between organic and non-organic chickens (p>0.05). The most common serotypes were *Salmonella* Kentucky (47%), Infantis (35%), Enteritidis (6%), Typhimurium (5%), and Blockley (4%). Enteritidis was associated only with non-organic chicken while Blockley recovered only from organic chicken. Typhimurium was more prevalent in organic chicken (10.53%) than non-organic chicken (2.19%). Ninety-one percent and 61% of the isolates were resistant to at least one antibiotic and multidrug-resistant, respectively. Resistance was often observed to tetracycline (82.8%), minocycline (42.3%), nitrofurantoin (40.3%), cefazolin (38.3%), and ampicillin (32.1%). Resistant to ceftriaxone in organic and non-organic chicken was 31.6% and 24.1%. All isolates were susceptible to antibiotic classes of fluoroquinolone, carbapenem, and glycylcycline regardless of the type of chicken.

Significance: The results of this study demonstrate a high prevalence of *Salmonella* contamination in organic and non-organic chickens and a significant number of these isolates were resistant to commonly used antibiotics.

T4-04 Prevalence of *Salmonella enterica* in Hatchling Chicks Sold in Vermont Agricultural Supply Stores in 2021

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Introduction: Backyard chickens are increasingly common in Vermont and across the U.S. Cases of salmonellosis associated with live poultry have also increased, especially during Covid-19. Studies indicate that that *Salmonella enterica* may be more common in chicks than adult poultry, but limited data exist. The National Poultry Improvement Plan's *Salmonella* monitoring program (NPIP-SMP) is a voluntary program intended to reduce the prevalence of *S. enterica* in hatcheries selling chicks to non-commercial farmers.

Purpose: The purpose of this study was to determine the prevalence of *S. enterica* in chicks sold at Vermont agricultural supply stores and determine whether hatchery participation in the NPIP-SMP affected *S. enterica* prevalence.

Methods: We collected shipping pads and/or soiled bedding to test at nine agricultural supply stores and three independent hatching/resale operations across VT in March-August 2021, recording hatchery, breed, and age of chicks. Hatchery NPIP-SMP participation was determined via hatchery websites. 25 gram samples were enriched in buffered peptone water for 4 hours at 37° C, followed by Rappaport-Vassiliadis and Tetrathionate enrichments for 24 hours at 42°C and 37°C, respectively. Positive samples were streaked onto XLT4 agar and incubated at 37°C for 48 hours. Identification was confirmed *hilA* PCR.

Results: 82/203 (40.4%) samples were positive for *S. enterica*. Chicks from hatcheries participating in the NPIP-SMP were significantly more likely to be positive for *S. enterica* (50.9%; 57/112) than chicks from non-participating hatcheries (27.8%; 22/72) (Chi-squared test, p <0.01). Hatchery positivity rates were 22-76%, with the lowest rate in the hatchery not participating in the NPIP-SMP.

Significance: *S. enterica* is common in chicks sold at agricultural supply stores, and chicks from hatcheries participating in NPIP's SMP had significantly higher rates of *S. enterica* infection than non-participating hatcheries. Consequently, purchasing chicks from NPIP-SMP participant hatcheries is not effective in reducing the risk of contracting salmonellosis from live poultry for poultry owners.

T4-05 Evaluating the Effect of Organic Matter on Peroxyacetic Acid Effectiveness Against *Salmonella* spp. in Raw Poultry Parts in Post-Chill Tanks

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Introduction: High volumes of water and antimicrobials are used by the poultry industry to comply with food safety performance standards; however, the impact on environment and occupational safety makes it necessary to improve sustainability of these treatments without compromising food safety effectiveness.

Purpose: To evaluate the antimicrobial efficacy of reused peroxyacetic acid (PAA) solutions for decontamination of *Salmonella* on chicken parts in simulated post-chill immersion tanks with added organic matter (OM).

Methods: Chicken thighs (average weight 208.7 ± 3.4 g) were inoculated with a five-strain cocktail of poultry-borne *Salmonella* (6.8 log10 CFU/ml per wing). Thighs were immersed into PAA solutions (500 or 1,000 ppm, 30 s) with different concentrations of OM (0, 5, or 10 g/L). OM was simulated using a chicken slurry made from water, thigh meat, and thigh skin. *Salmonella* counts were determined on XLD plates for neutralizing buffered peptone water rinsates (100 ml/thigh). Experiments were performed in triplicate (three thighs per treatment per replicate). Chemical oxygen demand (COD), total nitrogen (TN), and pH were used as water quality parameters of PAA solutions.

Results: Mean *Salmonella* reductions were 0.9 ± 0.1 log10 CFU/ml for 500 ppm PAA and 1.1 ± 0.0 log10 CFU/ml for 1,000 PAA, regardless of OM concentration. There was no significant effect of PAA concentration (p=0.244) or OM concentration (p=0.981) on reductions. COD ranged from 2,905 to 4,340 mg/L and from 5,525 to 6,290 mg/L for 500 ppm and 1000 ppm, respectively. TN was 42.5 ± 2.0 mg/L and 60.9 ± 8.3 mg/L for 5 and 10 g/L OM.

Significance: The OM concentrations tested did not affect the antimicrobial efficacy of PAA solutions against *Salmonella* on chicken thighs, which would allow these PAA solutions to continue to be reused. Studies are underway to determine OM levels that might significantly reduce the efficacy of PAA.

T4-06 Effect of Combined Action of *Nigella sativa* and Kefir on the Growth Performance and Health of Broiler Chickens

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❖ ***Developing Scientist Entrant***

Introduction: Coccidiosis and necrotic enteritis (NE) are two serious poultry diseases leading to high mortality rates as well as economic losses. The emergence of antibiotic resistance and increased demand for antibiotic-free poultry products stress an urgent need to explore natural products as substitutes.

Purpose: The project investigates *Nigella sativa* (black cumin seed) and authentic kefir alone or in combination in poultry feed or drinking water to inhibit coccidiosis and prevent or lessen NE in broilers.

Methods: An *in vitro* study was conducted to identify a black cumin seed (BCS) brand with strong anti-*Clostridium perfringens* (CP) activity, followed by an *in vivo* study consisting of 384 Cobb 500 male broiler chicks distributed in a randomized block experimental design. The animal trial included 5% BCS in feed, 20% kefir in drinking water, and their combination as treatments for birds challenged with coccidia and CP strain CP#4. After the coccidial challenge, the combined treatment received BCS only on day 14 and afterwards. Live broiler live performance, disease outcomes, and CP populations were measured for the trial.

Results: Black cumin seed, kefir, and their combination did not have a detrimental effect on broiler live performance parameters of feed intake, feed

conversion, and weight gain. The NE scores for BCS treated and combination groups were lower than the positive control and not significantly different from antibiotic control. There was no significant difference between the treatment groups and antibiotic control in mortality. Following CP infection, both the population of vegetative cells and spores of CP decreased for all treatments. Overall, the treatments successfully reduced CP infection and mortality, with no adverse impact on broiler growth performance and the combined treatment was the most effective in protecting broilers.

Significance: *Nigella sativa*, kefir, and their combination can be an alternative to commonly used antibiotic treatment in mitigating *C. perfringens* infection and mortality in poultry.

T4-07 Food Safety Verification in the Sampling Program Life Cycle: Raw Pork Sampling for *Salmonella*

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Introduction: The Department of Agriculture's Food Safety and Inspection Service (FSIS) verifies food safety in part through robust product sampling. Sampling programs have life cycles that drive FSIS priorities. Life cycles are driven by food safety verification, resource allocations, results, and ever-changing technological advancements. Raw pork sampling is an example: FSIS conducted a market hog baseline survey, as part of a risk assessment, but discontinued carcass sampling in 2011 since pathogen positive rate was low.

Purpose: Show a sampling program life cycle and how FSIS reevaluates sampling and refines policy. FSIS' raw pork sampling life cycle progression is depicted.

Methods: FSIS reviews sampling data, illness outbreak data, policies, industry practices, public comment, and other feedback to inform sampling programs and refine policy. FSIS evaluated *Salmonella* in pork product samples collected between 2015-2021 (3,896/22,557 [17.3%]) to assess prevalence, which is a weighted average based on production volume.

Results: Pork outbreaks and illnesses precipitated a return to exploratory sampling to identify ways to reduce *Salmonella*. In 2015, FSIS began sampling raw pork products for *Salmonella* and indicator organisms. Exploratory testing was conducted in phases for surveillance and to assess national prevalence (NP). During Phase I, FSIS analyzed 1,200 samples and 16.7% were positive for *Salmonella* (200/1,200). During Phase II, *Salmonella* percent positive and NP was highest in comminuted products (380/1,796 [21.2%]; 28.9%NP), followed by intact (97/1,170 [8.3%]; 5.3%NP), and nonintact (68/1,048 [6.5%]; 3.9%NP) cuts. FSIS continues to collect and analyze 8,500 samples annually. These data were used to develop *Salmonella* performance standards in raw pork products, which is currently under development.

Significance: The life cycle demonstrates how FSIS ensures food safety by evaluating data and implementing improvements to protect consumers: data refines sampling, policy is informed by the data, agency goals refined, and decisions made to improve public health outcomes.

T4-08 Analysis of Veal Cecal Samples Collected Under the NARMS Expansion Program during CY2020

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Introduction: National Antimicrobial Resistance Monitoring System (NARMS) takes a One Health approach to understanding antimicrobial resistance (AMR) across multiple animal species, humans, and the environment. USDA FSIS has been collecting and reporting AMR data since 2013. The agency explored additional commodities such as veal, Siluriformes, and goat/sheep/lamb as a potential reservoir of antimicrobial resistance in FY2020.

Purpose: The objective of this study was to expand surveillance for antimicrobial resistance of bacteria in the cecal contents of veal, previously not well-studied in a nationally representative manner.

Methods: Veal cecal samples were collected under 3 sampling programs: Animal-Calf-Bob Veal, Animal-Calf-Non-Formula-fed Veal, and Animal-Calf-Formula-fed Veal. A total of 378 Samples were analyzed for the presence of *Salmonella*, *Campylobacter*, *Enterococcus*, and generic *Escherichia coli*. Isolates recovered were characterized by phenotypic antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS). All data was compared to data from non-veal cattle cecal samples collected during the same time.

Results: For CY2020, the recovery rates of *Campylobacter*, *Salmonella*, generic *E. coli* and *Enterococcus* from veal cecal samples were 10.5% (32/306), 21.2% (80/378), 98.5% (197/200) and 93.0% (186/200) respectively. Most of the 80 *Salmonella* isolates (88.8%) recovered were Pan-susceptible, and 6.3% were multidrug resistant (MDR). Two rare genes, *bla-CTX-M-27* and *bla-CTX-M-14*, that impart resistance to ampicillin, ceftriaxone, ceftiofur were detected in *E. coli* isolated from veal cecal samples (4/197 [3 bob veal] and 1/197 [1 bob veal], respectively) but undetected *E. coli* from other cattle cecal samples collected during the same time period. Additionally, differences in the top *Salmonella* serotypes recovered between veal cecal (18.75% serotype Muenster, 8.75% serotype Infantis) and other cattle cecal (4% serotype Muenster, 0.75% serotype Infantis) samples were observed.

Significance: This data provides insights into the previously undescribed distribution of antimicrobial resistance in bacteria from veal cecal samples to support a One Health approach of expanding surveillance into additional commodities.

T4-09 Effect of High Pressure Processing on Shelf Life and Safety of Ground Beef Formulations Containing Different Sodium Levels

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Introduction: Salt reduction without loss of quality, safety and shelf-life is a major challenge to the meat industry. Although HPP has shown promise in enhancing the safety and shelf-life of sodium 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 to enhance shelf-life and safety of sodium-reduced ground beef formulations during storage.

Methods: Ground beef formulations with 3.0 % sea salt containing different NaCl levels (55, 65, 75, 99%) and a control (no salt) were subjected to pressures of 300 MPa for 3 min, 5 min and 600 MPa for 3 min. Samples were analyzed for total plate counts (TPC) and coliforms in uninoculated samples, and *L. monocytogenes* counts from inoculated (~6 log cfu/g) samples immediately after HPP and after 7 day storage at 4 and 7 °C.

Results: Reduction in sodium levels significantly increased TPC, coliforms and inoculated *L. monocytogenes* counts after 7 days of storage at 7 °C in all non-pressurized meats. Efficacy of HPP at 300 MPa for 3 or 5 min in lowering TPC was significantly reduced with increasing sodium levels. However, TPC recovery during storage at 4 and 7 °C was significantly inhibited by higher sodium levels. Pressurization at 300 MPa did not significantly effect inoculated *L. monocytogenes* counts. HPP at 600 MPa for 3 min, reduced TPC, coliforms and *L. monocytogenes* to below detectable levels in all meat formulations and the recovery of sub-lethally injured cells during storage was significantly inhibited by higher sodium levels.

Significance: HPP at 600 MPa for 3 min can be successfully used to enhance safety and shelf-life of NaCl reduced ground meat formulation.

T4-10 Identification of Dominant Factors Contributing to Persistence of Third-Generation Cephalosporin-Resistant *Salmonella* at a Beef Cattle Feedyard

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Introduction: Beef cattle are suspected to contribute to human 3GC-resistant *Salmonella* infections. Commensal third-generation cephalosporin (3GC)-resistant *Escherichia coli* are more frequently isolated than 3GC-resistant *Salmonella* at beef cattle feedyards and are theorized reservoirs of 3GC resistance.

Purpose: The goal of this research was to employ whole genome sequencing (WGS) to determine the contribution of 3GC-resistant *E. coli* to the occurrence of 3GC-resistant *Salmonella* at a beef cattle feedyard in Nebraska.

Methods: During each of 24 consecutive months 4 samples of pen surface material were obtained from 5 pens (N = 480) at a Nebraska feedyard. Illumina WGS was performed and susceptibilities to 14 antimicrobial agents were determined for 121 3GC-susceptible *Salmonella*, 121 3GC-resistant *Salmonella*, and 203 3GC-resistant *E. coli* isolates.

Results: 3GC-susceptible *Salmonella* isolates were predominantly Muenchen (70.2%) and Montevideo Clade 1 (23.1%). 3GC-resistant *Salmonella* isolates were predominantly Montevideo Clade 2 (84.3%). One *bla* gene type (bla_{CMY-2}) and the IncC plasmid replicon were present in 100% and 97.5% of the 3GC-resistant *Salmonella*, respectively. Conversely, 11 *bla* gene types distributed were distributed across 42 3GC-resistant *E. coli* multilocus sequence types. The bla_{CMY-2} gene and IncC plasmid replicon were present in 37.9% and 9.9% of the 3GC-resistant *E. coli*, respectively.

Significance: These results suggested that 3GC resistance in *Salmonella* was primarily due the persistence of a Montevideo Clade 2 sub-population with very minimal or no contribution from 3GC-resistant *E. coli* via horizontal gene transfer. These results suggest antimicrobial resistance in *E. coli* may not be a useful indicator of antimicrobial resistance in *Salmonella*. Intensive longitudinal WGS studies at commercial feedyards that include detailed records (cattle source, backgrounding processes, feed, and antimicrobial use) will aid the design, monitoring, and assessment of pre-harvest interventions effective against high-priority antimicrobial-resistant *Salmonella*.

T4-11 Utilizing *Salmonella* Dynamics between Beef Cattle and the Feedlot Environment to Determine Effective *Salmonella* Mitigation Strategies

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❖ ***Developing Scientist Entrant***

Introduction: Investigating *Salmonella* interactions between the feedlot environment and beef cattle, to identify pre-harvest environmental *Salmonella* mitigation techniques, will address the lack of post-harvest interventions for beef cattle lymph nodes harboring *Salmonella*.

Purpose: A longitudinal collection of beef cattle fecal and feedlot environmental samples, followed by an analysis of the isolated *Salmonella,* will determine if *Salmonella* serovars vary by season, sample type and feedlot pen to demonstrate environmental treatment plausibility.

Methods: Feedlot pens (n=30) were geographically distributed evenly across the West Texas A&M University Research Feedlot in Canyon, TX. Pen environment, water, feed and freshly voided fecal samples from a random selection of three beef cattle (n=360) per pen were collected monthly across June-December 2019. Cattle subiliac lymph nodes were harvested upon slaughter. Whole genome sequencing of all *Salmonella* DNA isolated from the lymph nodes (n=96), pen environment (n=145), water (n=34) and feed (n=13) and a random selection of *Salmonella* DNA isolated from feces (n=171) was completed. A bioinformatics pipeline was used to determine *Salmonella* serovars, sequence types, antimicrobial resistance (AMR) genes and phylogenetics were used to determine isolate relatedness.

Results: *Salmonella* serovars Montevideo (28.5%, n=131), Kentucky (28.3%, n=130), Anatum (20.3%, n=93), Lubbock (12.6%, n=58), Cerro (8.9%, n=41), Virginia (0.8%, n=4), Derby (0.2%, n=1) and Senftenberg (0.2%, n=1) were identified. Sequence type was consistent within serovars and all of the isolates were pan-susceptible except for the Senftenberg isolate which harbored multiple AMR genes. A multinomial logistic regression suggests the majority of *Salmonella* serovars varied significantly ($p<0.05$) by sample type and collection month. Phylogenetic trees display closely related *Salmonella* isolates comprised of similar sample type, pen location and season metadata.

Significance: Understanding *Salmonella* dynamics between the feedlot environment and cattle will help develop targeted mitigation techniques to address the incorporation of *Salmonella* harboring lymph nodes into beef products.

T4-12 Evaluating Droplet Digital PCR (ddPCR) as a Culture Independent Confirmation Method for Shiga Toxin-Producing *E. coli* (STEC) in Routine Beef Microbiological Samples

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Introduction: Current qPCR methods for screening STEC have common challenges of separating enrichments with linked and unlinked virulence, resulting in difficulty in further cultural confirmation.

Purpose: To explore the possibility of using ddPCR as a culture independent confirmation method, the objective of this study was to evaluate ddPCR for confirming STEC in routine beef samples following positive results from primary screening by qPCR.

Methods: Over a period of six months, 100 routine beef microbiological samples (MicroTally™ and beef trim) that were presumptive positive for *E. coli* O157:H7 via qPCR-based primary screening tests was collected from several beef production facilities from four different states (Texas, Kansas, California, and Colorado). Sample enrichments were processed for culture-independent confirmation using the ddPCR technology (dd-Check STEC Solution, Bio-Rad) and cultural detection and confirmation following the USDA MLG 5C.01.

Results: For the confirmation of linked virulence profiles (presence of *stx* and *eae* in a single cell), ddPCR and the MLG identified the presence of linked targets in 97 and 95 samples, respectively, indicating that ddPCR and the MLG reached a high level of agreement (98%) in confirmation of linked virulence profiles. Two samples that were positive for linked virulence via ddPCR produced colonies that were positive for *stx* and *eae*, but negative for the Big 7 serogroups when MLG was performed. This finding indicates the presence of *stx* and *eae* positive strains outside of the regulated Big 7 serogroups that could potentially pose risk to the public, the presence of which could be identified with ddPCR , but would be missed by MLG due to the specificity of its immunomagnetic steps.

Significance: With a high level of agreement in STEC confirmation between ddPCR technology and the MLG method, ddPCR technology proved useful for culture-independent confirmation following primary screening by qPCR assays. Additionally, ddPCR testing can be completed in a matter of hours as opposed to days with current cultural confirmation techniques.

T5-01 Differences in the Removal of Chemical and Bacterial Contaminants from Wastewater Effluent Using Sand-Based Filters with Zerovalent Iron Versus Biochar

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❖ ***Developing Scientist Entrant***

Introduction: As the use of treated wastewater for irrigation purposes increases, there is concern that chemical and bacterial contaminants persist in this recycled water. Cost-effective and renewable adsorbents, including sand-based filtration systems with zero-valent iron (ZVI) or biochar (BC), can remove these contaminants from wastewater effluent. However, these adsorbents have not been comprehensively compared in terms of their removal efficiencies.

Purpose: This research tested the efficacy of sand-based filters to treat wastewater effluent.

Methods: To test these filter media, we passed wastewater effluent samples through six types of sand filters containing BC, nanosilver amended BC (Ag-BC), ZVI, BC with ZVI, Ag-BC with ZVI, and sand-only across six different time points. Chemical contaminants were measured using HPLC/MS-MS and *E. coli* concentrations were enumerated using standard membrane filtration. Total bacterial community composition was characterized by Illumina HiSeq sequencing of the V3-V4 region of the 16S rRNA gene.

Results: All filters, except ZVI, significantly removed chemical contaminants from the wastewater. The Ag-BC filter was the most effective in removing *E. coli* compared to the other filters. Although bacterial diversity in sand filtered samples was the highest, there was no significant effect of filter type on overall bacterial diversity and composition. With significant temporal variation (ANOSIM R = 0.69; p<0.05) in beta diversity across the six-time points, bacterial community richness was higher during the warmer months compared to the colder months. Specifically, compared to the control filter, relative abundance of *C39* and *Methylocaldum* at time point 2 and that of *Methylosinus* at time point 4 was significantly lower in all amended sand filters.

Significance: Our data demonstrate that ZVI- and BC-amended sand filters are potential solutions to remove micropollutants and bacteria from treated wastewater. Future studies on the efficacy of differential amounts of these media in sand-based filters over longer time periods to treat similar water types are needed.

T5-02 Factors Affecting the Recovery of Low Levels and Isolation of *Salmonella enterica* from Surface Water: A Multi-Laboratory Evaluation of Methods

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Introduction: The presence of antimicrobial-resistant *Salmonella enterica* in water indicates environmental contamination and is an emergent food safety and public health issue. Identifying and developing effective and sensitive detection methods for *Salmonella* from surface water is a recent goal of the National Antimicrobial Resistance Monitoring System (NARMS), supported by EPA, FDA, and USDA.

Purpose: To compare methods of and identify factors that affect recovery of *Salmonella* from surface water.

Methods: Volumes of surface water were collected in fall 2021 on five different dates. Rainfall data, water temperature, turbidity, pH, and dissolved oxygen were recorded. Water was shipped and analyzed at CA, GA, NE, and MD locations for recovery of environmentally present *Salmonella* and an inoculated, fluorescent-labeled *S.* Typhimurium strain (ca. 30 CFU), which was added to 1 L samples. Each sample was subjected to one of three recovery/enrichment methods: bulk water enrichment (BW), vertical Modified Moore Swab (MM), and the modified Standard Method 9260.B3 (SM). Presumptive *Salmonella* colonies were confirmed by biochemical analysis (environmental) and/or fluorescence (inoculated). Data were analyzed using a Chi-squared test with recovery method (RM), laboratory location (LL), and *Salmonella* detection (D) as categorical values.

Results: Statistical analysis revealed that the RM, LL, and D were not independent ($p < 0.001$) of each other, and all concurrently influenced recovery/detection of environmental and inoculated *Salmonella*. Across all four LL, SM was the method that most frequently recovered environmental and low levels of inoculated *Salmonella* from water.

Significance: Recovery of low levels of *Salmonella* is dependent on the method used and the specific laboratory conducting the work. These data support the need to standardize and simplify the recovery methods to reduce variability from different scientists. The modified SM 9260.B3 more frequently recovered low levels of *Salmonella* from inoculated water samples and should be prioritized for *Salmonella* recovery from surface water in laboratory settings.

T5-03 Use of Bulk Refillable Dispensers for Alcohol-Based Hand Sanitizers Can Lead to Alcohol Evaporation and Loss of Antimicrobial Efficacy

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Introduction: During the COVID-19 pandemic, many grocery stores and restaurants adopted alcohol-based hand sanitizer (ABHS) for use. Many of these establishments utilized bulk refillable ABHS dispensers, which have been associated with loss of alcohol due to evaporation, which may lead to a loss of antimicrobial efficacy.

Purpose: The purpose of this study was to perform a set of controlled laboratory experiments to evaluate the effect of bulk refillable ABHS dispenser use on ethanol content and antimicrobial efficacy over time.

Methods: Eight refillable dispensers were filled with 750ml of a gel based ABHS containing 70% ethanol (v/v). To simulate misuse observed in real world settings, half of the dispensers had the dispenser cap removed. As a control, sanitary sealed dispenser refills containing a 70% ethanol (v/v) gel based ABHS were evaluated. Samples were stored under controlled conditions (40°C, 75% RH) for 6 weeks, and aliquots were collected every 2 weeks for analysis. Ethanol content was measured by gas chromatography. Antimicrobial activity was evaluated using ASTM E2315 (Time Kill procedure). Triplicate samples were challenged with *Staphylococcus aureus* ATCC 6538 and *Serratia marcescens* ATCC 14756 for 15s.

Results: All ABHS in sanitary sealed refills and capped bulk dispensers exhibited no ethanol loss for the duration of the study and demonstrated a >5 $\log_{10}$ reduction of both test organisms. Uncapped bulk dispensers lost ethanol at a rate of 7.0% (v/v) per week, ultimately dropping to 29.33% ethanol at week 6. By week 6, antimicrobial efficacy of these samples dropped to <1.0 $\log_{10}$ reduction for both test organisms.

Significance: Storage of ABHS in open and uncapped bulk refillable dispenser resulted in a significant decrease in alcohol content after 6 weeks, which correlated to a decrease in antimicrobial efficacy. Therefore, storage and dispensing of ABHS in open bulk dispensers is not recommended for restaurants, grocery stores, and other food establishments.

T5-04 Ready-to-Eat Meat Plant Characteristics Associated with Food Safety Deficiencies during Regulatory Compliance Audits, Ontario, Canada

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Introduction: Food safety deficiencies in ready-to-eat (RTE) meat processing plants can increase food-borne disease risks.
Purpose: The purpose of this study was to identify common deficiencies and factors related to improved food safety performance in RTE meat plants in Ontario.
Methods: Routine food safety audit records for licensed provincial free-standing meat processing plants (FSMP) and abattoirs that process RTE meats were analyzed in Ontario, Canada, from 2015 to 2019. Plant characteristics associated with achieving a higher audit item pass rate and an overall 'pass' (vs. conditional pass or fail) rating were evaluated using log-linear and logistic regression models, respectively.
Results: Audits of 204 unique meat plants were evaluated, with FMSP (81.9%, *n* = 167) being the major establishment type. Producing blood products (Incidence rate ratios [IRR] = 1.009, 95% confidence interval [CI] = 1.001, 1.017) and canning processing (IRR = 1.022, 95% CI = 1.013, 1.031) were associated with higher rate of passing audit items. In contrast, carcass aging and breaking (IRR = 0.991, 95% CI = 0.987, 0.995) and fabrication (IRR = 0.995, 95% CI = 0.991, 1.000) activities were associated with a lower pass rate. For the logistic model, FMSPs were more likely to pass audits than abattoirs (odd ratio [OR] = 4.207, 95% CI = 2.216, 7.989). Plants that engaged in carcass aging and breaking (OR = 0.347, 95% CI = 0.175, 0.689) and that produced jerky (OR = 0.471, 95% CI = 0.264, 0.840) were less like to achieve a pass rating. For each unit increase in the number of plant employees, the odds of achieving an overall pass rating decreased by approximately 0.9%.
Significance: The results found in this study can support and guide future inspection, audit and outreach efforts to reduce food-borne illness risks associated with RTE meats.

T5-05 Descriptive Analysis of the Most Common Types of Food Safety Infractions at Ready-to-Eat Meat Processing Plants in Ontario, Canada

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Introduction: Food safety inspections of meat processing plants and abattoirs that process ready-to-eat (RTE) meats have identified a lack of compliance with a good manufacturing practices.
Purpose: This study was undertaken to identify common food safety infractions in the RTE meat processing sector in Ontario through an analysis of historical audit records.
Methods: Annual food safety compliance verification audit records from 2015 to 2019 for free-standing meat processing plants (FSMP) and abattoirs that process RTE meats in Ontario were analyzed. The audits assessed up to 426 items across the following evaluation categories: facilities and surface, equipment, water, operation, personnel, meat product handling and processing, labeling, packaging, transportation, and records. Frequency of audit item result (pass or fail) was analyzed using descriptive statistics in RStudio.
Results: A total of 376,457 audit item results were evaluated across 912 unique audits of 204 different RTE meat plants. A nearly two-thirds overall item pass rate (64.4%; *n* = 242,478) was identified. Excellent compliance was found across audit items in the two highest risk categories (critical-high and significant-high), with no items exceeding a 0.8% infraction rate. In the high-risk category, the most common infraction was improper 'sanitary condition of the plant' (41.3% fail rate; *n* = 450). Across all other risk categories, the highest rates of infractions were observed in the 'maintenance of premises, equipment and utensils' (56.7%; *n* = 750), 'temperature and humidity control' (40.1%; *n* = 393) and 'pest control' (24.9%; *n* = 236) items. The overall item pass rate of was higher in FSMPs (93.1%) than abattoirs (64.1%), while pass rates gradually decreased from 93.4 to 81.4% cross the study period.
Significance: The results of this study have identified key areas for improvement in future inspection, audit and outreach with RTE meat processing plants.

T5-06 Developing *In Vitro* Dry Surface Biofilm Models of *Salmonella enterica* Serovar Typhimurium, *Listeria monocytogene*s, and *Pseudomonas aeruginosa* in Low-Moisture Conditions to Understand Microbial Interactions

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❖ ***Developing Scientist Entrant***

Introduction: S. enterica ser. Typhimurium, Listeria monocytogenes and Pseudomonas aeruginosa can survive in a desiccated state as dry surface biofilms (DSB) for prolonged periods of time and can serve as a processing environment contamination source of low-moisture foods (LMFs).
Purpose: To develop in vitro DSB models to understand bacterial interactions under dehydrated conditions in biofilms to further evaluate and improve mitigation strategies.
Methods: Mono-and mix-culture wet surface biofilms (WSBs) of S. enterica ser. Typhimurium ATCC 700720, L. monocytogenes FSL R8-5318, and P. aeruginosa ATCC 15442 were developed and harvested following standard EPA MLB SOP MB-19 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 similarly harvested at each time point. Serial dilutions were plated on selective agar for each species. Mean log10 CFU/cm2 were calculated and compared to control (WSB) using LSM of PROC GLM to fit linear models (a=0.05). Replications were done in triplicate.
Results: Approximately 7.8±1.1, 7.7±0.6, 7.2±1.6 mean log10 CFU/coupon were recovered from mono-culture S. enterica serovar Typhimurium DSB at 24, 48, and 72 h, respectively. S. enterica populations decreased to 6.0±0.1, 5.5±0.6, and 5.3±0.5 when co-cultured with P. aeruginosa. While log10 CFU/coupon for mono-culture L. monocytogenes DSB were comparatively lower (3.8±0.1, 3.4±0.6, and 2.6±0.3 at 24, 48, and 72 h), mean log10 densities/coupon increased to 5.6±0.3, 5.7±0.1, and 5.6±0.5 in presence of P. aeruginosa.
Significance: Considering emergence of LMFs as potential sources of foodborne outbreaks and recalls, our data will provide insights to existing conditions in LMF processing facilities that may harbor pathogens and models that support mitigation strategies.

T5-07 Identification of a Surrogate for *Salmonella* Enteritidis PT 30 in Physical Removal Experiments Relevant to Dry Sanitation

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Introduction: Dry sanitation activities commonly rely on physical removal to control *Salmonella* cross-contamination. However, a non-pathogenic surrogate for dry cleaning experiments has not been identified.
Purpose: The purpose of this study was to evaluate potential *Salmonella* Enteritidis PT 30 surrogates for their relative tolerance to physical removal from surfaces.
Methods: Non-pathogenic *Enterococcus faecium* NRRL B-2354, *Listeria innocua* ATCC 51742 and *Escherichia coli* ATCC 25922 were selected as potential surrogates for the cell physical removal of *S.* Enteritidis PT 30. Coupons (L*W*H: 3.5*2.4*0.48 cm^3) of different surface materials (stainless steel (SS), high density polyethylene (HDPE), rubber) were spot inoculated using 18 spots of 10 µL inoculum and then dried overnight in a biosafety cabinet. Physical removal of cells from coupons was simulated using a custom platform for consistent application of a paper towel across the surface. Following the simula-

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tion, cells 1) remaining on the surface and 2) transferred to the paper towel were enumerated after three passes of the paper towel. All experiments were conducted in triplicate. A two-way ANOVA was performed to evaluate the interaction effect between surface material and surrogate identity. All statistical analysis was performed in RStudio with a significance level of 0.05.

Results: The log reductions of *S. Enteritidis* PT30 on SS, HDPE and rubber surfaces after physical removal were 0.57±0.44, 0.37±0.52, 0.81±0.67 CFU/coupon, respectively. The corresponding log reductions of *E. coli* ATCC 25922 were 0.16±0.30, 0.00±0.10 and 0.42±0.11 CFU/coupon, respectively. *E. coli* ATCC 25922 was conservative as a surrogate in physical cell removal studies. The effects of surface material, surrogate identity, and the interaction with surface material were not significant on the removal of cells. However, surrogate identity had a significant effect on the cells transferred to paper towel.

Significance: *E. coli* ATCC 25922 may be used as a non-pathogenic surrogate in validation studies of physical removal using dry sanitation activities.

T5-08 Patented Organic Peracetic Acid and Hydrogen Peroxide-Based Sanitizing Solution Achieves > 5 Log CFU/g Reduction in *Salmonella* Surrogate *Enterococcus faecium* NRRL B-2354 on Peanuts at an Industrial Scale

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Introduction: Peanut contamination with *Salmonella* has caused product recalls and illness outbreaks, necessitating a pathogen control step. Studies evaluating pathogen control technologies have identified *Enterococcus faecium* NRRL B-2354 as a suitable *Salmonella* surrogate for shelled peanuts. In this investigation the efficacy of a commercially-available pathogen control technology involving application of a patented organic acid and hydrogen peroxide solution followed by drying against *E. faecium*-inoculated shelled peanuts was evaluated.

Purpose: The study objectives were to: 1) determine application and drying parameters for a > 5 log CFU/g reduction of *E. faecium*, and 2) validate these parameters in commercial equipment and assess sensory attributes.

Methods: Peanuts were inoculated with *E. faecium* (30 mL/kg overnight culture with food colouring added as a visual indicator). Inoculated samples were treated (110 mL/kg) and dried (112.78 °C for 5 min) in a pilot scale continuous dryer. Recovery was compared before and after treatment (5 x 45g samples, plated on Slanetz and Bartley agar).

For validation, 23 kg inoculated and dyed peanuts (30 mL/kg overnight culture) were mixed with fresh peanuts (totaling 705 kg), treated in a commercial applicator (105mL/kg), and conveyed immediately into a commercial fluidized bed dryer (112.78 °C for 5 min). Samples were collected every minute from the dryer exit. Inoculated and dyed peanuts were sorted out for enumeration (13 x 45 g samples). Recovery was compared to inoculated, untreated samples (10 x 45 g). Undyed, fresh peanuts treated at the same parameters were blindly assessed in smell, appearance, taste, and texture sensory attributes (5-point hedonic scale, 5 participants).

Results: A 5.33 and 5.15 log CFU/g reduction of *E. faecium* was achieved at lab and industrial-scale, respectively, with sensory attributes unchanged.

Significance: The industrial-scale system is a suitable intervention method, giving an average > 5 log CFU/g reduction of a *Salmonella* surrogate on peanuts, while maintaining sensory attributes.

T5-09 Assessment of *Listeria* Sampling Designs for Improving and Validating *Listeria* Control in Small Food Processing Facilities

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❖ ***Developing Scientist Entrant***

Introduction: Appropriate sampling schemes and effective cleaning and sanitation are necessary to control and eliminate *Listeria* from the processing environment. General guidance recommends performing environmental monitoring for *Listeria* at least 4 h into production (mid-operation), which is a key strategy for identifying sources of *Listeria* in the processing environment, but mid-operation sampling alone is not able to demonstrate the efficacy of cleaning and sanitation procedures at controlling *Listeria*.

Purpose: To assess whether sampling for *Listeria* both (i) after cleaning and sanitation and before production (termed pre-operation) and (ii) ~4 h into production (termed mid-operation) in eight small food processing facilities can improve the efficiency of identification of root causes of contamination.

Methods: For each facility, 18-50 environmental sponge swabs were collected from the same sites within the processing environment at pre-operation and mid-operation timepoints. Swabs were processed according to the FDA-BAM standard method for detection of *Listeria* in environmental samples. Presumptive positive isolates were confirmed as *Listeria* through PCR amplification of the 660 bp fragment of *sigB*. Sanger sequencing of *sigB* was performed for preliminary subtyping of *Listeria* isolates.

Results: *Listeria* was found in both pre-operation and mid-operation environmental samplings in 7/8 facilities, and there was no significant difference in the number of samples that were positive for *Listeria* in the pre-operation swabbing (37/237) compared to the mid-operation swabbing (31/237) (p=0.43). In addition, 6/8 facilities had at least one sampling site that was positive for the same *sigB* subtype of *Listeria* in the pre-operation swabbing and the mid-operation swabbing.

Significance: Our results suggest that routine cleaning and sanitation procedures in these small facilities were ineffective at controlling *Listeria*. Overall, for small food processors that are just starting to build their environmental monitoring programs, performing pre-operation and mid-operation environmental sampling for *Listeria* may provide a more direct route to identifying weaknesses in cleaning and sanitation compared to only performing environmental sampling mid-operation.

T5-10 Using Surrogate Viruses to Predict Human Norovirus Surface Sanitizer Efficacy: Time for a Change?

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Introduction: Human norovirus (hNoV) is the leading cause of foodborne disease and transmitted readily via surfaces. Many surface sanitizing products having anti-hNoV label claims are based on their ability to inactivate the cultivable surrogate, feline calicivirus (FCV). Unfortunately, FCV is often more sensitive to sanitization than hNoV.

Purpose: To evaluate the suitability of Tulane Virus (TuV) as a hNoV surrogate by using it to comparatively determine the viricidal efficacy of four food contact surface sanitizing products.

Methods: Four commercial food contact surface sanitizers [A (29.4% ethanol); B (0.06% dodecylbenzenesulfonic acid and 0.15% lactic acid)]; C (200ppm sodium hypochlorite); and D (400ppm quaternary ammonium compound)], some having hNoV label claims based on FCV, were evaluated. GI.6 and GII.4 Sydney-positive stool suspensions (20% in PBS), and semi-purified TuV cell culture lysates were used in viricidal surface assays using a modified ASTM E1053-11 protocol, on Formica™ coupons with 30 and 60 sec contact times. hNoV titer was determined using RNase pre-treatment, followed by RNA extraction and RT-qPCR, with results expressed as genome equivalent copies (GEC). TuV infectivity was determined by plaque assay using LLC-MK2 cells. Virus inactivation was calculated as the difference in titer between untreated and treated virus-inoculated coupons expressed as $\log_{10}$ reduction (LR). All experiments were repeated in triplicate.

Results: Product A significantly outperformed all other products, regardless of virus tested, achieving for GII.4 Sydney, 3.55±0.72 and 4.03±0.47 LR at 30 and 60 sec, respectively; and for TuV, 3.35±0.20 and 6.63±0.20 LR at 30 and 60 sec, respectively. Products B, C, and D all achieved <0.5 LR, regardless of contact time or virus tested. Similar results were observed for hNoV GI.6.

Significance: Despite all products having anti-hNoV label claims, there was wide variability in product performance. Data trends were similar when comparing hNoV to TuV, suggesting TuV may be a more relevant surrogate than FCV. Care should be taken in choosing products claiming efficacy against hNoV.

T5-11 Efficacy of Cleaning and Sanitation Methods Against *Listeria innocua* on Hard to Clean Food Contact Surfaces in Produce Packinghouses

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Introduction: Previous foodborne listeriosis outbreaks and recalls of fresh produce have been linked to cross-contamination with food contact surfaces (FCS) of packing equipment. Thus, effective sanitation practices are warranted in the short-term to compensate for FCS that do not have a hygienic design.
Purpose: To evaluate the efficacy of seven cleaning and sanitation methods against *Listeria innocua* populations on FCS common to apple packinghouses that have been found to have a higher prevalence of *Listeria* harborage.
Methods: Polishing brushes made of two different materials (100% nylon and horsehair mix), 100% polyethylene wash brushes, stainless steel and Teflon wrapped rollers, and interlocking conveyor belts were evaluated (n=6 per treatment). Surfaces were inoculated with *L. innocua* (9 log CFU/mL) and fouled with food-grade wax where appropriate. Treatments included the use of two sanitizers: chlorine, peroxyacetic acid (PAA), alone or in combination with an alkaline detergent or a degreaser, and the use of steam at 95 °C for 15 s. *L. innocua* was enumerated and the log reduction was calculated and compared to untreated controls.
Results: Horsehair mix polishing brushes were the type of surface with the lowest log reduction regardless of treatment applied ($p<0.05$). Compared to 100% nylon polishing brushes, where five treatments caused a > 3 log reduction, horsehair mix brushes only achieved this level of reduction when degreaser + PAA was applied. For both types of rollers and conveyor belt, an effective wax removal using a degreaser or detergent followed by sanitizer application caused the greatest *L. innocua* reduction (> 5 log reduction). The application of steam did not show a significant log reduction on any surface ($p>0.05$).
Significance: This study highlights that cleaning and sanitation strategies must focus on effective wax removal. In addition, 100% nylon polishing brushes could potentially offer a better hygienic design in produce packinghouses.

T5-12 TASKI Floor Cleaning Machine Effectively Eliminates *Staphylococcus aureus* Using a Combination of Sanitizers and Surface Cleaning Pads

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❖ ***Developing Scientist Entrant***

Introduction: Optimization of machine cleaning practices using a range of sanitizers and application methods is needed to reduce microbial loads on non-food contact surfaces (e.g., floors) in food handling facilities and healthcare systems; peer-reviewed data are limited.
Purpose: To determine optimal sanitizer and application method combinations to mitigate *S. aureus* using an automated floor cleaner.
Methods: Six products, (five sanitizers and one neutral product) were tested with three cleaning pads (Red, Twister and Wipeout pads) using TASKI floor cleaning machine (Diversey, Inc, USA). A Formica sheet was cleaned, rinsed with DI water, then disinfected with 70% ethanol. The sheet was inoculated in three zones with 3×10^8 CFU *S. aureus* and dried for 30min. Neutralizing swabs were used to sample multiple parts of the equipment and 25cm^2 of floor every 0.5m for 2m. Samples were serially diluted in PBS, plated on Mannitol salt agar, and incubated for 48h at 37°C. All testing was in triplicate (N=54); statistical analyses were completed in SAS.
Results: All product-pad combinations exhibited high bactericidal efficacy across all sample areas. Products were statistically significant ($P<0.001$), with the most efficacious being hydrogen peroxide and quaternary ammonium products, while the least was the neutral product. Pad types were marginally significant ($P=0.068$). Limited *S. aureus* was recovered across 2m of flooring; there were insignificant differences among 0.5m increments ($\alpha=0.05$). Wastewater had the most residual contamination (≥6 $\log_{10}$ CFU/ml), while cleaning pads had the least (0.7–3.4 $\log_{10}$ CFU/ml). Product-pad combination and level of cross-contamination on previously sanitized floors were insignificant ($P=0.372$). Quaternary ammonium products had the most variable disinfectant efficacy.
Significance: TASKI machine effectively eliminated *S. aureus* from the floor under multiple sanitizer-cleaning pad surface conditions with limited cross-contamination. Opportunities remain to improve SSOPs for floor cleaning equipment after use as they may serve as a cross-contamination source if not sufficiently cleaned and sanitized.

T6-01 A Colony-Based Confirmation Workflow for *Legionella* and *L. pneumophila* Serogroup1 for Same-Day Results

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Introduction: Rapid examination of *Legionella* is important in drinking water and In-process samples. There is a *Legionella* risk in tanks for the beer, mineral water or soft drink industry. Water use by the vegetable and fruit processing industry is essential to the washing and cooling of food products.
Purpose: Our study should show the performance of the MALDI Biotyper sirius system (MBT) with Gram-*negative Legionella* colonies. For our study we applied the German guidelines for the validation of species identifications using MALDI-TOF-MS in an individual laboratory (recently published by German authority BVL in 2021).
Methods: MALDI biotargets were used for applying of samples, or Bacterial Test Standard at a precise target position. One part of the MBT is a mass spectrometer equipped with smartbeam laser technology and a linear flight tube. The prepared MALDI target was introduced into the MBT, measured and identified by using the Bruker library. In parallel, samples have been applied to the IR Biotyper Kit (Bruker) and spotted on Silicon 96 microtiter plates for a second measurement with the IR Biotyper instrument based on Fourier Transform InfraRed (FT-IR).
Results: We tested >50 different strains of *Legionella* spp. and >30 NON-*Legionella* strains. Different media were used for cultivation of organisms. We have successfully identified *Legionella* spp. in all positive samples. In addition, we showed that differentiation of serogroups of the species *L. pneumophila* is possible using FT-IR with focus on differentiation of serogroup 1 (SG-1) from all other SGs.
Significance: The MALDI Biotyper sirius system can be applied for confirmation of *Legionella* spp. Starting with colony material of non-selective and selective media the user has little workload and significant better time-to-results for confirmation/identification. A combination of MALDI-TOF MS and FT-IR is a promising workflow for same-day results for confirmation/identification and serogroup differentiation.

T6-02 Development, Modernization, and Validation for the Determination of Ractopamine in Liver and Muscle Tissues for Porcine and Bovine through LC-MS/MS

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Introduction: USDA's Food Safety and Inspection Service (FSIS) laboratories conduct chemical residue analysis on animal tissues to prevent chemically adulterated food products from entering the food supply. Ractopamine is a veterinary drug administered to livestock by the meat production industry as a feed additive; however, studies have shown that ractopamine can have adverse effects on animal health with possible carryover effects to human health upon consumption.
Purpose: Validate an updated analysis method for ractopamine in muscle and liver tissues of porcine and bovine samples.
Methods: We utilized a protein precipitation extraction with Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) salts and LC-MS/MS analysis to quantitatively analyze ractopamine in spiked tissue matrices. For each tissue, samples were spiked with eight known concentrations of ractopamine.

Results: The validation demonstrated reproducible results and repeatability within acceptable Horwitz ratios for all levels examined in spiked tissue samples. The examined levels spanned low concentrations and high concentrations reflective of violative samples. Extraction recoveries ranged from 87–118% in the different tissue matrices. The validation data supports lowering the method detection level of ractopamine from 15 ppb to 3 ppb.

Significance: Upon implementation, the updated method will allow FSIS to lower the minimum level of applicability for ractopamine analysis, thereby lowering the minimum level of reportable regulatory results. As a result, FSIS will be able to monitor lower levels of ractopamine to address several countries' bans on the use of ractopamine and increasing public health concerns about the risks of ractopamine in livestock.

T6-03 Dual-Chromogenic Membrane Filtration Ampoule Medium for Enumeration of *E. coli* and Coliforms

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Introduction: A novel ready-to-use m-*E. coli*/Coliform (m-ECC) broth ampoule was developed for simultaneous detection and enumeration of total coliforms and *E. coli* in water samples through membrane filtration. m-ECC contains dual chromogenic substrates, which relies on the presence of ß-glucuronidase in *E. coli* that produces dark blue colonies and ß-galactosidase in coliform bacteria that produces pink to purplish pink colonies.

Purpose: To evaluate the performance of m-ECC ampoule medium for enumeration of *E. coli* and coliforms.

Methods: Recovery of *E. coli* and coliforms were performed with two lots of m-ECC and compared to m-ColiBlue24 ampoules. The data was analyzed using ANOVA. Growth of other inclusive and exclusivity organisms with m-ECC was also compared to m-ColiBlue24 ampoule broth.

Results: The recovery of the mixture of *E. coli* ATCC 25922 and *C. freundii* was 127, 120 CFU with two lots of m-ECC, and 100 CFU with m-ColiBlue24. The CFU recovery of *E. coli* ATCC 8739 was 48, 52 CFU with two lots of m-ECC, and 35 CFU with m-ColiBlue24. There was no significant difference (P = 0.118) in the recovery of the mixture of *E. coli* and *C. freundii*, however significantly higher recovery (P = 0.001) was found with m-ECC ampoules compared to m-ColiBlue24 for enumeration of *E. coli* ATCC 8739. All other inclusive cultures showed desired growth and color development with m-ECC. For exclusive organisms, *E. faecalis, B. subtilis* and *S. aureus* are completely inhibited with both m-ECC and m-ColiBlue24 broth. *Pseudomonas aeruginosa* is partially suppressed with m-ECC ampoule method, which produces beige to yellow colonies when it is recovered. Whereas *P. aeruginosa* forms red colonies with m-ColiBlue24, which is indistinguishable from coliform bacteria.

Significance: m-ECC broth ampoule medium has greater selectivity and provides more accurate enumeration relative to m-CoilBlue24 ampoule. Therefore, it enables greater reliability for monitoring *E. coli* and coliforms using membrane filtration systems.

T6-04 Validation of Hygiena™ Prep Xpress Liquid Handling Automation Platform for Pipetting Accuracy, Assay Performance, and Cross-Contamination in Comparison to Manual Pipetting

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Introduction: High-throughput laboratories require skilled technicians to perform long hours of repetitive tasks that ultimately determine food safety risk from results. However, as sample inputs increase, training and retaining labor becomes more difficult in today's environment.

Purpose: The purpose of this study was to verify that the utilization of the Prep Xpress automation platform performs equivalently to manual processes throughout BAX® System PCR methods to provide a reliable tool for high-throughput laboratories.

Methods: The BAX® System sample preparation steps were integrated into automation by executing modular protocols being, Bulk Lysis Fill, Sample to Lysis (5 or 20 µL), and PCR Hydration which can be utilized individually or in combination. Verification was performed in triplicate, across three instruments, to verify pipetting accuracy, assay performance, and cross-contamination for each protocol individually and in combination in comparison to manual processes. Pipetting accuracy was determined by liquid weight with standard margin of error < 20% established by the instrument manufacturer (Hamilton Robotics). Assay performance and sensitivity claims were verified by utilizing post-enrichment inoculated samples to create lysates and PCR tablet hydration. Cross-contamination was determined using a checkerboard pattern with 8.0 Log CFU/mL of bacteria and un-spiked media to perform all BAX® System sample preparation to PCR steps.

Results: The Hygiena™ Prep Xpress automation instrument performed equivalently compared to manual pipetting across all BAX® System PCR method steps. The variability for pipetting accuracy was <1% for the bulk lysis fill, 20 µL transfer and PCR tablet hydration. A 6.6% variability was observed for the 5 µL transfer protocols. All assay performance criteria and sensitivity claims were met with no cross-contamination utilizing the Prep Xpress for any protocol individually or in combination.

Significance: Integration of automation solutions that modulate laboratory procedures can increase lab efficiencies by reducing the burden on technicians from compounding repetitive processes, like pipetting, that require long-term, high levels of accuracy and precision.

T6-05 The Recently Described *Listeria sensu lato S*pecies Will Likely Not be Detected by Rapid Methods and Detection of the *Listeria sensu stricto* Species Varies by Species and Method

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Introduction: *Listeria* spp. rapid detection methods were designed for targets that are no longer universal to all species. The *Listeria* genus now consists of 10 *sensu stricto* species and 16 *sensu lato*.

Purpose: The objective of this study was to evaluate commonly used rapid detection methods for their ability to detect the recently described *Listeria* spp.

Methods: Pure cultures of the recently described *Listeria* species were serially diluted to a 10^6 CFU/mL concentration followed by screening using commercially available *Listeria* spp. rapid methods per the manufacturer's protocols. The methods evaluated included commercially available PCR (n=4) and ELFA (n=2) methods. The achieved CFU/mL levels were verified by pour plating. Two biological replicates were performed for each species-method combination.

Results: None of the methods (n=6) included in this study detected the recently described *sensu lato* species. The end-point PCR only reliably detected the *sensu stricto* species *L. farberi.* The real-time PCR methods were the only method type to detect all 10 *sensu stricto* species. Some of the PCR methods yielded weak positives (i.e., late Ct values) with some of the *sensu stricto* species (*L. cossartiae*, *L. immobilis*) suggesting low levels of these species may not be detected. The ELFA methods detected three of the recently described *sensu stricto* species; *L. immobilis* was not detected.

Significance: Recent studies have shown that several of the recently described *Listeria* spp. will grow to detectable levels in commonly used *Listeria* selective broths, but there were no data for whether they would be detected by rapid methods. Importantly, the growth data (*Listeria* spp. differ significantly with respect to growth levels) combined with this rapid method study indicate a potential for (i) true positives to be categorized as false positives, (ii) false negatives, and (iii) biased prevalence data.

T6-06 Comparison of Matrix-Assisted Laser-Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and a Biochemical Panel to Identify Bacterial Pathogens Directly from Plating Agar

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Introduction: Since 2007, the Food Safety and Inspection Service (FSIS) has utilized an automated biochemical assay to confirm the identity of target pathogens in meat, poultry, and egg products. Currently, many Microbiology Laboratory Guidebook (MLG) methods conclude with the confirmation of a bacterial isolate from a solid agar matrix. FSIS recently performed a comparative study to assess MALDI-TOF MS as a replacement for biochemical ap-

proaches.

Purpose: To compare MALDI-TOF-MS against biochemical assays to confirm identities of bacterial pathogens from agar matrices.

Methods: A total of 57 isolates representing target pathogens and frequently observed non-target isolates were selected to test MALDI-TOF MS systems. Biochemical panel results were compared to results from a MALDI-TOF MS system designed for bacterial identification to examine accuracy, sample preparation time, and time-to-result. A blinded study consisting of random isolates was also conducted to ensure reproducibility.

Results: FSIS found that the MALDI-TOF MS system was more accurate than biochemical panels in identifying target (100% versus 93%) and non-target (100% versus 89%) pathogens. Notably, the biochemical panels failed to identify non/weakly-hemolytic *Listeria monocytogenes* strains. Gram negative isolates required an average of 22.68 minutes per ten samples versus biochemicals, which required an average of 253.1 minutes per ten samples. Gram positive isolates were confirmed in an average of 23.04 minutes per ten samples while the biochemical panels required an average of 448.3 minutes. Additionally, previous biochemical panels employed by FSIS could not confirm the identity of potential *Campylobacter* isolates, while MALDI-TOF MS platforms identified all target pathogens tested for by the agency.

Significance: Since the adoption of MALDI-TOF MS, this confirmation process provides significant advantages for bacterial confirmation, including improved accuracy, significant time savings (one day faster for some methods), a methodology that works for every organism for which FSIS currently tests, and the ability to identify pathogens incompatible with previous biochemical methods.

T6-07 Rapid Identification of Foodborne Bacteria Using Single-Cell Raman Spectroscopic Analysis Combined with a Conditional Generative Adversarial Network

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❖ ***Developing Scientist Entrant***

Introduction: Rapid detection of bacteria at an early stage is crucial to ensure food safety. Raman spectroscopy has been widely used in the detection and identification of foodborne bacteria as it is reliable, label-free, and easy to perform. However, Raman spectra for single-cell analysis are hindered by relatively low signal-to-noise ratio (SNR) and analytical speed.

Purpose: This study aimed to identify different foodborne bacteria using Raman spectroscopy at single-cell level and accelerate the detection using a conditional generative adversarial network (CGAN).

Methods: Five major foodborne bacteria were investigated, including *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica*. A total of 1000 single-cell Raman spectra of these bacteria were collected using a homebuilt confocal Raman spectroscopic system with a 671-nm laser. A CGAN was developed to improve the SNR of single-cell Raman spectra and reduce the spectral collection time. The bacterial classification was performed using a convolutional neural network.

Results: SNR of single-cell Raman spectra increased from 3.35 to 20.27 after spectral recovery using CGAN and the processed Raman spectra recovered most spectral features. An identification accuracy of 94.9% was achieved for the classification of 5 major foodborne bacteria using CGAN-recovered single-cell Raman spectra, compared to 60.5% using unprocessed spectra. CGAN can accelerate data acquisition time by one order of magnitude (i.e., 30 s v.s. 3 s) by improving the SNR by a factor of ~ 6.

Significance: We proposed a rapid and reliable approach for the identification of foodborne bacteria using single-cell Raman spectroscopy combined with CGAN, providing a powerful tool for epidemiological surveillance of foodborne pathogens in the agri-food chain.

T6-08 Evaluation of Viability of Cells of *Listeria innocua* with Raman Microspectroscopy after Incorporation of Heavy Water (D2O)

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Introduction: *Listeria innocua* is a Gram-positive ubiquitous bacteria, widely distributed in a range of environments and in food-processing environments. Different environmental stresses can induce the viable but non-culturable (VBNC) state of this bacteria during food processing, such as cleaning and disinfection procedures. Bacteria in the VBNC state have very low metabolic activity and do not divide. Consequently, VBNC cells do not grow on culture media but retain the ability to recover. To detect these VBNC bacteria, an innovative approach is to use Raman microspectroscopy coupled with deuterium isotope probing (Raman-DIP). This technique allows evaluating the metabolism of bacteria, but requires to feed bacterial cells with deuterated nutrient medium. Since biochemical reactions are slower with deuterated molecules, such a treatment may cause a metabolic stress that can alter the state of bacteria from Viable Culturable (VC) to VBNC or dead state. If we want to further investigate Raman-DIP for the detection of VBNC, it is of crucial importance to assess the harmlessness of DIP.

Purpose: We evaluated the impact of heavy water incorporation on the viability state of *L. innocua* cells (VC, VBNC, dead).

Methods: We exposed the *L. innocua* bacterial suspension to different heavy water concentrations (0%, 25%, 50% and 75%) during different times of incubation (from 0h30 to 72h). For each heavy water concentrations, total, viable (VC and VBNC) and VC populations were quantified by qPCR, PMA-qPCR and plate count agar respectively. In parallel, we analyzed heavy water absorption by Raman microspectroscopy.

Results: We showed that exposure to heavy water does not affect the viability of *L. innocua* cells. In parallel, we quantified metabolic probing through measuring deuterium incorporation by Raman microspectroscopy. The height of the C-D peak was monotonously correlated with the fraction of D_2O in the medium. Nevertheless, a maximum level of labelling was reached after 2 hours, whatever the D_2O fraction.

Significance: We can further investigate Raman-DIP for the detection of VBNC of foodborne pathogens.

T6-09 Impact of Disinfectant Neutralizing Buffers Used for Sampling Methods on the Viability of *Listeria monocytogenes* Cells in Monospecies Biofilm

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Introduction: The ready-to-eat products can be contaminated during processing by pathogen and/ or spoilage bacteria, which persist in the industrial environment. To check the bacterial contamination present on the surfaces in the food processing plants, the professionals must regularly use surface sampling methods (sponge, swab, gauze pad…) to detect the pathogen such as *Listeria monocytogenes*. Due to the presence of disinfectant residues on the surface, many sampling methods are moistened in a nutrient broth combined with a neutralizing buffer to inactivate disinfectant residues that can have a slight deleterious impact on bacterial cells. This could be a source of false negatives.

Purpose: the objective of this study was to evaluate the impact of the neutralizing buffer on the viability of *L. monocytogenes* after sampling.

Methods: In this study, biofilms of *L. monocytogenes* were cultivated on stainless steel for 24 hours at 8°C or 20°C. The biofilms were treated with two different disinfectants or with sterilized water (control) and then were neutralized with 6 different commercially neutralizing buffers. The bacterial populations were detached by swab and analyzed directly after sampling and after 24 hours of incubation at 8°C to simulate the transport time before samples

analysis (EN ISO 18593 standard, 2018). The analyses included agar enumeration to quantify the viable culturable (VC) population and qPCR and PMA-qPCR assays to quantify the p dead and viable populations of *L. monocytogenes*.

Results: This study showed that in our conditions tested, neutralizers have a variable effect depending on the type of biocide used (quaternary ammonium or hydrogen peroxide), the culture temperature and the type of neutralizer. No neutralizer systematically allowed us to enumerate only the VC population of *L. monocytogenes*.

Significance: The Dey-Engley and sponge neutralizer were the most suitable neutralizers in the majority of the conditions tested to enumerate the maximum of the VC cells of *L. monocytogenes* in monoespecie biofilm.

T6-10 Application of an Engineered Enzyme to Detect *Listeria monocytogenes*

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Introduction: Biofilms that are not remediated during routine cleansing and disinfecting procedures can serve as reservoirs for pathogens, leading to contamination of food, packaging, equipment, and processing facilities to cause foodborne illnesses.

Purpose: Applying engineered enzymes that disrupt biofilms can enhance the detection of foodborne pathogens adsorbed on abiotic and biotic surfaces by releasing them from biofilm, providing a more accurate assessment of the level of contamination present.

Methods: We demonstrated previously that an engineered enzyme could act to remove a wide range of biofilms from gram-positive and -negative pathogens. In this study, we applied this enzyme to improve the detection of biofilm-embedded pathogens. First, the minimum inhibitory concentration (MIC) of the enzyme against *L. monocytogenes* was determined. Next, the impact of treatment duration was assessed quantitatively using microbiological plating, quantitative PCR (qPCR), and crystal violet assays. Finally, biofilms were grown on RTE meat products and evaluated using parameters consistent with the Microbiology Laboratory Guidebook (MLG-8) as set forth by the USDA Food Safety and Inspection Service (FSIS).

Results: The results indicate that the effective concentration of enzyme needed for *L. monocytogenes* biofilm disruption (0.1 mg/mL) was at least 10-fold lower than the MIC. Crystal violet staining, 6x6 plating enumeration, and qPCR demonstrated that biofilms treated with enzyme for longer than 4 hours resulted in a significant increase ($p<0.05$) in the number of cells released into the media as compared to PBS-treated controls. Moreover, in the absence of microbial enrichment, the pathogens were detectable by qPCR from pathogen-contaminated RTE meat after a 6-hr enzyme treatment while PBS-treated controls remained undetectable. These results are in agreement with measurements obtained via the 3M Molecular Detection System as defined in the MLG.

Significance: This work demonstrates the potential of engineered enzymes targeting biofilm to improve the detection of biofilm-embedded pathogens that are otherwise difficult to detect and eliminate or shorten microbial enrichment protocols to achieve rapid detection.

T6-11 Modernizing *Campylobacter* Analysis at FSIS – Decreasing Time to Result

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Introduction: FSIS ensures food safety through inspection which includes product sampling and testing. FSIS continuously modernizes laboratory methods and procedures, providing timely and accurate results. While negative and potential positives are routinely reported in 24h for *Salmonella* testing in poultry, initial results for the paired *Campylobacter* analysis are not available until 48h.

Purpose: To bring initial *Campylobacter* reporting times in line with *Salmonella*, FSIS laboratories evaluated multiple *Campylobacter* enrichment media. Overall sensitivity and specificity were examined at 24h incubation in comparison to the current method found in the Microbiology Laboratory Guidebook.

Methods: Hunt Broth, blood-free charcoal broth, 3M™ *Campylobacter* enrichment broth, Food Pathogen Enrichment broth, ASK broth, and Bolton's broth (current FSIS *Campylobacter* media) were used to enrich chicken parts rinsate samples inoculated with *C. jejuni* at fractional recovery levels (n=60). Media were incubated for 24h with the exception Bolton's broth (48h) and analyzed. Media with fractional recovery rates (20-80%) were used to enrich all poultry product sample types within scope of MLG Chapter 41 and again analyzed at fractional recovery levels (n=130).

Results: Data analysis indicated samples enriched in Hunt Broth for 24h showed the best overall performance for FSIS poultry samples. This was the only broth statistically better than the current method. The sensitivity (97%) and specificity (97%) of Hunt Broth was the highest of the investigated media across all product types.

Significance: Validating an enrichment broth for *Campylobacter* analysis with a 24h enrichment step allows FSIS to report results one day sooner to inspection and industry. A sample that is screened negative for *Campylobacter* can be reported in one day rather than two, while a positive sample can be fully confirmed and reported in 4 d instead of 5 d. FSIS is exploring the effects of this method improvement on any *Campylobacter* performance standards that are currently proposed or in development.

T6-12 An Electrochemical Biosensor for Rapid Detection of *Campylobacter jejuni*

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Introduction: *Campylobacter jejuni* is a common food pathogen found in poultry that can cause severe life-threatening illnesses in humans. It is important to detect this pathogen in food to manage food-borne outbreaks. Electrochemical biosensors provide several advantages for the diagnosis of *C. jejuni* due to their high specificity and ability to provide rapid responses.

Purpose: This study is aimed to develop an impedimetric electrochemical biosensor platform to detect *C. jejuni* at concentrations of 100 CFU/mL.

Methods: A multiwall carbon nanotube-based impedimetric biosensor platform modified with a phage protein, namely FlaGrab as bioreceptor, was developed for selective detection of *C. jejuni*. The electrochemical impedance spectroscopy (EIS) technique was used to measure resistance changes of the sensor upon interaction with *C. jejuni*. The sensitivity of the phage protein immobilized electrode was evaluated using suspensions of different concentrations of *C. jejuni* NCTC 11168 (10^1-10^9 CFU/mL). Furthermore, the selectivity of the biosensor towards *C. jejuni* NCTC 11168 was analyzed using non-target bacterial cells such as *Salmonella enterica* subsp. *enterica* serotype Typhimurium 291RH and *Listeria monocytogenes* Scott A. Selectivity experiments were carried out using the single cells' bacterial species. All the bacterial cells were suspended in phosphate-buffered saline.

Results: The biosensor gave a linear response from 10^2 to 10^7 CFU/mL, with good reproducibility. The detection limit was determined to be 10^2 CFU/mL. Further, the detection was highly selective toward *C. jejuni* NCTC 11168 as no signal was observed for non-target bacterial cells. The developed biosensor was found to be stable for a week.

Significance: We have developed a novel impedimetric biosensor to detect *C. jejuni* using a phage protein. The proposed biosensor exhibited a low limit of detection and high specificity. Furthermore, the phage protein-based biosensors developed in this work would be ideal for rapid diagnostic applications.

T7-01 Modernizing Approaches to HACCP Training to Build an Effective Food Safety Guiding Coalition

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Introduction: Improving food safety culture is not about inventing a new HACCP-like program but about instilling food safety as business critical. HACCP's role can be building an expert guiding coalition that is competent in food safety continuous improvement in both culture and systems.

Purpose: To investigate whether a novel HACCP-based development program can build a proficient guiding coalition and instil risk-based food safety education as a business-critical tool to improve and sustain food safety culture.

Methods: The study ran April-December 2020, using a published HACCP proficiency scale administered through Qualtrics. HACCP team members (n=27) from four manufacturing sites of one US-based food company completed the test before engaging with the novel HACCP-based coaching and development program. Team members were segmented into three learning groups (foundation, practitioners and coaches) based on initial test results. The learning program was designed to improve knowledge and competency for foundation and practitioner segments and to engage the coaches in building a guiding coalition for their manufacturing site. Participants repeated the HACCP proficiency test following completion of the learning program. Descriptive statistics were calculated via Microsoft Excel to allow comparison of pre- and post-learning results.

Results: Initial tests revealed a high degree of variation in HACCP knowledge area scores amongst HACCP team members, both within plants and across the company, suggesting opportunities in upgrading HACCP learning and communication. Substantial improvement was seen during the retest across all sites. Basic HACCP knowledge had the most progress, and knowledge topic areas on CCPs and their control, hazard analysis, and HACCP maintenance received the highest scores. One common observation among participants was their limited skill to apply the knowledge, indicated by questions asking about scenarios.

Significance: Novel HACCP training methods enhancing clarity, engagement, making personal connections, and utilizing employees' feedback are critical considerations for maximized knowledge delivery and retention. Effective, and timely proficiency assessment and setting clear objectives based upon results are crucial in tracking for continuous progress.

T7-02 Driving a Cultural Change in Produce Safety through the Use of a Novel Confidential Data Sharing Platform

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Introduction: Western Growers is leading an industry-wide data sharing initiative to develop a platform and methodology, using artificial intelligence and predictive models, to enhance best management practices and safeguard public health.

Purpose: This proof-of-concept (POC) will securely gather highly confidential data from multiple stakeholders to enable preventive actions for food safety across various regions, commodities and production practices.

Methods: Five initial stakeholders across the specialty crop supply chain, including growers, grower-shippers, and processors were identified. The technology company, Creme Global, performed a review of data required to develop machine learning algorithms to predict and prevent food safety issues for this complex, high-volume industry.

In summary, >100 data types including water, tissue, adjacent land use, etc. were identified as relevant. >1,000,000 data points, spanning three years have been gathered including confidential stakeholder data, weather, etc.. A novel means of gathering and anonymizing data was also developed.

Results: Highly interactive dashboards were developed to enable users to access insights required to drive meaningful change to their food safety culture.

This initiative also addresses the challenges of data standardization and digitization. Most produce farming, harvesting, packing, and processing operations rely on paper-based systems. This Platform addresses data capture in a comprehensive and efficient manner.

Significance: Produce is one of the few foods typically not thermally processed before consumption, thus increasing the potential for pathogens to persist. The short shelf-life creates difficulty in identifying contamination risks and sources. The need for prediction and prevention is paramount.

Through this food safety data sharing initiative, we are bringing produce operations on a revolutionary journey where their data enables smarter decisions on risk mitigation. This project seeks to demonstrate that by aggregating anonymized data, industry-wide collaborative benchmarks can be established to drive real improvements in food safety.

T7-03 Growth and Survival of *Listeria monocytogenes* and *E. coli* O157:H7 in Soy Protein-Based Meat Analogue during Storage at Refrigerated and Abuse Temperatures

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Introduction: As the consumption of plant protein based meat analogue is increasing among the consumer population, understanding the growth behavior of pathogens in this product is important.

Purpose: To determine the growth and survival potential of *Listeria monocytogenes* and *E. coli* O157:H7 in the product.

Methods: Challenge studies with 5 strains of *Listeria monocytogenes* and *E. coli* O157:H7 was conducted according to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) guidelines. Experiments were conducted at two temperatures 4°C (refrigerated) and 10°C (abuse) for 18 days storage period with three replicates (n=3). Cold adaptation of pathogens at 7°C for 7 days was carried out before start of experiments. Selective agars such as MOX agar and CT-SMAC agar were used for enumeration of *Listeria monocytogenes* and *E. coli* O157:H7 respectively. Limit of detection of the study is 1 log CFU/g. To detect low levels of pathogens, enrichment procedure was carried out. The inoculum level of *Listeria monocytogenes* organisms was 2.06 log CFU/g. The inoculum level of *E. coli* O157:H7 organisms was 2.34 log CFU/g. The sampling dates were 0, 3, 6, 9, 12, 15 and 18th day. The pH of product ranged from 6.0 to 6.5 and water activity was 1.0. During experimental period, pH, water activity, growth of background microflora was also determined.

Results: Results showed that cold adapted *Listeria monocytogenes* grew to 6.03 log CFU/g at 4°C and 5.08 log CFU/g at 10°C on 18th day storage period. The cold adapted *E. coli* O157:H7 showed decrease in count at 4°C with 1.36 log CFU/g on 18th day, whereas at 10°C *E. coli* grew to 3.40 log CFU/g on 18th day.

Significance: The product supports the growth of *Listeria monocytogenes* at both 4°C and 10°C but supports the growth of *E. coli* O157:H7 at only 10°C.

T7-04 New Active Edible Food Packaging Films Entirely from Citrus Peel Wastes

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Introduction: Minimizing environmental impacts of synthetic packaging and reducing food losses throughout the production chain are pressing concerns that have recently attracted significant attention of low-income communities to the urge of adopting sustainable development and circular bio-economy principles for identifying greener sources of food packaging materials alternative to petrochemical-derived sources.

Purpose: Fabricating innovative active/edible food packaging films exclusively based on low cost/abundantly available agro-based citrus wastes.

Methods: The casting method was employed to prepare active films by incorporating grapefruit peel methanolic extract (GFPE) and maltodextrin-en-

capsulated lemon peel extract (MD-LPE) as bioactive compounds (BACs) into grapefruit-extracted pectin (GFPec) matrix with PEG_{400} as a plasticizer. Biological, physicochemical, mechanical and optical properties of active films were studied. Film-wrapped cherry tomato samples artificially contaminated with a four-strain cocktail suspension of *E. coli* O15:H7 were stored at 4°C for 10 days to determine the efficiency of active films.

Results: Compared to commercial pectin, high-methoxyl GFPec powder demonstrated superior properties regarding solubility, methoxylation degree, galacturonic acid and moisture contents, supporting its consideration as the polymeric matrix. Simultaneous incorporation of the BACs generated thermally stable films with unique antioxidant/antimicrobial capacity without compromising their transparency. Significantly ($P < 0.05$) improved tensile strength/elongation, water vapor permeability, soil biodegradability, and UV-light barrier properties of active films were associated with excellent miscibility, even distribution of MD-LPE nano/microcapsules and strong intermolecular bonding of GFPE within the pectin matrix. After 8 days of refrigerated storage, growth of *E. coli* O157:H7 populations was reduced in active film-wrapped samples by 2 log CFU/g, whereas unwrapped samples supported an increase in population counts of 1.5 log units.

Significance: To our knowledge, this is the first study on mobilizing three value-added compounds from agro-industrial citrus peel wastes to fabricate novel and cost-effective active edible films with multiple functionalities. Results provided evidence of potential applicability of active films under real-life conditions as candidates for packaging light-sensitive foods, pathogens elimination, and extending the shelf life of fresh-cut produce.

T7-05 Humidity Controlled Thermal Inactivation of *Salmonella* Enteritidis in Black Peppercorns

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Introduction: Traditional method utilizes steam to pasteurize black peppercorns, but the process causes condensation, which leads to quality losses and energy cost from post-drying. Humidity controlled thermal treatment could be an alternative method.

Purpose: Develop a test unit to prove the concept of using humidity-controlled hot air to pasteurize low-moisture foods. Study the inactivation of *Salmonella* on black peppercorns using this concept.

Methods: The test unit consists of two drum-shaped sample holders, a mechanical tumbling system, air circulation fans, temperature & humidity sensors, and a self-opening lid. The test unit inside a controlled atmosphere chamber allows duplicated samples to be treated in a dry heat environment (up to 90°C) during the humidity come-up time of the chamber, and then exposing the samples to controlled relative humidity (RH) heating (up to 90 %).

Two 4-g black peppercorn samples (a_w 0.43) inoculated with *Salmonella* Enteritidis PT 30 (*S*. Enteritidis) were treated in the test unit at 80°C and different RH levels (60, 70, or 80 %RH). Survival populations of *S*. Enteritidis were enumerated for the control, after dry heating, and after controlled RH heating (3 time-lengths). The experiments were triplicated.

Results: The results indicate that a 15-min dry heating (80°C, RH < 40%) caused no reduction of *S*. Enteritidis, while the controlled RH treatments, i.e., 80% & 3min, 70% & 9min, and 60% & 25min, at 80°C caused 5.4, 6.2, and 6.1 log-reductions, respectively. The thermal death curves were nearly linear (slightly concave). The estimated times for a 5-log reduction treatment were 2.9, 9.1, and 25.6 min for 80, 70, and 60% RH treatments at 80°C, respectively. No condensation was observed on all of the treated samples.

Significance: This study demonstrated the potential of using short-time high RH treatments to control pathogens in low-moisture foods, without the need for post-treatment drying.

T7-06 Advances in High-Pressure Pasteurization of Wild-Type and Pressure-Stressed Bacterial Pathogens and Endospores by Synergism with Bacteriocin and Bactericidal Compounds

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Introduction: Utilization of high-pressure processing is gaining increasing importance and momentum in food manufacturing as the technology has minimal effects on organoleptic properties of various commodities and assist entrepreneurs to manufacture products with clean labels.

Purpose: Current oral presentation provides summary of new studies conducted in the Public Health Microbiology laboratory for pressure-based pasteurization of wild-type and pressure-stressed phenotypes of planktonic pathogens and bacterial endospores. Effects of bacteriocin and plant-based bactericidal compounds will additionally be discussed for augmenting decontamination efficacy of the treatments.

Methods: Presented studies are complete randomized block designs with each study composed of two biologically independent replications as the blocking factor. Effects of a selection of bacteriocin and bactericidal compounds will be presented on decontamination of non-typhoidal *Salmonella* serovars, *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, *Cronobacter sakazakii,* and four bacterial endospores of significance to the food industry. Studies are statistically analyzed using Tukey-adjusted ANOVA at a type I error level of 5%.

Results: Vast majority of planktonic bacterial pathogens were reduced (P <0.05) by up to >5 log CFU/mL due to pressure-based treatment at 450 to 650 MPa in presence of carvacrol, caprylic acid, nisin, malic acid, protamine, and defensin. Pressure-based treatments at 650 MPa augmented with nisin led to a reduction (P <0.05) of bacterial endospores with effects in order of *B. amyloliquefaciens* > *B. atrophaeus* ≥ *A. acidoterrestris* > *G. stearothermophilus.*

Significance: An optimized pressure-based pasteurization synergized with bacteriocin and/or bactericidal compounds could ensure decontamination of >5 logs CFU/mL of bacterial pathogens and up to 99.9% reduction in counts of bacterial endospores of significance to the food industry. Utilization of antimicrobial compounds augments the decontamination efficacy of the pressure-treated commodities and assist in cost optimization due to the need for less intense pressure treatments. Providing residual antimicrobial effects during shelf-life is an additional co-benefit for the utilization of this synergism.

T7-07 Inactivation of *Staphylococcus aureus* and *Clostridium sporogenes* in Modified Atmosphere Packaged Pizza Crusts by Targeted Directional Microwave Technology

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Introduction: Post-process contamination of heat-treated products with pathogenic or spoilage microorganisms can pose risks to consumers and cause economic loss for processors. Furthermore, commercial modified atmosphere packaging (MAP) applications can enhance the survival of anaerobic bacteria due to reduced amount of oxygen. Targeted directional microwave technology (TDM) applied in packaging can help to reduce post-process microbial contaminants.

Purpose: Determine the effectiveness of in-package TDM application to reduce *S. aureus* and *C. sporogenes* during shelf-life of partially baked organic pizza crusts in retail MAP.

Methods: Pizza crust samples packaged in 70% CO_2, 29% N_2, 1% O_2 were separately challenged with three-strain cocktails of *S. aureus* (n=81) and *C. sporogenes* (n=81) overnight cultures and stored at room temperature until predetermined pull dates. Samples were individually subjected to TDM at 7.125 kW for 32.2 s and cooled under ambient conditions. After the treatment, samples were stored at room temperature, pulled at 26 different time points during 19 w and analyzed in triplicates. Untreated controls for each target microorganism were also analyzed in triplicates. One pizza crust (70 g) was aseptically removed from packaging and homogenized in Butterfield's phosphate-buffered diluent. Homogenates were enumerated by spread-plating on Baird-Parker agar for *S. aureus* and differential reinforced clostridial agar for *C. sporogenes*.

Results: Initial levels of inoculum for *S. aureus* and *C. sporogenes* were 6.3 and 6.0 $\log_{10}$CFU/g, respectively. *S. aureus* showed 4.8 $\log_{10}$CFU reduction ($p<0.05$) right after the treatment and 5.6 $\log_{10}$CFU reduction during consecutive pull dates after day zero as all the plate counts were below the limit of detection (0.6 $\log_{10}$CFU/g). *C. sporogenes* was reduced by 2.0 $\log_{10}$CFU ($p<0.05$) right after the treatment and a maximum of 3.78 $\log_{10}$CFU reduction was observed during the shelf-life tests.

Significance: Results indicate that TDM can help to control post-process contamination in food products to reduce public health risks and economic losses.

T7-08 Survivability of *Escherichia coli* O157:H7 and *Enterococcus faecium* in a Hydrocolloid Gel Model and Military Ration Bar Under Vacuum Microwave Drying and Storage

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Introduction: Vacuum Microwave Drying (VMD) is being explored by the military to produce shelf-stable, dehydrated ration components. There is little information regarding the effects of VMD processing on pathogen survival.
Purpose: To provide information regarding the microbial response to VMD, evaluate if VMD met the standard 5-log reduction for food processing technologies, and evaluate the effect of macronutrient composition on pathogen survivability.
Methods: *Escherichia coli* O157:H7 ATCC 43888 and *Enterococcus faecium* ATCC 8459 were subjected to VMD processing with sampling timepoints; pre-VMD, post-VMD, 10 days and 1 month storage at 25°C. A sodium alginate hydrocolloid gel (SA) bar model was used to assess pathogen survivability without influence of a food matrix. Peanut butter banana (PB) bars were formulated to various macronutrient concentrations (high fat, carbohydrate, protein, and control of equal concentrations) to assess the impact of macronutrients on pathogen survivability. Moisture content, water activity, and microbial counts on selective and non-selective media were obtained for each sample. Statistical analysis was done via SAS mixed model ANOVA.
Results: The standard 5-log reduction was not met for either pathogen with maximum colony forming unit (CFU) log loss of 2.56 and 0.26 for *E. coli* in the SA bar model and PB bar model respectively. There were statistically significant differences ($p < 0.05$) in pathogen loss between PB bar types. The fat bar showed more loss than control and protein bars during processing (0.26 CFU log loss), carbohydrate had higher loss than the control, fat, and protein after 10 days' storage and 1-month storage (1.19 and 2.69 CFU log loss, respectively), and control had more loss than protein at 1-month storage (1.79 CFU log loss).
Significance: These data suggest that safety measures should be taken when processing foods with VMD technology and that pathogen survivability to the process may be impacted by the macronutrient composition of the food. APPROVED FOR PUBLIC RELEASE, PR2022_12138

T7-09 Phage Biocontrol of *Salmonella* on Raw Poultry Products

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Introduction: Poultry processors are constantly battling contamination by the foodborne pathogen *Salmonella enterica*. The steady rise in antimicrobial resistance as well as consumer preference for natural, chemical-free food choices highlight the need for new, natural antimicrobial treatments to reduce contamination of raw poultry during processing.
Purpose: We tested the efficacy of a new formulation of SalmoFresh™, a commercial bacteriophage preparation made of three naturally occurring, lytic bacteriophages targeting *Salmonella*, against *Salmonella* contamination of raw poultry, using raw chicken breast as a representative test matrix.
Methods: *Salmonella* Enteritidis strain SE949 was spray-inoculated on chicken breast portions at 5 log CFU/g and allowed to attach for 15 min before spray-application of $1x10^8$ PFU/g phage or PBS at an application rate of 4 mL/lb. Peracetic acid (PAA) is a common food processing aid that may negatively impact phages, so phage survival was evaluated by applying up to 800 ppm of PAA followed by phage application after a 0-, 5-, 15-, or 30-min interval on chicken breast.
Results: Phage application resulted in a significant (2.0 log CFU/g, $P<0.01$) reduction of *Salmonella* on chicken breast in 1 h post treatment. A dose-dependent reduction of *Salmonella* was observed with a linear relationship ($R^2_{adj}=0.997$); application of $2x10^{10}$ PFU/g phages resulted in a 5-log reduction in *Salmonella* on chicken breast. The phage preparation was further evaluated for its efficacy against three recent outbreak *Salmonella* strains of serotypes Hadar (FSIS12139005), Schwarzengrund (FSIS21926306), and Enteritidis (FSIS12141157) individually inoculated onto chicken breast. A significant reduction of ~1.0 log CFU/g ($P<0.01$) in the levels of these strains was observed in 1 h and 24 h post-treatment. No significant effect ($P>0.05$) of PAA application on phage viability was observed for any dwell time.
Significance: Together, these data show phage biocontrol is an effective intervention for managing *Salmonella* contamination of raw poultry and can be used in conjunction with a commonly used antimicrobial PAA in a multi-hurdle approach.

T7-10 Detection of Zoonotic Bacteria in Commercially Available Red Swamp Crayfish

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Introduction: Studies on Red Swamp Crayfish (*Procambarus clarkii*) outside of the United States confirm a variety of zoonotic pathogens, but in the $200 million dollar U.S.A commercial crayfish industry it is unknown if these same pathogens occur, demonstrating a need to evaluate this consumer commodity.
Purpose: This study evaluated the presence of *Escherichia coli*, *Salmonella, Staphylococcus aureus,* and *Vibrio spp*. in Red Swamp Crayfish and their shipping materials from commercially available populations in the U.S.A (Alabama & Louisiana).
Methods: During a two month period, four shipments of crayfish (18.15 kg total) were obtained from two farms. Each shipment had 34 samples collected: 30 from live crayfish and 4 from shipping environment. 15 samples were allocated to *Vibrio spp*. and 15 were allocated to *E. coli, Salmonella*, and *S. aureus*. A total of 600/811 crayfish were swabbed, with one sample consisting of five individual crayfish exteriors swabbed to focus on risks associated with pre-cooking food handling. Microbial analyses followed the Food and Drug Administration's Bacteriological Analytical Manual recommendations for identification of pathogens in food samples and Pearson chi-square or Fischer's exact tests were used to determine if there was statistical differences between farms.
Results: A total of 3/60 samples returned *E. coli* growth on MacConkey agar with no statistical difference ($p=0.5536$) between farms. In contrast, 60/60 samples returned suspect *Vibrio spp.* growth on TCBS agar with three morphologically distinct colonies present on most plates. One phenotype's presence, a colorless non-sucrose fermenter with a dark green center, demonstrated statistical difference ($p=0.0101$) between farm A (4/30) and farm B (14/30). *Vibrio spp.* presence in environmental samples (13/16) showed no difference ($p=0.5218$). No growth occurred for *Salmonella* or *S. aureus*.
Significance: This data suggests *Vibrio spp.* and *E. coli* as potential pathogens associated with pre-cooking food handling hazards in commercial crayfish in the U.S.A.

T7-11 Population Genetic Structure of *Listeria monocytogenes* Strains Isolated from Salmon and Trout Products and in Food Plants in France

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Introduction: *Listeria monocytogenes* is a ubiquitous pathogenic bacterium. Salmon and trout have been considered to be at risk from this pathogen. In 2018, the salmon production sector was affected by a multi-country outbreak of 22 listeriosis cases caused by *Listeria monocytogenes* sequence type 1247. Clonal complex (CC) 8 has been identified through whole genome sequencing (WGS) in five EU countries. Several patients have died due to the disease.

This has led to several questions about the occurrence of hypervirulent or persistent CC in salmon and trout production. Knowledge of the genetic diversity of strains circulating in the salmon and trout production sector is necessary to assess the risk associated with this pathogen. Until now, no typing data have been available on strains isolated through the salmon and trout production chain.

Purpose: We have analysed here the genetic structure of the population of more than 700 strains of *L. monocytogenes* isolated from 2006 to 2017 imported on the French market and coming from different salmon and trout European producers.

Methods: The genetic structure has been described on the basis of CC of Multilocus sequence typing (MLST). Most of the CCs were obtained by mapping the PFGE profiles of the strains. Another small part of the CCs were determined in the GENOLISTERIA project. The distribution of CCs was first compared between the strains in the study and then with bibliographic and database data.

Results: Eleven CCs were identified with variable distribution depending on the producing country and the processing company. Overall, the two most common CCs in the salmon and trout compartments were CC121 and CC9. Two CCs (CC1 and CC6) considered as potentially dangerous because characterized as hyper-virulent were identified. No CC was exclusively associated with the salmon sector.

Significance: This project allowed us to evaluate the diversity of CCs of *L. monocytogenes* in the salmon and trout industry and to provide strains for the GENOLISTERIA project.

T7-12 Incidence and Pathogenic Potential of *Shewanella* Species in Oysters and Seawater Collected from the Chesapeake and Maryland Costal Bays

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❖ ***Developing Scientist Entrant***

Introduction: *Shewanella* species are flesh-eating and food spoilage bacteria that can be transmitted through contaminated water and seafood. Immunocompromised individuals are at greater risk of infection if raw or lightly cooked oysters are consumed, or if cuts in the skin have been exposed to contaminated marine environments. Adequate information is not available on the abundances of these bacteria in oysters (*Crassostrea virginica*) and seawater from the Chesapeake and Maryland Coastal Bays.

Purpose: The objective of this study was to evaluate the incidence and pathogenic potential of *Shewanella* species in the Chesapeake and Maryland Coastal Bays.

Methods: From June 2019 through December 2020, oyster (*n*=63) and seawater (*n*=64) samples were collected monthly from three sites in the Chesapeake Bay and one site in the Maryland Costal Bays. Seawater temperature, salinity, dissolved oxygen, turbidity, pH, chlorophyll-a, and precipitation was also recorded during each sampling time-point. All samples were plated on iron agar and confirmed using 16S rRNA sequencing. Confirmed isolates were tested for hemolytic activity using blood agar plates.

Results: Sixty-eight percent of oysters and 19% of water samples tested were positive for *Shewanella* species. The counts ranged from non-detectable to 1.3×10^4 CFU/g in oysters and non-detectable to 4.3×10^2 CFU/mL in seawater. The levels of *Shewanella* were significantly higher ($p\geq0.05$) in oysters than in seawater. The top four species isolated from oyster and water samples were *S. amazonensis, S. marisflavi, S. lohica* and *S. algae*. Forty-four percent of the oyster and water isolates were beta hemolytic. There was a moderate positive correlation (r=0.5-0.6) between *Shewanella* counts and seawater temperature and turbidity.

Significance: This is the first comprehensive study that provides insight on the diversity and pathogenic potential of different *Shewanella* species recovered from oysters and seawater from the Chesapeake and Maryland Coastal Bays.

T8-01 Ranking Produce Safety Priorities of Fresh Produce Industry Stakeholders in the United States

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Introduction: A comprehensive understanding of stakeholder food safety priorities around fresh produce does not currently exist. Such information is essential to improve food safety knowledge and practices effectively and efficiently throughout the fresh produce industry.

Purpose: To identify and rank stakeholder produce safety priorities in the United States.

Methods: Survey questions were designed and approved by food safety experts to rank 24 produce safety priorities. The anonymous survey was distributed online via Qualtrics™ to all sectors of the produce industry from November 2020 to February 2021. A score was calculated for each priority by summing weighted ranking scores across responses. Descriptive statistics and logistic regression were used to determine frequencies and distribution of response and identify factors (e.g., role in food safety system, size/location of organization) that influenced priorities.

Results: A total of 281 respondents played fourteen different roles in the fresh produce industry, with the majority of respondents (39.5%) identifying as growers. Operations were distributed across the U.S. and annual produce sales ranged from below $25,000 to over $5,000,000. Health and hygiene, training, postharvest sanitation, traceability, and harvest sanitation were the top five food safety priorities. The top five food safety priorities were health and hygiene, training, postharvest sanitation, traceability, and harvest sanitation. Compared to growers, buyers (OR = 3.66) and regulators (OR=2.24) were more likely to prioritize health and hygiene, whereas extension professionals were more likely to prioritize training. Small farms (OR=2.47) were more likely to prioritize training when compared to large farms. Health and hygiene and training were most frequently a priority in the North Central followed by Northeast and Western regions.

Significance: These findings provide insight into stakeholder food safety priorities around fresh produce and can be used to inform intervention efforts, ranging from specialized training for produce growers and packers as well as scientifically validated mitigation strategies.

T8-02 Examining Contamination of Romaine Lettuce with *Escherichia coli* O157:H7 through Tissues Damaged by Exposure to Freezing Temperatures

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Introduction: Since 2018, at least seven outbreaks associated with *Escherichia coli* O157:H7 of leafy greens have been reported. While contamination likely occurs pre-harvest, specific environmental and cultivation factors contributing to this contamination are not well understood.

Purpose: To examine the potential contamination risk to freeze and frost-induced damaged tissues of romaine lettuce by *E. coli* O157:H7.

Methods: Lettuce (*Lactuca sativa* L.) plants from a single cultivar 'Green Towers' were grown from commercial seed and maintained in the NCSU BSL-3P phytotron greenhouse. Plants of various maturities were moved into an environmental growth chamber and exposed to frost conditions [5 nights (4 h/night) of alternate -4°C or 0°C] or -10°C for 15 min (freeze). Leaves displaying evidence of tissue damage were identified and marked for inoculum placement. *E. coli* O157:H7 contamination was introduced via direct application at ca. 6.4 $\log_{10}$ CFU/wound. Leaf, head, and core samples of mature romaine (42–72d) were processed and enriched for *E. coli* O157:H7 in accordance with FDA BAM methods. Data were analyzed for prevalence of contamination (single inoculated leaves versus head versus core samples).

Results: For those samples harvested from *E. coli* O157:H7-inoculated leaves damaged from frost exposure (n=24), 91.7% (22/24) leaves, 20.8% (5/24) heads, and 16.7% (4/24) core samples were contaminated with O157. Similarly, for those samples harvested from inoculation of leaves damaged by exposure to -10 °C for 15 min (n=45), 95.6% (43/45) leaves, 17.8% (8/45) heads, and 4.4% (2/45) core samples were found to harbor O157.

Significance: Leaf damage caused by exposure to freezing temperatures may contribute to the contamination of *E. coli* O157:H7 on the edible portions of romaine lettuce. Future studies should address the degree of product damage and pathogen persistence under conditions of low temperature-induced tissue damage.

T8-03 Colonization and Internalization of Cantaloupe Fruit with *Escherichia coli* O157:H7 through Blossom Inoculation

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Introduction: Although cantaloupes are typically associated with outbreaks involving *Salmonella* and *Listeria*, it is important to examine the role female blossoms may contribute to colonization with other enteric pathogens, including *Escherichia coli* O157:H7.

Purpose: To examine the pre-harvest contamination risks to cantaloupe fruit when plant blossoms are exposed to a leafy green-associated outbreak strain of *E. coli* O157:H7.

Methods: Cantaloupe plants (*Cucumis melo* 'reticulatus') from a single cultivar 'Primo' (Western variety) were grown from commercial seed and maintained in the NCSU BSL-3P phytotron greenhouse. *E. coli* O157:H7 contamination was introduced via blossoms at two inoculum concentrations, ca. 6.1 or 2.3 $\log_{10}$ CFU/blossom. Surface and internal samples of mature fruits were processed and enriched for *E. coli* O157:H7 in accordance with modified FDA BAM methods. Data were analyzed for prevalence of contamination [surface and inside (blossom versus stem side)].

Results: Of the cantaloupe fruit harvested from *E. coli* O157:H7-inoculated blossoms at ca. 6.1 $\log_{10}$ CFU/blossom (n=45), 97.8% (44/45) were contaminated and 84.4% (38/45) had evidence of internalization into the fruit (n=38); the majority, 68.9% (31/38), harbored O157:H7 within the entire fruit while the remaining fruits were contaminated with the inoculated strain only within the stem side [15.8% (6/38)] or blossom side [2.6% (1/38)] of the fruit pericarp. In comparison, for fruit harvested from *E. coli* O157:H7-inoculated blossoms at ca. 2.3 $\log_{10}$ CFU/blossom (n=6), 83.3% (5/6) were colonized on the surface and 33.3% (2/6) had O157:H7 internalized into the fruit, with 100% (2/2) harboring O157:H7 within the entire pericarp.

Significance: These results identified blossoms as a route by which *E. coli* O157:H7 can colonize and internalize cantaloupe fruit at a high percentage, even at a low inoculum concentration. Blossoms may serve as an important route for pathogen contamination in atypical commodity-pathogen pairs.

T8-04 Plant Growth-Promoting Rhizobacterium *Pseudomonas* spp. Shifts Plant Phytochemical Profiles, Affecting *Salmonella enterica* Association with Baby Kale Leaves

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❖ ***Developing Scientist Entrant***

Introduction: Our previous research has shown that drought, plant age and plant growth-promoting rhizobacteria (PGPR) can restrict *Salmonella* association on kale. How these processes influence *Salmonella*-kale interactions is not understood.

Purpose: Evaluate the effects of PGPR, plant developmental stage and drought on phytochemical profiles of kale and assess how these changes relate to *Salmonella* association.

Methods: Greenhouse-grown kale 'Improved Dwarf' (23°C, 16h L:8h D) were root-inoculated twice with *Pseudomonas* spp. (10^8 CFU/mL) (PGPR+) or 0.1% peptone water (PGPR-, negative control), 2-days and 9-days post-germination. Plants were watered for 5 (baby kale) or 47 (mature kale) days then subjected to drought, for 6 or 3 days depending on age, or regularly watered (RW, control). Leaves were flash-frozen, ground and extracted in methanol/formic acid for biochemical analyses. *Salmonella* Newport (10^6) were inoculated onto leaves and enumerated 24 hours post-inoculation. Data were analyzed using JMP 14.

Results: PGPR- 20-day-old RW baby plants supported higher *Salmonella* growth than 59-day-old mature RW plants and had lower antioxidant capacity and accumulation of flavonoids (p<0.05). In the PGPR- group subjected to drought, baby kale leaves had higher antioxidant capacity and flavonoid, phenolic and glucosinolate levels (p<0.05) and supported lower *Salmonella* populations (p<0.05) than the RW group. PGPR+ baby kale that were watered regularly had lower *Salmonella* counts than PGPR- kale (p<0.05) but had higher antioxidant capacity and phenolic and flavonoid levels (p<0.05). Drought did not affect *Salmonella* populations on PGPR+ juvenile plants but this group had higher antioxidant capacity, flavonoids and phenolics than PGPR+ RW baby plants (p<0.05). *Salmonella* counts on 59-day-old kale were not impacted by drought or PGPR inoculation. However, glucosinolate content in PGPR+ RW mature kale was higher than PGPR+ drought-subjected kale (p<0.05).

Significance: PGPR may be a promising strategy to enhance food safety by shifting phytochemical profiles that may limit *Salmonella* association with baby kale.

T8-05 Polydimethylsiloxane (PDMS) Topomimetic Artificial Leaf Surfaces to Study the Influence of the Produce Surface Waterscape on Foodborne Pathogen Dispersion and Adhesion

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❖ ***Developing Scientist Entrant***

Introduction: The produce surface waterscape, termed the phyllotelma, is dynamic and multiple factors, including microscale surface topography, impact its size and shape. It remains unclear how the phyllotelma and its alterations influence the dispersion and adhesion of microbial contaminants.
Purpose: Engineer and validate polydimethylsiloxane (PDMS) replicasts (topomimetic artificial leaf surfaces) of produce with variability in surface macro- and micro-scale topography to evaluate the dispersion and adhesion of foodborne viruses in relation to the phyllotelma.
Methods: PDMS replicasts of 15-, 45-, and 75-days old leaves of spinach and Romaine lettuce plants were fabricated in a two-step molding process. Adaxial and abaxial sides of Romaine lettuce and spinach leaves were used as templates. Control replicasts without surface topography were generated with glass slides as a template. The macro-scale topographical complexity of replicasts was determined by assessing percentage venation using light microscopy. The micro-scale topography of replicasts was compared to produce using scanning electron microscopy. To compare recovery rates of Tulane virus from replicasts to spinach leaves, 500 µl of Tulane virus (4.97 Log_{10} PFU/ml) was applied to adaxial surfaces of 15-days old spinach leaves in triplicate and incubated for one hour. Virus was eluted in 5 ml PBS supplemented with 0.05% Tween20 and infectious virus was enumerated by plaque assay.
Results: PDMS replicasts of Romaine lettuce and spinach leaves of various ages were generated. Percentage venation increased with leaf age and was more pronounced on abaxial surfaces than adaxial surfaces. The recovery rate of Tulane virus from spinach replicasts was 53.2% and 55.2% from fresh spinach leaves.
Significance: Replicasts can be foundational tools for studying the influence of the phyllotelma on pathogen dispersion and adhesion on produce surfaces. Results indicate significant differences in macro-scale topography between leaves of different ages and recovery of a human norovirus surrogate from replicasts was similar to that of spinach leaves.

T8-06 Survey of Aquaponics and Hydroponics Systems in the Commonwealth of Virginia

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Introduction: Due to demographic changes and food insecurity associated with urbanization, urban agriculture including aquaponics and hydroponics has been gaining attention for its health, environmental, economic benefits, and increased food security for urban populations. However, potential implications of food safety in aquaponics and hydroponics systems are not well established in the Commonwealth of Virginia.
Purpose: The purpose of this study was to assess water and microbiological quality in current aquaponics and hydroponics production in VA.
Methods: A total of 176 samples (i.e., fresh produce, produce tank water, fish tank water, biofilter, sludge tank, and fish skin) were collected from six schools, three residential sites, and one correctional department between December 2019 and March 2020. Following AOAC-approved or performance-tested methods, microbiological testing was performed for the prevalence of *E. coli*, *Listeria*, *Salmonella*, *Aeromonas*, and *Streptococcus*.
Results: Water obtained from the systems had the following qualities; pH (6.5-8.5 and 6.5-8.5), alkalinity (17.1-102.6 and 17.1-68.4 ppm), hardness (17.1-530.1 and 444-684 ppm), NO_2 (0-11.9 and 0.0-0.4 ppm), total ammonia (0.0-7.5 and 0.2-0.8 ppm), and temperature (8.1-25.4 and 14.7-20.1°C) for aquaponics and hydroponics, respectively. For aquaponics systems, the aerobic mesophilic bacterial counts level was the lowest for fish skin (1.6±1.5 log CFU/g) and the highest for produce (6.5±0.6 log CFU/g). For hydroponics systems, the counts ranged with the lowest and the highest for produce tank water (4.5±0.8 log CFU/g) and produce (6.4±2.5 log CFU/g), respectively. A total of 37 opportunistic pathogens consisting of 11 (6.25% of samples tested) *E. coli*, 2 (1.14%) *Listeria*, and 1 (0.57%) *Aeromonas* were detected in the samples.
Significance: Findings demonstrated a potential health hazard arising from aquaponics and hydroponics system-acquired food products. This study validated the need to investigate further the prevalence of fish and foodborne pathogens on samples at more sites, as little research has previously been done in this area.

T8-07 Fate of *Listeria monocytogenes* Strains on Different Whole Apple Varieties during Long-Term Simulated Commercial Storage

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❖ ***Developing Scientist Entrant***

Introduction: Since 2014, multiple recalls and outbreaks have resulted from apples contaminated with *Listeria monocytogenes* (*Lm*).
Purpose: This 2-year study assessed *Lm* survival on apples as affected by apple cultivar, growing season, storage atmosphere, and type of inoculum (planktonic/biofilm).
Methods: Unwaxed Gala (G), Granny Smith (GS) and Honeycrisp (HC) apples obtained from multiple growers during two growing season were dip-inoculated in an 8-strain *Lm* cocktail of planktonic- or biofilm-grown cells to contain ~105 CFU/apple. Thereafter, the apples were dried and subjected to air or controlled atmosphere (1.5% O_2, 1.5% CO_2) storage at 2 °C. At monthly intervals, the peelings from six apples were combined, stomached in PBS, appropriately diluted, plated on Modified Oxford Agar, and incubated (37 °C/48 h) for *Lm* enumeration.
Results: After 90 days at 2 °C, no significant difference in *Lm* survival ($P > 0.05$) was seen for either storage condition. *Lm* populations decreased over time; however, some apples still had populations of 4.6 log CFU/apple after 7 months of storage. The year of harvest significantly ($P < 0.05$) impacted *Lm* survival, with greater survival seen in year 1 compared to year 2. Apple cultivar significantly impacted *Lm* survival ($P < 0.05$) during both harvest years. During year 1, GS apples exhibited a significantly lower reduction in *Lm* ($P < 0.05$) compared to G and HC. During year 2, HC had a significantly lower reduction in *Lm* ($P < 0.05$). Regardless of harvest year, G exhibited significantly greater reductions in *Listeria* ($P < 0.05$). The type of inoculum also impacted *Lm* survival ($P < 0.05$), with cells previously grown as a biofilm surviving significantly better ($P < 0.05$) compared to planktonically grown cells.
Significance: These findings should aide apple packers in the development of strategies to minimize *Lm* survival on apples during long-term storage.

T8-08 Effect of Type of Mulch and Raw Manure Application Technique on Microbial Food Safety Risk on Cucumbers Irrigated with Contaminated Water

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❖ ***Developing Scientist Entrant***

Introduction: Use of raw manure in agriculture improves plant health, however it also increases the risk due to microbial and pathogenic presence.
Purpose: This study examined the effectiveness of different mulches and raw manure application techniques to minimize microbial risk from contaminated water used for irrigation of cucumbers.
Methods: A plot of 360 ft^2 with 54 beds (30 ft long) covered with five types of mulch (maize, biodegradable, conventional plastic, lightweight and heavyweight paper) and raw manure applications (tilled, no-till and no raw manure) were planted with Dasher 2 Variety cucumbers. Soil samples were collected for first five weeks and stored at 4˚C prior to examining naturally present *E. coli*/coliforms with petrifilms. Well water contaminated with or without nalidixic acid resistant *E. coli* (8 Log CFU/mL) was used for irrigation 7 days before harvesting. Cucumbers harvested were stored at 4°C prior to plating in VRBA

supplemented with nalidixic acid.

Results: Prior to irrigation of contaminated water, naturally present *E. coli*/coliforms in soil from mulch beds in tilled or non-tilled treatments were in the range of 1.92-3.51 Log CFU/g and 4.01-4.95 Log CFU/g respectively. *E. coli* levels on cucumbers from plots irrigated with contaminated water from lightweight paper and tilled plots (1.35 Log CFU/cm^2) were significantly higher ($P<0.05$). Samples from plots irrigated with non-contaminated water resulted in *E. coli* levels at or below the detectable limit. Total coliforms levels from cucumbers harvested from contaminated plots were higher in no-mulch no-till samples (3.71 Log CFU/cm^2) while samples from conventional tilled treatments had the lowest levels (1.56 Log CFU/cm^2). A significant die-off of inoculated *E. coli* was observed on cucumbers within 1 day (>1.47 Log CFU/cm^2) and (>2.30 Log CFU/cm^2) after 4 days.

Significance: Tilled plots with no-mulch, lightweight or heavyweight paper were the least effective in minimizing *E. coli* contamination on cucumbers from contaminated irrigation water.

T8-09 Evaluating the Risks Associated with Utilization of Modified Washing Machines in the Processing of Leafy Greens.

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❖ ***Developing Scientist Entrant***

Introduction: About 46% of U.S. foodborne illness incidents are attributed to produce, of which leafy greens are responsible for most. Small and medium-sized leafy green growers commonly retrofit washing machines to dry triple-washed leaves, utilizing their spin cycle, effectively turning them into large salad spinners. However, the potential for this practice to cause microbial contamination and the degree to which it can pose a risk to cause foodborne illnesses has not been explored.

Purpose: This project aims to investigate the risk of cross-contamination associated with the practice of drying leafy greens with retrofitted washing machines to help inform best practices to mitigate risks.

Methods: *Listeria innocua* at 10^3-10^6CFU/ml was inoculated and dried onto spinach prior to washing, after which leaves were dried in a retrofitted washing machine. After drying, three contact surfaces of the machine in different locations (loading basket, internal chamber, water collection chamber) were sampled using sterile microbial swabs and the spinach was stomached to enumerate the relative levels of the bacterial transfer, with 3 swabs per region.

Results: There was an 90%+/-5% of 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. Additionally, *L. innocua* from contaminated loading baskets were transferred to non-inoculated spinach during the spin-drying process with a microbial recovery of 10^1-10^2 CFU/ml. This microbial transfer to the produce did not occur when the immediate contact surface of the washing machine drying basket was clean but the water collecting layers below were contaminated.

Significance: These results that these machines commonly used by small and medium-sized processors could pose a risk of contamination, validates the need for establishing cleaning and sanitation guidelines to improve the safety of processing leafy greens in this manner.

T8-10 What We Know about How Consumers Handle and Wash Raw Produce: Findings from Two Observation Studies

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Introduction: Consumers are advised to wash and scrub fruits and vegetables under running water and follow other recommended practices to help prevent foodborne illness.

Purpose: To provide quantitative data from observation studies conducted in test kitchens on consumer handling and washing of different types of produce.

Methods: Participants prepared a meat or poultry product and a salad in test kitchens while being videotaped. For study 1, participants (n = 196), prepared a chicken product and a salad made with canned black beans, frozen corn, and cucumber. For study 2, participants (n = 66) prepared hamburgers and a salad made with bagged lettuce, apples, and carrots and 50 additional participants prepared guacamole. Coders viewed the videos to determine adherence to recommended food safety practices.

Results: Many participants did not follow the recommended practice of scrubbing fruits and vegetables under running water. Many participants simply rinsed the produce or did not attempt to wash it. For example, for study 1, 48% of participants washed and rubbed the cucumber surface using their hands under running water as recommended, while 25% did not attempt to wash the cucumber and the remaining participants simply rinsed it. For study 2, about 44% of participants washed and rubbed the surface of the carrot and apple using their hands under running water, while about 39% did not attempt to wash the carrot and apple and the remaining participants simply rinsed it. Twenty percent of participants washed the bagged salad which is not recommended. For participants who prepared guacamole, many did not attempt to wash the avocado (78%) or cilantro (76%). No participants washed their hands after handling the avocado, as recommended.

Significance: Observational data highlighted gaps in practices. Additional consumer outreach is needed to educate consumers on proper handling and washing of produce and to motivate consumers to follow recommended practices.

T8-11 Simulation of Bacterial Cross-Contamination from Farmers Markets Fomites to Produce and Hands

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Introduction: Consumers demand for locally grown fresh produce is often tied to the need to support local famers and enhance environmental sustainability. Previous studies have shown that practices in farmers market are associated with re-use of produce containers and handling multiple fomites at the same time (money and food contact surfaces).

Purpose: This study aimed to investigate microbial cross-contamination between farmers' markets fomites (e.g. credit card or cellphones) to produce and hands under laboratory simulation conditions.

Methods: Farmers markets fomites (plastic, molded pulp fiber, wicker) coupons, credit cards, and cellphones were inoculated with a cocktail of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus* (10^7 CFU/ mL) and left for one hour at 23±2 °C. The mean log CFU/cm^2 of bacterial transfer from fomites to produce and hands were analyzed. The mean log CFU/cm^2 of bacterial transfer from credit cards and cellphones to hands-produce were also assessed at 23±2 °C by placing produce or hands in contact with inoculated fomites.

Results: The mean log CFU/cm^2 of *Salmonella* from artificially contaminated fomites to produce and hands ranged from 2.1 to 2.8 log CFU/ cm^2, and from credit cards and cellphones to hands and produce ranged 2.2 to 2.8 log CFU /cm^2 respectively. The mean log CFU /cm^2 from fomites to produce and were ranged from 1.7-2.9, 0.8-1.9, and 2.1-3.3 for *E. coli*, *L. Monocytogenes*, and *S. aureus*, respectively. Means log CFU/ cm^2 from credit cards and cellphones to hands-produce were ranged 2.5-3.1, 1.6-2.1, and 2.4-3.0 for *E. coli*, *L. Monocytogenes*, and *S. aureus*, respectively. The results indicated that fomites used at farmers' markets, credit cards, and cellphones could lead to cross contamination challenges.

Significance: The findings of this study could be used by food safety educators and extension agents to design effective farmers market food safety training curriculum.

T8-12 Metabarcoding Sequencing Reveals the Bacterial and Fungal Communities in Edible Flower (*Torenia fournieri* F. Land.) Cultivated in Organic System

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Introduction: Edible flowers have been used in foods worldwide. Torenia (*Torenia fournieri* F. Lind.) is widely consumed fresh in salads and desserts. It stands out due to the velvety texture and sweet taste. Few studies assessed the microbiota composition of edible flowers from organic cultivation, which is the classical system used in their production.

Purpose: To evaluate the microbiota of torenia flowers from organic cultivation system by 16S rRNA and 18S rRNA amplicons sequencing analyses.

Methods: The flowers were cultivated in a field experiment following international procedures for organic cultivation. Samples were collected in maturity commercialization stage. The DNA was extracted using a MoBio Power Food DNA isolation kit. 16S rRNA and 18S rRNA libraries were sequenced using the MiSeq Sequencing System (Illumina Inc., USA) with the V2 kit, 300 Cycles for assignment of bacteria and fungi communities, respectively. The readings of the demultiplexed pairs were joined using the FLASH command and the cut quality using PRINSEQ. High-quality readings were analyzed using QIIME 1.9.1. Greengenes database was used to collect operational taxonomic units (OTUs). Statistical analyzes were performed using XLSTAT software 2020.1.3, considering $p<0.05$.

Results: Six bacterial phyla were identified in torenia flowers, with higher abundance of *Proteobacteria* (82.7%) and *Cyanobacteria* (14.3%) in indigenous microbiota. The fungal main phyla found were *Basidiomycota* (79.69%) and *Ascomycota* (19.37%). *Gammaproteobacteria* (71.8%), *Oxyphotobacteria* (14.4%) and *Alphaproteobacteria* (10.9%) corresponded to the bacterial classes with higher abundance in torenia. On the other hand, *Tremellomycetes* (78.7%) and Dothideomycetes (14.0%) were the dominant fungal classes. *Enterobacteriaceae* (66.3%) and *Oxyphotobacteriaea* (14.1%) showed the higher abundance among the identified bacterial families. *Trimorphomycetaceae* (76.8%) and *Cladosporiaceae* (9.4%) showed higher abundance among fungal families in torenia. *Pantoea* (61.4%) and *Saitozyma* (68.4%) were the dominant bacterial and fungal genera identified in torenia, respectively. None of the identified genera indicated risks for human consumption.

Significance: Results characterize for the first time the microbial composition in edible organic torenia flowers.

T9-01 Maintaining Impactful Food Safety Trainings in a Virtual World

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Introduction: In-person training offers active engagement, hands-on learning and easily fosters discussion to enrich food safety learning. In the wake of the COVID-19 pandemic, many traditional in-person training needed to enter the virtual world. We deployed a food safety program as an in-person (pre-pandemic) and real-time virtual program. We studied the overall program ratings and perceived preparedness on key food safety competencies.

Purpose: Evaluate the program delivery of in-person vs. real-time virtual food safety training programs customized to small and emerging food businesses (SEFB).

Methods: Pre- and post-program evaluation surveys were deployed to participants before the course, immediately after the course, and >3 months later to measure overall program impact and perceived preparedness specific to key food safety competencies. Surveys were administered via paper (in-person) and digitally via SurveyMonkey (virtual). Analysis of descriptive statistics (e.g., frequencies, percent's, means, and standard deviations), one-way ANOVA followed by Scheffe's post hoc procedure, correlations, and t-tests determined statistical significance between means.

Results: Program evaluations reported no statistical differences in overall program evaluations, self-rated level of understanding, and general preparedness (N=87-89) between workshops delivered in-person and virtually. Overall program evaluation ratings reported that participants would apply the knowledge and skills learned in the course to their food application (4.5 ± 1.0, n=90, scale:5-point Likert scale: 1=Strongly disagree to 5=Strongly agree). Participants were more prepared to implement key food safety practices, including implementing GMPs, records and assessing and controlling food safety hazards after attending the food safety training.

Significance: While COVID-19 has made significant food safety technical support challenges, virtual training programs can increase food safety knowledge and enhance skills (preparedness) similar to traditional in-person training for small and emerging food businesses. Additionally, the virtual platform allowed participants from any U.S. geographical region to attend, enhancing programming capacity.

T9-02 Small-Scale Processor Self-Identified Barriers to Effective Food Safety Training Programs

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❖ ***Developing Scientist Entrant***

Introduction: Small-scale processors make up 20% of all food processors in the United States and struggle adapting to the evolving criteria for food safety management, including effective employee food safety trainings.

Purpose: Identify barriers to employee food safety trainings for small-scale processors and assess their perception of different training formats.

Methods: The IRB-approved study targeted 30 one-hour interviews with food safety managers from small-scale food processors. The questions were developed on four topic areas including (1) current food safety trainings; (2) perceived of employee trainings and training formats; (3) challenges to comply with food safety regulations; (4) suggestions for future trainings. Interview questions were reviewed by multiple industry and Extension professionals, and piloted tested with an experienced food safety manager. Interviews were transcribed by undergraduate researchers and independently checked by a reviewer. Data were managed and analyzed using NVivo.

Results: In-person training was: (1) highly preferred, (2) perceived to be highly adaptable and (3) enabled instructors to visually infer trainees' understanding. training was perceived as convenient but often lacks interaction and few resources exist to create trainings. With limited time and high turnover, many businesses doing only annual trainings face an issue where new employees can go a long time without receiving in depth food safety training. Language barriers have presented themselves as a challenge to some participants. Most of the business have non-native English speakers and translating trainings can be problematic as some terms get lost. Participants find that it helps to have multi-lingual trainers, but there are still some issues. Lastly, employees often enter with varying levels of background knowledge which can make it harder for trainers. Overall, to improve future training development, participants suggested: using in-facility pictures, concrete examples, and smaller groups

Significance: Findings will inform educators and Extension specialists to create more effective trainings for small-scale food processors.

T9-03 Utilising Triangulation to Assess Current Food Manufacturing Training Approaches and Employee Preferences for Future Learning Purposes

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Introduction: Food safety training is an essential aspect of safe food production, but content and quality may also be demonstrative of the underlying organisational food safety culture. For example, generic 'off the shelf' training programmes delivered online or in classrooms may not always lead to desired behaviour. Determining hand hygiene compliance (as an indication of basic food safety awareness) in combination with assessing training approaches and evaluating employee training preferences may be necessary to inform future learning strategies.

Purpose: To assess training approaches, hand hygiene compliance and employee training preferences in food manufacturing and processing environments to inform future learning improvements.

Methods: Training-related data gathered from management interviews (*n*=23), all-staff survey (*n*=75), procedural review (*n*=8) and focus group sessions (*n*=3), supported by observational analysis (55 hours), were triangulated to assess the effectiveness of current hand hygiene training approaches.

Results: Food handling and preparation actions/tasks recorded (*n*=2082) during observational analysis revealed inadequate (98%) hand hygiene practices during production. Nevertheless, when interviewed, senior management perceived food safety and hand hygiene behaviour across food production sites as *"pretty good"*, led by *"common sense"*, remarking that company training (online/classroom) and supporting documentation were effective. The document review highlighted that food safety and hand hygiene instructions were vague/ambiguous with technical staff during focus group discussions recognising that procedures contained *"many grey areas"*. While production managers in the survey indicated a preference for continuing classroom-based training, food production employees perceived such training to be *"extremely tedious" and "boring"*, preferring short, hands-on, interactive training sessions within work environments more frequently instead.

Significance: Hand hygiene compliance did not accord with senior management perceptions of established food safety training programmes which were not well-received by food production employees. Involvement of the intended audience is necessary in the co-creation of targeted training interventions to address food safety and hand hygiene malpractices.

T9-04 Content Analysis of Food Safety Information in Dried Apple Recipes on Youtube, Blogs, Cookbooks and Extension Materials

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❖ ***Developing Scientist Entrant***

Introduction: Dried fruits have growing food safety concerns after multiple *Salmonella* and *E. coli* outbreaks associated with low-moisture foods. Given dried fruit's popularity, many consumers follow drying recipes. However, little is known about the food safety information on those recipes.

Purpose: To evaluate the food safety information and apple-handling practices presented in dried apple recipes from YouTube, blogs, cookbooks and university extension materials.

Methods: A systematic search of dried apple recipes from YouTube videos, blog articles, cookbooks, and extension publications were conducted. Recipes followed this inclusion criteria: 1) English 2) contains instructions to dry apples at home 3) <20 minutes (videos) 4) >500 views (videos) 5) created by University State Extension (extension materials). This resulted in 112 blog articles, 97 videos, 20 cookbooks, and 22 extension publications. Two researchers coded the recipes for drying process information while a third researcher checked their codes.

Results: Handwashing instructions were excluded in videos and cookbooks, 97% blogs and 91% extension materials. Potential cross-contamination events like handling apples after touching non-food-contact surfaces and drying raw meat with apples were observed in 12% videos. When selecting fresh apples, 16% videos, two blogs and five cookbooks used bruised or damaged apples. Recommendations of pre-treatment were lacking in over a third of blogs (37%) and videos (36%). If pre-treatment was mentioned, it was predominantly to minimize browning with almost no mention of antimicrobial benefits. Average drying temperatures varied by drying methods, with sun-drying being the lowest. Most temperatures were at levels insufficient for recommended pathogen reduction for safe consumption. Drying temperatures were missing in many recipes (cookbooks 35%; videos 41%). It was a common suggestion to use subjective indicators instead of unit measurements when slicing apples and checking for doneness. Only two cookbooks and one extension publication provided storage temperatures, while no recipes provided storage humidity levels.

Significance: Findings reveal the need for drastic improvement of food safety information dissemination to home apple dryers and recipe developers.

T9-05 Evaluating the Awareness of Food Safety Messages on Flour and Baking Mix Packages Using Eye-Tracking Technology

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Introduction: Wheat flour and baking-mix have been associated with foodborne outbreaks and recalls. Many consumers are unaware of the repercussions of consuming raw cookie dough and batter.

Purpose: To assess the accessibility of flour safety messages on packages and to identify consumer barriers to processing these messages.

Methods: Eye-tracking technology was used to track 47 participants' eye movements to assess their time to fixation (TTF) on the flour safety messages. Messages that were longer than one sentence were considered "long" messages. Ten packages were selected from the local supermarket, representing short and long messages (S1-S5 and L1-L5). Participants had 20 seconds to look for the message. Font size of the messages were then measured by researchers.

Results: All participants were primary meal preparers and used wheat flour/baking mixes at least once a month. Over half of the participants were 25-34 years old and 66% had over 5 years of meal preparation experience. On average, the eye-tracker measured participants TTF to be 9.78s for short messages and 9.22s for long messages. Only two participants (4.3%) found all the messages on the ten packages. High accessible messages did not result in highly preference among participants. Most of the participants (98%) found the message on the S4 package which correlated with the lowest TTF of 7.08s. However, only 15% of those who found the S4 message chose it as their preferred message. Most participants (93%) found the long message on L1, and this message also had the most preference (34%). Most people preferred the long messages over the short messages. The letters for the messages were 1.5 to 2.5mm in height which did not correlate with TTF or percentage of identification.

Significance: Flour safety messages on the current packages required almost 10 seconds to be seen if noticed at all. More science-based messaging strategies need to be developed to provide guidance to flour safety communication.

T9-06 The Impact of Digitizing Training Management Process, Audit, and Assessment on Approval of Food Safety Training Center and Trainers in Dubai United Arab Emirates

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Introduction: The Food safety department has mandated two trainings for food handlers working in food establishments in the Emirates of Dubai. To conduct these training, the food permits unit of food safety department approves training centers based on the criteria set by the department. Annually more than 100,000 food handlers get trained on best practices while operating in the food establishment, which is crucial for ensuring that the food is not contaminated at any point across the food chain

Purpose: The purpose of this study is to ensure the training center's provide impactful training with proper training materials and the trainers are competent to deliver training to enhance the knowledge of food handlers, therefore significantly reducing the number of violations that correlate to personnel behaviors.

Methods: Food safety department introduced an online digital platform called "Foodwatch" to digitalize food safety training management, approval of the training center, and the trainers. The system has completely digitalized registration, attendance, time management, location tracking etc as part of this system For approval of trainers, an assessment process have been introduced;

Results: The results are divided into two main factors:

Trainers with incompetent profile: 12.6% of applicants previously approved were without appropriate or prior food industry experience, in addition to 15.3% percent trainers that lacked educational background.

Incompetency of training centers: the training centers lacked proper management that lead to ineffective trainings. The main contributing factors for this were observed to be ,30 % training were conducted in area that lack proper training facilities,15 % training had trainees with different languages clubbed in one class conducted in one language and 25% trainings were conducted with reduced training hours.

Significance: The findings can be a significant enabler of future works towards improving the quality of training and compliance of training centers and trainers to significantly enhance the knowledge and competency of food handlers to ensure reduction in foodborne illness in the Emirates of Dubai.

T9-07 Food Safety Implementation and Culture Costs for Small- and Medium-Sized Food Processors Complying with Preventive Controls

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Introduction: Small and medium-sized processors (SMPs) encounter barriers that inhibit Preventive Controls (PC) compliance. SMPs may be deterred from compliance due to a lack of information regarding the overall cost of food safety implementation (cost of new equipment, procedural changes, training regimens, etc.), and culture development (food safety engagement amongst employees). For SMPs that are required to comply with the PC Rule, existing training resources are often insufficient in meeting their needs of successfully identifying costs that are involved in food safety implementation and food safety culture development.

Purpose: This study conducted a qualitative evaluation of verbal testimonials to determine culture and implementation costs of small and medium-sized processors from differing backgrounds, resource access and team size within the Northeast as supported by the Preventive Controls for Human Foods Rule.

Methods: Food safety cost assessment evaluations were distributed to 10 participants. The evaluations broke down food safety costs into food safety implementation and culture. The survey consisted of 15 questions; participants ranked how much they have spent on developing and implementing food safety into their facilities (time, monetary equivalence, etc.) in the past year. Cost assessments were comparatively analyzed to determine a food safety cost range depending on what food products respondents produced.

Results: Qualitative results reported an average cost for a shared-use facility to implement food safety was approximately $25,500+. For businesses with 1-2 team members associated with the business, costs averaged approximately $16,100+. SMPs with 1-2 team members had more difficulty analyzing cost associations for culture development than those with established employee bases due to lack of stimulated culture in very small processing environments. SMPs processing in shared-use facilities were more aware of the cost associations of food safety due to programs available through their facility.

Significance: The survey results indicate that cost is a barrier to adopting food safety plans and codifying food safety culture.

T9-08 Beef-Handling Practices of Consumers in the U.S. Virgin Islands

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Introduction: U.S. Virgin Islands (USVI) has over 106,000 residents in three islands (St. Croix, St. John, and St. Thomas). There are no recent food safety studies about consumer meat handling practices in USVI.

Purpose: The purpose of this study was to identify the beef handling practices used by consumers in the USVI.

Methods: Printed and online surveys were disseminated on all three islands through extension agents and local media sources. The questionnaire had 33 questions on practices including purchasing, storage, hygiene, sanitation, and cooking practices, and demographics. Consumers (n = 334) completed the survey, and later frequencies and Pearson Chi-square tests of independence were performed.

Results: Majority of participants bought beef from grocery stores (92%) and removed beef from shelves immediately after entering the store (55%). More than one-third of respondents either "always" or "often"; checked the expiration date (70.6%), separated beef from other foods (62.4%), used insulated bags (46.9%), and took less than one hour to return home after shopping (83.13%). At home, 295 (92%) participants took less than 30 minutes to store groceries, and 98% stored beef in either freezers (≤ 12 months) or refrigerators (≤ 30 days). During power outages, 133 (45.1%) consumers maintained cold temperatures of beef. Most participants (69%) washed hands using soap, water, and paper towels. Majority (72.3%) washed for more than 10 seconds, and duration depended on ethnic background (Chi-square = 25.24, $p < 0.05$). More than half of respondents cleaned cutting knives (56.2%) and kitchen sinks (53.8%) using soap and water. The frequency of cleaning kitchen sinks was based on level of education (Chi-square = 37.25, $p < 0.05$). Thawing frozen beef mostly was done in refrigerators (39.7%). When cooking, 254 (80.1%) participants did not check the temperature of beef for doneness, and 34 cooked hamburgers below 160°F.

Significance: Future consumer food safety education initiatives in the USVI should emphasize temperature control of food, sanitation, and hand-hygiene.

T9-09 Consumer Food Safety Knowledge, Attitude and Self-Reported Practices with Particular Reference to COVID-19 Hygiene Barriers and Lockdown Measures in Mauritius

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Introduction: Mauritius national measures to fight the COVID-19 pandemic included two lockdown periods and sustained, forceful communication to educate the population on effective hygiene barriers to break transmission of coronavirus. Such education measures are also relevant to hygiene aimed at controlling food safety hazards.

Purpose: To investigate consumer food safety behaviour during the pandemic to assess knowledge, attitude and self-reported practices and contribute to the formulation of evidence-based policies and implementation of population education actions.

Methods: A retrospective online (English) and telephone-administered (Kreol Morisien) survey of specifically formulated knowledge, attitude and self-reported practice statements was administered between May and July 2021. Recruitment was via online and social media advertising with purposive sampling of Mauritian citizens resident in Mauritius who were responsible for household food purchasing and preparation; 291 completed surveys were received. Study demographics were monitored with reference to current official statistics in Mauritius. Quantitative data were analysed using the Statistical Package for Social Sciences (SPSS) to compute descriptive and inferential statistics. Qualitative data on understanding of food safety were evaluated using thematic network analysis.

Results: Results established a significant relationship between level of education and food safety knowledge ($p<0.05$). Participants had a positive attitude towards food safety and the majority of household participants (76.2%) claimed that they washed hands according to COVID-19 national guidelines more often during pandemic lockdowns. However, at least half of the respondents stated that implementation of specific food hygiene practices, including handwashing before food handling, were about the same during COVID-19 lockdowns and COVID-safe period. Consumer understanding of food safety revealed a thematic network of 17 basic themes and 8 organising themes.

Significance: This consumer food safety study was implemented in an unprecedented context and adopted a novel approach. Survey findings will be disseminated to stakeholders to inform educational initiatives and future research to measure impact on food safety culture.

T9-10 Nothing Works! The Mediating Effects of Consumers' Perceived Safety/Risks on Patronage Intention and Restaurants' Marketing Strategies during COVID-19 Pandemic Crisis

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Introduction: The Coronavirus Disease 2019 (COVID-19) pandemic is affecting the nation's food and agricultural systems in a unique way. Every echelon or level of the food supply chain, from production to consumption, is exposed to the pandemic. Particularly, its impact on the restaurant industry has been well publicized. The restaurant industry is seeing promising recovery through various marketing strategy, while facing serious challenges.

Purpose: This study aimed to investigate the mediator or moderator effects of consumers' perceived safety risks on patronage intention and restaurants' marketing strategies during the pandemic.

Methods: A questionnaire was designed consisting of three constructs – restaurant marketing practice, consumer patronage intention, and consumer perceived safeties/risks. The study identified 18 widely suggested "best marketing practices" from multiple sources. Consumers' restaurant patronage intentions during the pandemic were measured with five items. Items in the perceived safety/risks sections were adapted from literature. All variables were measured using a 7-point Likert scale. Demographic questions included participants' age, gender, ethnicity, education, income, employment status, and marital status. After instrument validity testing, responses were collected via Qualtrics®.

Results: All constructs included in the research model show sufficient validity and reliability for the following R-square and structural path analysis. A total of 593 responses were collected. PLS-structural equation modeling (PLS-SEM) is used to analyze the research data. Results showed that during a crisis like Covid-19 pandemic, consumers' perceived safety/risks mediated the relationship between consumers' patronage intentions and the suggested marketing strategies completely.

Significance: The findings contributed to the literature on effective marketing strategies during a crisis like the pandemic and offer practical implications for the restaurant management team during their recovery.

T9-11 Consumers' Food Safety Perception of Fresh Produce from Small- and Medium-Sized Farms

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❖ ***Developing Scientist Entrant***

Introduction: Small- and medium-sized farms struggle between potential profit and increasing regulatory requirements on food safety; however, consumers expect their produce to be safe for consumption.

Purpose: To assess consumers' perception of fresh produce from small- and medium-sized farms.

Methods: An online national survey was developed and distributed to a representative US sample (N=916) October–November 2021. Prior to the administration of the survey, it was pilot tested among 50 consumers to ensure reliability and readability. Screening criteria required participants to be primary grocery shoppers for their household and to have purchased fresh bell peppers, spinach, or kale in the past month. The survey was quota-controlled for participants' sociodemographic characteristics to be nationally representative. The topics included food safety knowledge, attitudes, perception, and willingness to pay for food safety from small- and medium-sized farms.

Results: Most consumers knew that on-farm food safety practices could reduce the risk of produce contamination (83%) and considered food safety to be a minimum quality standard for their fresh produce (85%). However, rural residents were less likely (Odds Ratio 0.39; Confidence Interval 0.23, 0.65) to consider food safety as a minimum quality standard, compared with urban residents. Compared to produce from large farms, consumers perceived produce from small- and medium-sized farms as being "fresher" (57%) and "higher quality" (53%). However, most consumers did not consider produce from small- and medium-sized farms to be "safer to eat," with only 37% selecting this attribute. Nevertheless, the majority of consumers (72%) reported they would pay more for fresh produce from small and medium-sized farms if the produce bears a food safety inspection label.

Significance: The findings provide guidance for policymakers and food safety educators. The findings also shed light on food safety cost internalization for small- and medium-sized farms.

T9-12 Validation of a Food Safety Survey for Older Adults

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Introduction: Older adults are more susceptible to foodborne illness and are more likely to experience severe illness and hospitalization. A validated survey is needed to characterize food safety risks among older adults in order to inform future consumer education efforts.

Purpose: The purpose of this study was to develop and validate a food safety survey for older adults to measure food consumption and handling practices as well as behavioral constructs which might be related to safe food handling practices among older adults.

Methods: The survey was developed to capture food handling and consumption practices related to foods that could be contaminated with *Salmonella*, *Campylobacter* or *Listeria*. Survey questions were also developed to measure behavioral constructs from the Health Belief Model. The survey was admin-

istered to 350 adults 55 years of age and older. Factor analysis was used to assess construct validity. Internal consistency was assessed using Cronbach's alpha. Two weeks after initial administration, the survey was administered again to a subset of 150 individuals from the original sample to evaluate test-retest reliability

Results: As a result of factor analysis and Cronbach's alpha, some questions were dropped or reworded. The final survey consisted of 50 questions. Four categories of questions were identified relating to the Health Belief Model and 3 categories were identified relating to food handling behaviors. Cronbach's alpha for these subscales ranged from 0.62 – 0.77. Test-retest reliability varied between items; however, most HBM and food handling scales demonstrated moderate to good test-retest reliability.

Significance: The final survey will allow us to determine current food consumption and handling practices among older adults. It will also allow us to determine behavioral factors which may be related to safe food handling practices among older adults. The survey will be administered to a representative sample of older adults from across the United States.

T10-01 Sample-Initiated Retrospective Outbreak Investigation of *Salmonella* Weltreveden Linked to Imported Shrimp

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Introduction: *Salmonella* serovar Weltevreden rarely causes foodborne illness in North America, but is sometimes associated with seafood, particularly from Asia. *Salmonella* spp. have long survival times in aquatic environments and have been recovered from a variety of tropical shrimp culture systems. In 2021, FDA and CDC investigated a cluster of nine *Salmonella* Weltevreden illnesses associated with frozen, precooked shrimp imported from India.

Purpose: An investigation was initiated following the identification of *Salmonella* Weltevreden in Ready-to-Eat (RTE) shrimp, sampled at import by the FDA, and genetically linked to clinical isolates.

Methods: Samples of shrimp product were collected as part of the Imported Seafood Safety Program and the enhanced border surveillance under the Food Safety Modernization Act. Whole genome sequencing analysis of shrimp samples was conducted by FDA. Case-patient food histories were collected using routine interviews and targeted food exposure questionnaires. Traceability documents were obtained for each case-patient with a confirmed purchase date of shrimp product from consumer purchase data.

Results: *Salmonella* Weltevreden was detected in samples collected from imported RTE shrimp in January 2021. In April 2021, FDA noted three clinical isolates in the public database that were within 0 SNPs from product isolates. In total, nine case-patients from four states were part of the outbreak. Epidemiologic data was available for seven case-patients, all reported exposure to RTE shrimp. Traceback analysis identified one common supplier in India for two out of three legs and confirmed the manufacturer of the imported shrimp product supplied shrimp products to retail locations reported by case-patients. Based on product sampling, epidemiologic data, and traceback analysis, the manufacturer issued two recalls of RTE shrimp.

Significance: This investigation highlights the importance of product surveillance and utility of sample-initiated retrospective outbreak investigations in identifying sources of foodborne illness.

T10-02 The Advantages of Using WGS to Detect Foodborne Illnesses Clusters Potentially Associated with FSIS-Regulated Products

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Introduction: Since May 2019, FSIS laboratories transitioned from pulsed-field gel electrophoresis (PFGE) to whole genome sequencing (WGS) to characterize foodborne pathogens from FSIS samples and compared the WGS features with clinical isolates to identify potential foodborne illnesses.

Purpose: To compare foodborne illness clusters detected by WGS with PFGE to assess precision for the two technologies

Methods: Using FSIS foodborne illness cluster surveillance data, we compared illness clusters detected by PFGE during May 1, 2017 and April 30, 2019 (Period 1) to clusters detected by WGS during May 1, 2019 and April 30, 2021 (Period 2). Variables include the number of illnesses per cluster, cluster recognition interval CRI (the period between the first case and the cluster was coded by PulseNet), pathogens distribution, FSIS isolates in the clusters, and FSIS regulatory actions

Results: During period 1, 154 clusters were monitored, the case count ranged 1 to 436 with a mean of 33 per cluster. The CRI was 2 to 345 days with mean of 65 days. 108/154(70%) were *Salmonella*, 22/154(14%) STEC, 12/154(8%) *Listeria monocytogenes*, 8/154(5%) *Campylobacter*, and 4/154(3%) unknown pathogens. 71/154(46%) included FSIS isolates. During period 2, 150 clusters were monitored, the case count ranged 1 to 445 with mean of 22. The CRI ranged from 1 to 161 days with mean of 61 days. 96/150 (64%) were *Salmonella*, 32/150(21%) STEC, 15/150(10%) *Listeria monocytogenes*, and 7/150(5%) *Campylobacter* and 76/150(51%) clusters included FSIS isolates. The mean case count in period 2 was significantly lower than the period 1 (Unpaired *t* test: p=0.05). The difference in the mean CRI was not statistically significant (Unpaired *t* test: p=0.6523).

Significance: The clustering criteria is usually within 0-5 alleles for WGS and "indistinguishable" for PFGE pattern. WGS uses allele range is more flexible than PFGE. WGS excludes unrelated cases that leads to earlier, more efficient foodborne illness cluster detection and helps to prevent further illnesses.

T10-03 Temporal Changes in the Proportion of *Salmonella* Outbreaks Associated with Twelve Broad Commodity Classes in the United States

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Introduction: Using data from twenty years of *Salmonella* foodborne outbreak and illness counts, where each outbreak had an identified food vehicle, this study tests for trends in the proportion of outbreaks associated with 12 broad commodity classes.

Purpose: To determine if significant trends exist in the proportion of outbreaks associated with FSIS-regulated foods.

Methods: Two analyses were conducted. First, a variable selection process was used to determine the best variable for assessing changes in the proportion of outbreaks associated with each commodity. Second, analyses were conducted to determine if significant changes were observed in consumption patterns for FSIS-regulated products and the proportion of outbreaks attributed to any of the 12 commodity classes.

Results: Outbreak counts have a stronger trend signal than outbreak illness counts. The number of outbreaks with an identified food vehicle increased significantly between 1998 and 2000, followed by a 10-year period when the number of outbreaks decreased. The number of outbreaks increased significantly between 2010 and 2014, and then remained unchanged for the remainder of the study period. During 1998–2017, the proportion of outbreaks associated with eggs, pork and seeded vegetables changed significantly. Of note for FSIS was the consistent and significant increase in the proportion of pork associated outbreaks. No significant changes were observed in the remaining nine commodity classes.

Significance: Pork ranks as the third most frequently consumed meat commodity in the United States, yet only the chicken and the fruits-nuts commodities are responsible for a higher average proportion of outbreaks in the later years of the dataset. Regulatory agencies benefit from analyses that elucidate illness trends attributable to the products under their jurisdiction. Results from this trend analysis can be used to inform the development and assessment of FSIS policies and programs.

T10-04 Geographic Origin of Cattle and *Salmonella* Presence in Beef at Processing

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Introduction: The relationship between the geographic origin of cattle and the presence of Salmonella in beef has not been established on a nationwide basis.

Purpose: The objective of this analysis is to explore the relationship between the geographic origin of cattle and Salmonella presence in beef.

Methods: Results from FSIS' Salmonella sampling from 2015-2021 were matched to individual ground beef and trim production lots for five large production facilities in the USA. The origin state of cattle slaughtered within a three-day window included in the FSIS-sampled lots was determined and aggregated by lot. The rate of Salmonella-positive (SPR) lots per 10,000 head of cattle was compared between states using a Gamma conjugate prior and numerical integration.

Results: Approximately 23.5 million cattle from 33 states were matched to 3,146 FSIS-sampled lots from five processing plants located in the West, Midwest, and Southwest regions, representing approximately 12% of the domestic beef production. Most cattle came from Texas (6M/25.6%), followed by Kansas (5.4M/23.0%), Nebraska (4M/17.0%), and Iowa (3M/12.8%). Salmonella-positive lots involved 3.2% (752k) of all cattle. The SPRs (SPR:95%CrI) for Tennessee (9.46: 1.93-22.8), New Mexico (5.25: 3.45,7.37), Arizona (5.17: 2.48, 8.84) and Oklahoma (3.41: 2.26, 4.80) were higher than that of all other states, but not significantly different from each other. All other states had SPRs under 2.0 and were not significantly different from each other.

Significance: By understanding how geographic origin of cattle relates to Salmonella presence in beef, regionally specific interventions to reduce Salmonella can be considered at pre-harvest and processing levels, thereby reducing the public health burden associated with beef-attributed Salmonellosis. This exploratory analysis will inform planned further multi-level modeling that adjusts for cattle type, season, and plant effects.

T10-05 Incidence of Selected Foodborne Pathogens in Hospital Stool Samples in Ethiopia, 2018 – 2020

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Introduction: Diarrhea is a significant public health concern and a major cause of morbidity and mortality globally. Infectious causes of diarrheal illness are often unidentified, and surveillance is limited, especially in low- and middle-income countries. Foodborne bacterial pathogens are a major contributor to diarrheal illness globally and estimating the level of exposure is necessary for prevention of illness and reductions in mortality rates.

Purpose: The objective of this study was to estimate the incidence of *Salmonella* and *Shigella* in fecal samples analyzed at three clinical laboratories in Ethiopia.

Methods: A retrospective analysis of laboratory results from stool samples collected at Yekatit 12 Hospital (Addis Ababa), University of Gondar Hospital (Gondar), and Hiwot Fana Hospital (Harar) between 2018 and 2020 was undertaken. Descriptive statistics and chi-square tests were used, respectively, to summarize the number of stool samples collected and the pathogens detected and compare trends across regions. Incidence was calculated dividing the number of positive results by the number of samples for bacterial pathogens.

Results: Data was obtained for 19,363 stool samples analyzed at the study sites in Addis Ababa (n=701), Gondar (n=16,891) and Harar (n=1,771). The number of samples collected varied by month, with peaks during the rainy season, and by year. Of the stool samples collected, 30.75% were tested for microbial pathogens. Overall, *Salmonella* and *Shigella* were detected in 0.01% and 0.10% of samples tested for microbial pathogens, respectively. The incidence of Salmonella was 0.14% in Addis Ababa, 0% in Gondar and 0% in Harar. The incidence of Shigella was 0.29% in Addis Ababa, 0.38% in Gondar and 0% in Harar.

Significance: Results showed a marked regional difference in detection rates for two foodborne pathogens that warrant further investigation.

T10-06 An Evaluation of Diagnostic Practices Around *Salmonella* spp., *E. coli*, *Campylobacter* spp., and Norovirus at a Large Tertiary Pediatric Hospital

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Introduction: Each year in the United States, an estimated 9.4 million people fall ill with foodborne diseases of known etiology. Understanding how these illnesses are diagnosed is critical for their control and prevention.

Purpose: The purpose of this study was to examine diagnostic practices around norovirus, *Salmonella spp.*, *Campylobacter spp*., and shiga toxin-producing *E. coli* (STEC) at a large tertiary pediatric hospital in Columbus, Ohio.

Methods: Demographic data (age, gender, race), encounter data (encounter date, diagnostic codes, insurance type, outcome), and lab data (order date, test type, results) were obtained from the electronic medical records of all patients who had a diagnostic code (ICD-9/10-CM) or a positive stool sample for the pathogens of interest between 2016 and 2020. Descriptive statistics were used to examine the agreement between diagnostic codes and laboratory test results overall and by pathogen. Logistic regression was used to examine differences between pathogens.

Results: Over the 5-year period, 957 unique patients were diagnosed 1,066 episodes of illness with norovirus (582), salmonellosis (207), campylobacteriosis (168) and STEC (160). Of these, X (Y%) had a stool sample collected. The majority of episodes (80.2%) had both a diagnostic code and positive corresponding test result. Of the remaining episodes, 15.1% were diagnosed without a corresponding positive laboratory results and 4.3% had a positive laboratory result but were not diagnosed. The proportion of episodes with both a diagnostic code and lab result was lower for episodes of *E. coli* (71.9%, OR: 0.57, p<0.01) and *Campylobacter spp.* (73.8%, OR: 0.64, p=0.02), and higher for norovirus (87.1%, OR: 2.64, p<0.01) when compared to *Salmonella spp.* (78.4%). The proportion was also lower for patients aged 6-10 (73.4%, OR: 0.66, p=0.04) when compared to patients aged 0-5 (80.7%).

Significance: Discrepancies between patient diagnostic codes and laboratory test results suggest that education programs on proper diagnosis of foodborne diseases are needed.

T10-07 Detection of Gastrointestinal Pathogens in Stool Samples Using a Rapid Multiplex PCR Test at a Large Tertiary Pediatric Hospital

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Introduction: Rapid multiplex PCR tests, such as BioFire FilmArray Gastrointestinal (GI) Panel, can quickly test multiple pathogens associated with gastroenteritis simultaneously. Co-detection of bacterial and viral pathogens can be common; however, the clinical significance may vary, and current trends are not well understood.

Purpose: This study examined co-detection rates among 20 pathogens included on the GI Panel for patients who had stool samples tested at a large tertiary pediatric hospital in Columbus, Ohio.

Methods: Electronic medical records were obtained for patients with stool samples tested using the GI Panel between January 1, 2016 and December 31, 2020. Demographic (patient age, sex, race), encounter (encounter date, insurance type, diagnostic codes, outcome), and laboratory data (order date,

lab name, lab result) were extracted and summarized using descriptive statistics. Incidence rates were calculated by dividing the number of positives by the number of stool samples tested. Correlation analysis was conducted to identify associations between pathogens.

Results: A total of 12,907 patients had at least one stool sample tested during the study period; 60% had no pathogen detected, 33% had one pathogen detected and 7% had more than one pathogen detected. The most commonly detected pathogens were: *Clostridioides (Clostridium) difficile* (9.7%), norovirus (7.6%), sapovirus (5.1%), rotavirus (4.7%), and adenovirus (3.9%). The pathogens with the highest co-detection rates were: enterotoxigenic *Escherichia coli* (ETEC) (73.4%), *Plesiomonas shigelloides* (59%), *Giardia lamblia* (54.1%), *Yersinia enterocolitica* (53.8%), and astrovirus (44.6%). The most common co-detected pathogens with regards to total number of co-detections were: norovirus (12.7%), sapovirus (11.7%), *C. difficile* (10.7%), rotavirus (8.8%), and astrovirus (8%).

Significance: The two most common pathogens detected through GI Panel and co-detected with other pathogens were *C. difficile* and norovirus. These results will help inform clinical management of patients with gastrointestinal illness and expand our understanding of the epidemiology of foodborne illness.

T10-08 Impact of Seasonal Variation in Soil Bacterial Microbiome of Dairy Farms and Risks Associated with Pathogen Transmission

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❖ ***Developing Scientist Entrant***

Introduction: Soil is an extremely heterogenous environment harboring all sorts of microorganisms, including pathogens. Soil microbial ecology depends on various factors such as composition, season, moisture contents, and deposition of biomaterial from soil inhabitants. Further, soil microbes play critical role in transmission pathogens to humans and animals.

Purpose: To investigate the impact of seasonal variation on the microbial ecology of traditional dairy farm soil for evaluating the practices in animal husbandry and crop production.

Methods: A total of 40 soil samples (~1kg of soil/sample, from multiple locations) were collected during the summer and winter seasons from the grazing field of 3 dairy farms in Maryland, USA. Bacterial genomic DNA was extracted from 300mg of collected soil. Metagenomic analysis of the extracted DNA was performed by targeting 16S rRNA genes and the acquired data were analyzed to identify the variation of abundance at phylum and genus-level. Total species diversity in a single community was analyzed by calculating Simpson (D) and Shannon (H) indices. Student's t-test was applied to determine statistical significance between the seasons.

Results: A significantly (p<0.05) higher abundance of Actinobacteria (15.97%), Gemmatimonadetes (2.64%), and Chloroflexi (6.81%); whereas lower abundance (p<0.05) of Verrucomicrobia (2.42%) was documented during the summer. A numerically higher abundance was observed in Proteobacteria (26.70%) and Acidobacteria (13.32%). A complete difference in the predominance of bacterial genus was documented due to seasonal variation. In summer, a notable presence of *Gp6, Sphingomonas, Nitrolancea,* and *Bacillus* was observed. Whereas, in winter the abundance of *Spartobacteria, Parcubacteria,* and *Saccharibacteria* became predominant. Surprisingly, we documented a comparatively lower abundance of common foodborne pathogens (e.g., *Escherichia, Campylobacter,* and *Salmonella*) during summer. Though, Simpson (D) and Shannon (H) indices did not reveal any significant difference (p>0.05) of species diversity between the seasons.

Significance: Seasonal variation impacts microbiome prevalence which might also influence pathogen transmission and safety of food products.

T10-09 Comparison of an Ultrafiltration Concentration Method for Viruses in Fresh and Frozen Produce with the Reference Method ISO 15216: 2017-1

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❖ ***Developing Scientist Entrant***

Introduction: In recent years, the presence of hepatitis A virus (HAV) and noroviruses (NoV) has been actively monitored through surveillance plans on fresh and frozen produce by US and Canadian governmental bodies. Since concentration of viruses is usually necessary to detect them on food, the detection methods used are often long and tedious.

Purpose: The aim of this study was to validate the limit of detection (LOD) of an ultrafiltration (UF) method to concentrate viruses and to test its effectiveness on various fresh and frozen produce in comparison to the ISO reference method.

Methods: The LOD of HAV, NoV GII.4 and GI.7 (10^5 to 10^1 genome copies in deionized water/sample) was measured on fresh and frozen strawberries (25g). The recovery rate of these viruses (10^4) was evaluated on fresh and frozen raspberries and blackberries, lettuce, and green onions. Viruses were then eluted, concentrated to 1 ml, and purified according to the ISO reference method or an UF protocol based on a commercial UF system. Viral RNA was extracted and then detected with RT-qPCR. All experiments were replicated three times.

Results: On fresh strawberries, a difference of one log of detection between the two methods was observed for NoV GII.4 (10^3 vs 10^4) and NoV GI.7 (10^2 vs 10^3) in favor of UF, while HAV was detected at 10^2 for the two methods. No difference was observed between the two methods with frozen strawberries for each virus. The recovery was not significantly different between the two methods for most of the different matrices regardless of the virus studied ($p<0.05$, Šídák's multiple comparisons test), except for fresh raspberries where recovery rate was lower with UF for NoV GI.7 ($p = 0.0306$).

Significance: The UF method has fewer steps and is shorter than the ISO reference method, it has interesting potential during routine analysis through food production and surveillance.

T10-10 Optimization of CDC's *Cyclospora cayetanensis* Genotyping Workflow Yields More Accurate Genetic Clustering Results

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Introduction: The U.S. Centers for Disease Control and Prevention (CDC) pioneered a *Cyclospora cayetanensis* genotyping system; however, best practices for *Cyclospora* genetic clustering are yet to be established.

Purpose: To improve genetic clustering accuracy of CDCs *Cyclospora* genotyping software.

Methods: The software includes three modules involved in genetic cluster detection: 1) haplotype identification, 2) pairwise genetic distance calculation of haplotypes from Module 1, and 3) cluster identification using distances from Module 2. User-defined parameters in Modules 2 & 3 influence genetic clustering results. A key parameter in each of these two modules was evaluated to define optimal settings. Performance was evaluated by calculating sensitivity, specificity, and accuracy using epidemiologic clusters as a gold-standard reference. For Module 2, we generated distance matrices from a reference dataset (n=1137 specimens) using several distance computation methods (including CDCs novel Heuristic method) to determine which yielded clusters that most closely match the gold-standard reference. Next, we optimized Module 3's stringency parameter (defined as the percentage of within-cluster distances falling below a reference-agnostic cutoff), by testing values between 95% and 99.5% in 0.5% increments. A distance matrix (n=2023 specimens) generated using CDCs heuristic method was used to compare stringency settings.

Results: Module 2's Heuristic algorithm displayed the most robust clustering performance (sensitivity = 90.8%, specificity = 99.9%, accuracy = 99.5%). Module 3's 97% stringency setting performed best (sensitivity = 95.0%, specificity = 99.8%, accuracy = 99.6%). Lower stringency values had higher sensitivity, but lower specificity and accuracy, while higher stringency values had similar specificity to 97% stringency but sacrificed sensitivity and accuracy.

Significance: Highly complex food supply chains mean appropriate identification of genetically-related cyclosporiasis clusters is of paramount importance for informing outbreak investigations. CDCs optimized *Cyclospora* genotyping workflow (heuristic algorithm & 97% stringency) improves clustering performance, which should facilitate investigation of potential exposures associated with cyclosporiasis outbreaks.

T10-11 Two Genetic Lineages of *Cyclospora cayetanensis* Cause Human *Cyclosporiasis* in the USA

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Introduction: From 2018 to 2020, *C. cayetanensis* genotyping was attempted on 3,715 fecal specimens at the US Centers for Disease Control and Prevention (CDC), including specimens from the USA, Canada, Mexico, Guatemala, and China: clustering of genotypes supported that two distinct genetic lineages cause outbreaks of cyclosporiasis in the USA.

Purpose: To characterize the two genetic lineages of *C. cayetanensis* (lineages A and B) and investigate potential epidemiologic implications.

Methods: Clustering of genotypes detected using CDC's genotyping system revealed two major populations. Genotypes of isolates belonging to each population were examined to determine loci driving their separation. Epidemiologic data for genotyped isolates was examined to understand case-patient demography, time of illness onset, and food consumption history; statistical comparisons (Fisher's exact test) were made to identify differences that may exist between lineages; P-values <0.05 were considered significant.

Results: Two loci were identified as driving the separation of lineages A and B. One locus possessed two major alleles; one almost exclusive to lineage A and the other to B. Six alleles existed at the second locus; two almost exclusive to lineage A, three to lineage B, and the sixth to a Chinese strain. Epidemiologic associations between lineage and geographical distribution and temporal associations were observed. Most lineage A infections were identified in patients from Midwestern states: Iowa ($p<0.001$), Illinois ($p=0.010$), Wisconsin ($p<0.001$), Minnesota ($p<0.001$), and Nebraska ($p=0.013$). Conversely, patients from Florida ($p<0.001$) and Texas ($p<0.001$) saw higher percentages of lineage B. Across the months, lineage A made up the highest percentage (84%) of cases with illness onset in May ($p<0.001$); lineage B made up the highest percentage (71%) in August ($p<0.001$).

Significance: Two lineages of *C. cayetanensis* cause seasonal cyclosporiasis outbreaks in the USA. Epidemiologic data support biological differences between the lineages based on temporal and geographic distinctions.

T10-12 Stability and Infectivity of SARS-CoV-2 in Foods and Common Beverages

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Introduction: COVID-19 patients develop gastrointestinal symptoms in addition to respiratory disease. Given that SARS-CoV-2 can infect human oral and gastrointestinal cells, ingestion of virus-contaminated foods needs to be investigated as an alternative route of infection.

Purpose: To determine the potential for food to serve as a potential source of infection, the stability and infectivity of SARS-CoV-2 was assessed in common foods and beverages.

Methods: Following inoculation of SARS-CoV-2 ($1x10^4$ PFU, Isolate USA-WA1/2020) to 13 foods and 34 beverages, virus was recovered by rinsing immediately with 1mL Dulbecco's Modified Eagle Medium (DMEM) to assess maximum infectious virus recoverable from each food and beverage (0h). Inoculated foods were refrigerated (4°C) and infectious virus quantified at 1h, 24h, 7d, 14d, and 21d post-inoculation by standard plaque assay (detection limit =0.7 log PFU/mL). Inoculated beverages were held at room temperature for 1h; milk samples were also refrigerated for 7d post-inoculation. Viral RNA was quantified in selected foods and beverages by RT-qPCR. Virus quantities were compared using ANOVA.

Results: SARS-CoV-2 remained infectious on meats, seafood, and some produce for at least 21 days. However, survival of infectious SARS-CoV-2 was significantly reduced ($p<0.05$) in processed meats, such as salami (3.3 log PFU/mL at 0h to 1.3 log PFU/mL at 24h), and avocado pulp (3.9 log PFU/mL at 0h to 1.0 log PFU/mL at 24h). Although SARS-CoV-2 survived well in beer, energy drink and light soda for 1h and in milk for 7d, other beverages such as tea, wine, grape juice, and hard liquor instantly inactivated the virus (0h).

Significance: Although further studies are needed to determine if infection can occur following ingestion of virus-contaminated foods and beverages, the survival and high recovery of SARS-CoV-2 from certain foods and drinks highlight the importance of safe food handling practices and the potential for food products and beverages as potential carriers of SARS-CoV-2.

T11-01 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.

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.

T11-02 Analysis of Cashew Allergen Cross-Contact in Shared Frying Oil

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Introduction:

Food allergen cross-contact in shared frying or oil-roasting operations is a challenge for allergen management due to substantial data gaps.

Purpose:

To quantitatively evaluate cashew allergen cross-contact in shared frying oil and compare the effectiveness of oil cleaning strategies on allergen removal.

Methods:

Fifteen batches of cashews (100 g/batch) were oil-roasted in 1 L soybean oil using low-temperature-long-time (LTLT, 138 C for 10 minutes) or high-temperature-short-time (HTST, 168 C for 3 minutes) conditions. Oil samples collected after every five batches were analyzed for cashew protein concentration using a targeted LC-MS/MS method. The remaining oil from the LTLT experiments was homogenized and used to analyze the effectiveness of oil cleaning methods on cashew allergen removal. The cleaning methods tested included gravity separation (settling, 50 g, and 2000 g centrifugation), filtration (pore sizes of 11 to 500 microns), and filter paper with filter aids (diatomaceous earth or magnesium silicate). A total of ten treatments were analyzed, each with three treatment replicates (35 mL oil/treatment), three sample preparation replicates (1 g oil/replicate), and two MS injection replicates.

Results:
The results of the cashew frying experiments indicated that cashew protein concentration was 126.7 ± 30.5 ppm (mean ± standard deviation, n = 12) after frying 15 batches of cashew nuts using LTLT treatment and 73.8 ± 10.8 ppm (n = 12) using HTST treatment. In terms of oil cleaning methods, all except 500-micron mesh demonstrated a significant reduction of cashew protein concentrations (adjusted $p<0.0001$, n = 18). The commercial filter paper combined with diatomaceous earth, 11-micron filter, and 25-micron filter were the most effective treatments, reducing cashew protein concentrations from 200 ppm to less than 5 ppm.
Significance:
The study provides data that can be used to develop and implement improved food allergen preventive controls and protect allergic consumers.

T11-03 Regulatory Policies for Heavy Metals in Spices – A New York Approach: An Update

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Introduction: Over the years, toxic metals have been the focus of ecological, health, and agricultural concerns. The New York State Department of Agriculture and Markets (NYSAGM), the primary food safety regulatory authority in New York State, has been monitoring the presence of adulterants, primarily toxic metals, in spices since 2014. In 2018, NYSAGM and the New York State Department of Health's (NYSDOH) Bureau of Toxic Substance Assessment (BTSA) formed an interagency collaboration to determine actionable limits of toxic metals commonly found in spices.
Purpose: The purpose of this collaboration was to evaluate the presence of lead (Pb), cadmium (Cd), and inorganic arsenic (iAs) in spices and derive health-based guidance values for Pb, Cd, and iAs in spices used in food preparation.
Methods: Inspectors collected samples using two sampling approaches: 'for cause' and commodity-based targeted assignments. Samples were shipped to the laboratory via overnight courier. Toxic metals were analyzed by ICP-MS.
Results: From 2014-to-date, more than 1500 spices were tested for toxic metals by NYSAGM. In 2016, based on laboratory findings of lead chromate in spices and absent a federal action level or federal guidance on toxic metals in spices, NYSAGM devised two State Class recall action levels: 1 ppm (Class II) and 25 ppm (Class I). Using these action levels, from 2016-to-date, over 150 spices were recalled. Based on the NYSDOH-BTSA assessment and observed background levels, NYSAGM is reducing the State's Class II recall action levels by an approximate 5-fold factor. Prior to implementing these new action levels, NYSAGM will work closely with stakeholders to ensure the reasons for the updated action levels are well understood and using a phased, targeted approach, will ensure spices most consumed are targeted during the first implementation phase.
Significance: New York is the first State to establish action levels for heavy metals in spices.

T11-04 Estimating Maternal Fumonisin Exposure Level and Risk of Neural Tube Defects during Pregnancy in Guatemala

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❖ ***Developing Scientist Entrant***

Introduction: Consumption of food contaminated with fumonisins by pregnant women has been associated with congenital malformations, such as neural tube defects (NTDs), in children. High fumonisin intake levels among reproductive-aged women have been found in populations where maize is a dietary staple, such as Guatemala.
Purpose: The objective of this study was to estimate maternal fumonisin exposure levels and risk of neural tube defects during pregnancy in Guatemala.
Methods: Two studies were conducted in the departments of Alta Verapaz and Guatemala from 2013 to 2014. A study of 109 cases and 436 controls was conducted to evaluate the association between maternal dietary risk factors and neural tube defects. A cross-sectional study of 775 reproductive-aged women was conducted to assess dietary and socioeconomic risk factors for fumonisin exposure as measured by urinary fumonisin B_1 levels (uFB_1). Nearest neighbor propensity scores were used to match women in the case-control study with women in the cross-sectional study based on age, language, education level, and number of household members and assign an estimated uFB_1 level to each case-control woman. Logistic regression was used to assess the relationship between NTDs and estimated maternal fumonisin level.
Results: Of the 545 women in the study, 57.25% (312) had estimated exposure levels to uFB_1 that exceeded 0.5 ng/mL. The estimated mean level of fumonisins among those who had a child with NTDs exceeded the Joint FAO/WHO Expert Committee on Food Additives provisional maximum tolerable daily intake (JECFA PMTDI) of > 2µg/kg bw/day at 6.06µg/kg bw/day (SD: 10.77). Odds of having a child with NTDs did not vary with estimated exposure level (OR=1.011, 95% CI: 0.66, 1.55).
Significance: These findings suggest that pregnant women in Guatemala are exposed to levels of fumonisins that are similar to or exceed the JECFA PMTDI. Research on interventions to reduce exposure to fumonisins during pregnancy is needed.

T11-05 Cassava Consumers Exposure to Cyanide in Burhinyi Chiefdom, D.R. Congo and Optimized Reduction Technologies

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Introduction: In Burhinyi chiefdom, cassava is prepared and consumed in various forms and the main form is stiff porridge known as *ugali* and eaten with a diversity of accompaniments. Consumption of cassava with high levels of cyanide causes different levels of disease and could even be fatal. In DRC, *konzo* is attributed to inadequate cassava processing and consumption of cassava meals with little protein.
Purpose: To determine the cassava consumers exposure to cyanide in Burhinyi Chiefdom, D.R.Congo and optimized reduction technologies.
Methods: this study involved a survey with a sample size of 240 households using a previously pre-tested structured questionnaire in the eight sub-locations in Burhinyi. The information on the traditional cassava processing methods used in the environment was collected and then the collection of 90 samples of *ugali*, 160 samples of fresh cassava and 100 samples of cassava flour samples for subsequent analysis for cyanide and moisture content.
Results: The results showed that most of *konzo*-affected people were women at 56.7% and the population of Burhinyi belongs in the lower socioeconomic class at 93.4%. The prevalence of konzo in Burhinyi is 35.4%. Sun drying without fermentation is the most applied method by the population of Burhinyi is at 94.5%. Cyanide exposure to the population of Burhinyi ranged from a mean of 26 mg/kg bw/day to 271mg/kg bw/day at the 95th percentile through consumption of cassava-based *ugali*. Cassava leaves and cassava flour are the most forms of cassava consumed Burhinyi at 97.9%, but unfortunately, they do not consume protein-based food at 92.95%. HCN was reduced with the sun drying and mold application during the 4 days of drying.
Significance: The results of the study will help the communities to improve the traditional cassava processing methods, in order to reduce cyanide below the tolerance levels and protect population from Konzo disease.

T11-06 Nitrite Lowers Transcription of Staphylococcal Enterotoxin C and Triggers the *Sigb* Regulon

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Introduction: Staphylococcal food poisoning is caused by staphylococcal enterotoxins including SEC. While *Staphylococcus aureus* is easily inhibited in foods by the surrounding microflora it can outgrow them under stressful conditions. Such conditions occur when additives such as nitrite are added.

Purpose: In this study, we aimed to assess the effect of nitrite on the *sec* expression and its control by regulatory elements.

Methods: We investigated the influence of 150 mg/L nitrite on SEC expression at mRNA level by qPCR and at protein level by ELISA in seven strains expressing different SEC variants (three independent biological replicates). Regulatory knockout mutants (*Δagr*, *ΔsarA*, *ΔsigB*) of high SEC producing strain SAI48 were investigated at mRNA level under nitrite stress and control conditions. In addition, whole genome sequences were generated by Illumina sequencing. For statistical analysis, a mixed effect linear model was fitted on the $\log_{10}$-transformed fold change, with a full three-way interaction between reference gene, strain, and time effects. To determine whether individual mRNA levels were increased, we used lsmeans to perform a one-sided effect test, with Holm-Bonferroni-corrected *p*-values. Protein data was analyzed via two-way ANOVA and post-hoc Tukey's multiple comparisons.

Results: Our findings suggest that nitrite lowers *sec* mRNA transcription (statistically significant decrease in 5/7 tested strains for at least one time point), but can increase SEC protein expression. While *Δagr* mutants exhibited lower *sec* mRNA transcription levels than wt strains, this response was not stress-specific. *ΔsigB* mutants displayed the opposite behaviour under stress conditions compared to control conditions. WGS analysis of the strains revealed a defective *agr* element in one of the seven strains (SAI3) that did however not influence *sec* expression.

Significance: SEC synthesis can be induced by nitrite stress and *sigB* regulates nitrite stress response. Our findings are not consistent with previous studies investigating SED, suggesting that staphylococcal enterotoxins differ in stress response.

T11-07 Fate of Aflatoxin B1 during Manufacture of Wheat Artisanal Beer Made with Contaminated Wheat Malt

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Introduction: Wheat grains can be contaminated with aflatoxin B_1 (AFB_1), in the field or during storage. This means that mycotoxins may be present in the final product if contaminated raw materials are used. The stability of AFB_1 during artisanal beer production is unknown.

Purpose: This study evaluates the fate of AFB_1 during the manufacture of artisanal wheat beer.

Methods: AFB_1 contaminated (1.2 ng/g) milled wheat malt was mixed with milled barley malt (1:1). Mashing followed this time temperature profile: 43 °C (15 min), 52 °C (20 min), 66°C (40 min) and 78 °C (5 min). Spent grains were separated and the filtered wort was heated to 100 °C. Magnum hops were added after 10 min, and the mixture was boiled for 60 min. Sweet wort was cooled (21°C) and inoculated with *Saccharomyces cerevisie* WB-06 (10^6 CFU/mL). The yeast (spent yeast) was removed after eight days at 21°C and the green beer was matured for 5 days at 0 °C resulting in finished beer. AFB_1 was extracted using vortex assisted solid phase matrix dispersion (MSPD) in solid samples (wheat grist, mash, spent grain) and liquid-liquid partition extraction (PLL) in liquid samples (sweet wort, green beer, spent yeast, beer). Samples were analyzed by High Performance Liquid Chromatography, quantified based on calibration curves in the matrix with a quantification limit of 0.08 ng/g or 0.08 ng per g or mL.

Results: AFB_1 levels was1.0 ng/g after mashing. Spent grains and spent yeast contained 0.71 ng/g and 0.11 ng/mL of AFB_1 respectively. The sweet wort contained 0.83 ng/mL of AFB_1, and 0.30 ng/mL remained in the green beer. In the final beer AFB_1 level was 0.22 ng/mL.

Significance: The findings highlight the stability and transfer of AFB_1 mycotoxin to artisanal beer when contaminated milled wheat malt is used.

T11-08 Global Meta-Analysis of Cheese Microbiomes

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Introduction: As global cheese consumption and production rises, understanding the determinants of cheese microbiome diversity is crucial in determining the final product's value, consumers' enjoyment, quality and safety, and shelf-life. However, the growing research in the microbiology of cheese has been localized and often focused on single cheese types.

Purpose: To this end, we present the first meta-analysis of cheese microbiomes on a global scale.

Methods: First, we surveyed the cheese microbiology literature to determine the techniques and physicochemical parameters that are generally reported in the field. Then, we collected 16S rRNA gene amplicon sequence data from studies which had publicly available data to examine patterns of microbiome diversity across cheeses.

Results: Of all studies surveyed, 75% focused on lactic acid bacteria rather than the whole cheese microbiome. We collected sequence data for 824 samples from 27 studies, spanning 58 cheeses types and 16 countries. We found a consistent, positive relationship between microbiome richness and pH, and a consistently higher microbiome richness in cheeses derived from goat milk. However, we found no relationship between pasteurization, geographic location, or salinity and richness. In contrast, we found that both experimental variables associated with sequencing (i.e., the molecule sequenced, the extraction kit and the sequencer used) and environmental variables (animal milk type, location, and pasteurization) explained over half of the variation in microbiome community composition. Through cluster analysis, we identified four different groups of cheese microbiomes, driven by distinct distributions of dominant taxa (*Lactococcus*, *Streptococcus,* and *Lactobacillus* genera). Notably, we found one cluster of cheeses predominantly from the Americas had a consistently higher proportion of Lactococcus genera, and exhibited a higher community evenness.

Significance: This study presents the first global survey of cheese microbiome diversity, and reveals notable patterns driven by geography and local environmental variables.

T11-09 Thermal Inactivation of *Lactobacillus parabuchneri* in Cheesemilk to Reduce Incidence of Histamine in Alpine-Style Cheeses

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Introduction: *Lactobacillus parabuchneri* is found in raw milk and is able to survive and metabolize during aging of alpine-style cheeses made with raw milk. Strains with histidine decarboxylase activity are responsible for accumulation of histamine in the cheese, which can cause headaches, urticaria, rashes, and dizziness. Thermization has been proposed to reduce the risk of *L. parabuchneri* in cheeses made from unpasteurized milk, but the effective time/temperature parameters have not been established.

Purpose: To determine D- and z-values of *L. parabuchneri* in whole milk at thermization temperatures of 57.2, 60.0, and 62.8°C.

Methods: Aliquots (100 ml) of non-homogenized, pasteurized whole milk were pre-heated in flasks to target temperatures and inoculated with 8-log CFU/mL *L. parabuchneri* (1% inoculum). Duplicate 1 mL samples were removed from heating at predetermined time intervals, immediately chilled in buffer, and surviving *L. parabuchneri* enumerated on De Man, Rogosa and Sharpe (MRS) agar. Triplicate trials were conducted and survival data was used to cal-

culate thermal inactivation rates. The inverse slope of the linear regression data was used to calculate D values at each temperature. Log of D-values were plotted for each temperature and z-value calculated from the inverse slope of the linear regression of those data points.

Results: D-values of 267.4+ 25.2, 140.1+ 21.6, and 26.1+ 2.2 sec at 57.2, 60.0, and 62.8°C, respectively, were observed, corresponding to a 3-log reduction in 13:22, 9:21, and 1:18 (min:sec), and an overall z-value of 5.49°C (R^2 = 0.948; T-ref 60.0°C).

Significance: The thermal inactivation of *L. parabuchneri* in this study was similar to the inactivation rate of another gram-positive microbe, *Listeria monocytogenes*, in cheesemilk (T-ref 60.0°C, $D_{60°C}$=2.28 min, Z=6.7°C). These data can be used to establish thermization processes for milk to reduce *L. parabuchneri* populations and prevent cheese handlers and consumers from harm caused by histamine poisoning.

T11-10 Impact of the Addition of Lactic Acid Bacteria with Antimicrobial Activity on the Growth of *Listeria monocytogenes* in Frescal and Semi-Hard Artisanal Minas Cheeses

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Introduction: Listeriosis has been linked to consumption of contaminated artisanal cheeses. Milk lactic acid bacteria (LAB) may inhibit the growth of foodborne pathogens in cheese. Little is known about the the growth potential (δ) of *Listeria monocytogenes* in Frescal and semi-hard Minas cheeses added of milk LAB.

Purpose: To determine the δ of *L. monocytogenes* in the presence of a pool of LAB with antimicrobial activity in Frescal and semi-hard Minas microcheeses.

Methods: Frescal and semi-hard Minas microcheeses were produced with pasteurized and raw milk, respectively. *L. monocytogenes* (CLIST 3974, CLIST 3969, and CLIST 4162) was inoculated in the milk used for cheesemaking (10^3-10^4 CFU/mL in frescal; 10^5-10^6 CFU/mL in semi-hard cheese). A pool of LAB [*Lactobacillus brevis* 2-392, *Lactobacillus plantarum* 1-399, and 4 strains of *Enterococcus faecalis* (1-37, 2-49, 2-388 and 1-400); 10^6-10^7 CFU/mL)] was also inoculated in the milk. *L. monocytogenes* and LAB were enumerated after cheese production and after 15 days of storage (4 and 7 °C) in frescal cheese or after 22 days of ripening (22 °C) in semi-hard cheese. The δ of *L. monocytogenes* and LAB was calculated by the difference between counts at the end of storage and the beginning of the experiment. pH and aw values were determined.

Results: In frescal cheese, LAB addition reduced the *L. monocytogenes* δ (up to 1.10 log CFU/g) and in semi-hard cheese, LAB caused inactivation (~1.74 log CFU/g). Changes in pH were observed in cheeses added of LAB and the pH reduction varies with the cheese type, strain added and storage temperature. The a_w did not change in frescal cheese, regardless the LAB addition but reduced in semi-hard cheese, particularly in those inoculated with *L. monocytogenes*.

Significance: Results show bacteriostatic effects of LAB in frescal cheese and inactivation of *L. monocytogenes* by LAB in semi-hard Minas cheese. LAB with antimicrobial properties are a promising strategy to enhance the safety of cheeses.

T11-11 Effect of Suspended Solids in Milk on UV-C Dose-Response of *Listeria monocytogenes*

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Introduction: Although use of UV irradiation to pasteurize milk has shown promise, the effect of physicochemical and optical properties of opaque liquids such as milk on the efficacy of UV irradiation to inactivate *L. monocytogenes* has not yet been characterized. This information is critical to designing appropriate UV irradiation systems for milk pasteurization.

Purpose: Identify the effect of suspended milk solids on UV-C (254 nm) absorption (μa) and scattering (μ's) in milk, and develop an improved method to calculate UV fluence required to inactivate *L. monocytogenes* in milk, taking μa and μ's into account.

Methods: The composition and particle size of five milk samples were analyzed using MIR Spectroscopy and Mastersizer 2000, respectively. Milk optical properties were measured using UV-VIS spectrophotometer and used in the μa and μ's calculation by Inverse Adding-Doubling program. Correlations between physicochemical and optical parameters were analyzed using ordinary least squares. The μa and μ's were taken into account on the UV-C fluence rate (E'avg) for calculating exposure time for UV-C dose 0, 2, 4, 6, 8, and 10 mJ/cm^2 using multiphysics engineering simulation software. *L. monocytogenes* inactivation in milk and buffer using a collimated beam device were compared. All experiments were done in triplicate.

Results: Bigger particles significantly increase the μa, while the presence of smaller particles strongly increases the μ's. The impact of protein content on the reduced scattering coefficient in milk is greater than fat content. E'avg in skimmed, 2%, 3.8% cow milk,2%, 3.8% goat milk were 0.0319, 0.0227, 0.0206, 0.0193, and 0.0155 mW/cm^2, respectively. The E'avg calculation method could estimate similar UV fluence up to 8 mJ/cm^2 between milk with various optical properties, but requires improvement to estimate higher UV fluence in milk.

Significance: The Inclusion of the optical properties of milk is critical for accurate UV fluence calculations.

T11-12 Commercial Bacteriophage Preparations for the Control of *Listeria monocytogenes* in Raw and Pasteurized Milk

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* remains a major challenge for dairy processors due to the potential presence in raw milk and post-processing contamination, yet antimicrobial interventions are limited by the Standards of Identity for milk and milk products.

Purpose: This study determined the host range of a commercial bacteriophage preparation against *L. monocytogenes* and the antimicrobial effect in raw and pasteurized milk.

Methods: Phage titers were determined via a standard agar overlay assay. Inhibitory phage/pathogen ratios (PFU/CFU) or multiplicities of infection (MOI) were determined using a microtiter plate-based assay with doubling dilutions of phage in wells containing 2 log CFU/mL of *L. monocytogenes* (n=17 strains) in tryptic soy broth and incubated at 37°C for 24h. Raw and pasteurized milk (25mL) were inoculated with 8 log PFU/mL of phage and 2 log CFU/mL of *Listeria* (n=3 strains) and incubated at 7°C. Milk without phage served as the control. *Listeria* were enumerated at 1, 4, and 24h and every 24h through 96h. All experiments were repeated at least twice.

Results: Titers with strain 2014L-6025 were higher than 5/17 tested (P<0.05) with all others similar. Mean MOIs were highest for LM1567, 2014L-6025, F4260, LM367, and Scott A (3.11-3.59 log PFU/CFU), which did not differ. MOIs for 11 other strains did not differ (2.53-2.98 log PFU/CFU). SLCC-2482 was not inhibited. Significant reductions in Scott A (2.25), ATCC-51414 (1.93), and Blue Bell (1.64 log CFU/mL) were observed in pasteurized milk with phage at 1h. Counts remained unchanged throughout storage while controls increased 1.18-1.93 log CFU/mL. Counts of the same strains in raw milk with phage did not differ from control but *Listeria* counts did not increase during storage in either case.

Significance: Phage was effective across a broad host range. Bacteriophage addition to pasteurized milk at an MOI of 10^6 reduced *L. monocytogenes* counts but were not effective in raw milk.

T12-01 Review of Select Historical Outbreak Investigations of *Salmonella* Infections Associated with Cashews and Cashew-Based Food Products

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Introduction: During 2013–2021, four U.S. salmonellosis outbreaks were associated with the consumption of cashews and cashew-based food products. These recurring outbreaks, combined with continued increases in sales of cashew products, underscore the need for identifying contributing factors that could inform prevention strategies.

Purpose: To share the results from salmonellosis outbreak investigations coordinated by the Food and Drug Administration (FDA) associated with cashews and cashew-based food products.

Methods: Historical investigation information for salmonellosis outbreaks associated with cashew and cashew-based food products from FDA and Centers for Disease Control and Prevention databases was reviewed. Investigational challenges, industry knowledge gaps, and firm compliance with the applicable regulatory requirements were considered and identified.

Results: Four Salmonella outbreaks were linked to cashews or cashew-based products, resulting in 74 illnesses and 14 hospitalizations. For three outbreaks, cashews were the suspected source; for one outbreak, cashews were the confirmed source. Products linked to outbreaks included cashew-based cheese alternatives (2 outbreaks), a cashew-based milk alternative, and raw cashews (1 outbreak each). Of the four identified outbreaks, implicated cashews were imported from one or a combination of three countries: Vietnam, Indonesia, and Côte d'Ivoire. Implicated firms that processed cashews had not established or implemented adequate sanitation controls or process controls (e.g., kill steps) or supply-chain programs for biological hazards, and there were general knowledge gaps regarding food safety practices and regulations. One firm was unregistered with FDA.

Significance: While definitive sources of contamination were not identified during these outbreak investigations, adequate controls for biological hazards were not implemented by the cashew importers and processors. Importer and processor compliance with existing regulations would alleviate some issues encountered during the outbreak investigations. Studies evaluating Salmonella prevalence and levels in the imported cashew supply and persistence during the production of cashew-based products could inform prevention strategies, such as targeted outreach to industry, to decrease the potential for foodborne illness associated with these products.

T12-02 Evaluation of Enverify™, A Microbial Positive Control, as a Hands-on Training Tool for Environmental Monitoring

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Introduction: There are many factors that can impact the results obtained from environmental monitoring of surfaces, one of which is the operator performing the sampling. Adequate operator training and qualification is of great importance to ensure that environmental contaminants are detected.

Purpose: Determine if Stratix Labs Enverify™, a viable microbe positive control, can be used to identify operator training opportunities that result in improved compliance with proper environmental sampling techniques.

Methods: A positive control was produced with a preserved quantity of *Escherichia coli* ATCC® 25922™ dried onto the transfer region of the device. 3M™ Swab-Sampler (1mL Letheen Broth) and 3M™ Petrifilm™ RAC plates were used for swabbing and enumeration of samples. The first experiment compared two swab techniques (n=4); technique 1 (minimal swab pressure, 1 pass of swab over device) and technique 2 (higher swab pressure and 3 passes over device). The second experiment involved four operators sampling the positive control devices to determine the percentage recovery of each operator (n=2).

Results: The positive control device had an average of 198 CFU/device. There was a significant difference in the CFU recovered ($P<0.001$) for the two sampling techniques. The average CFU/device recovered for technique 1 and technique 2 was 15 (7.6% recovery) and 93 (47.0% recovery) respectively. For experiment two, the average CFU/device for each of the 1-4 operators was 19 (9.6% recovery), 14 (7.1% recovery), 33 (16.7% recovery), and 44 (22.2% recovery) respectively. If a performance threshold were set at 10% recovery, this would indicate a training opportunity for operators 1 and 2.

Significance: A quantitative positive control may be a valuable training tool to drive compliance with proper environmental monitoring techniques and lead to improved detection of pathogens in food production.

T12-03 Protective Effects of Freshwater Microalgae Biomasses Toward Probiotic Cultures during Freeze-Drying, Storage, and *In Vitro* Digestion

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Introduction: Probiotics used in food production are generally supplied as freeze-dried powders. Polysaccharides and oligosaccharides may prevent dehydration and cell membrane rupture, increasing the stability of probiotics. Freshwater microalgae biomass contains large amounts of fibers and oligosaccharides. Little is known about the potential of microalgae biomass as probiotic protectants.

Purpose: To evaluate the protective potential of freshwater microalgae biomasses towards probiotics during freeze-drying, refrigerate storage and *in vitro* digestion.

Methods: Fresh *Lactobacillus acidophilus* 05 (La-5) and *Lacticaseibacillus casei* 01(Lc-1) suspensions (10 mL; 10 log CFU/mL) were mixed with 1 g of *Chlorella vulgaris* (CV) and *Lagerheimia longiseta* (LL) biomass. Samples were freeze-dried at 55 ± 2°C (vacuum pressure 138 μHg; 1 mm/h) for 40 h, sealed in polypropylene bags, and stored at 4°C for 4 months. Lyophilized probiotics without microalgae were used as controls. The resistance to gastrointestinal conditions was assessed through a semi-dynamic *in vitro digestion* system (stomach, duodenum, and ileum). After 1, 7, 15, 30, 60, 90, and 120 days probiotics were enumerated by plating on MRS agar with a detection limit of 1.5 log CFU/g.

Results: Counts of La-5 and Lc-1 freeze-dried with CV or LL did not decrease until 90 days of storage, while control samples decreased up to 2 log CFU/g in the same period. Over the 120 days of storage, Lc-1 freeze-dried with CV showed a lower reduction than LC-1 freeze-dried with LL. The opposite was observed for La-5. The *in vitro* digestion reduced 2 and 3 log CFU/g the La-5 freeze-dried with CV, and LL, respectively, and 1 and 2 log CFU/g the Lc-1 freeze-dried with CV, and LL, respectively.

Significance: Freshwater microalgae biomasses exert cryoprotective effects on probiotics and could increase their survival during *in vitro* digestion, however, the protection varies with the microalgae species and strain tested.

T12-04 Evaluation of *Listeria monocytogenes* Biofilms Attachment and Formation on Different Surfaces Using a CDC Biofilm Reactor

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial attachment to surfaces and biofilm formation can be influenced by the physicochemical properties of the environment, surface characteristics and microbial motility. *Listeria monocytogenes* can adapt, persist, and form biofilms in a variety of environments, therefore representing a challenge for food safety.

Purpose: The purpose of this study was to evaluate *L. monocytogenes* ability to attach and form biofilms on different food-contact surfaces.

Methods: Multi-strain *L. mono*cytogenes biofilms were grown in a CDC reactor for up to 96-hrs on wood, nylon, and polycarbonate coupons at 20± 2˚C. The initial attachment was measured determining surface properties. Wettability and hydrophobicity were characterized by static contact angle and interfacial tension measurements using the standard sessile drop technique with deionized water and formamide. Biofilm structures and degree of growth were evaluated by Laser Scanning Confocal Microscopy (LSCM) after 2, 24, and 96-hrs. All experiments were done in triplicate for each material, respectively, and the results were analyzed for statistical significance.

Results: Coupon material, incubation time, and type of solvent significantly affected the hydrophobicity, interfacial tension, and wetting properties of biofilms (P<0.05). Biofilm growth and incubation time resulted in increased hydrophobicity and contact angle, and in decreased surface energy and interfacial tension. The highest and lowest interfacial tensions were recorded as 16.65 N/m for the polycarbonate coupon (96-hr incubation) and 3.87 N/m for the wood coupon (2-hr incubation). Wood coupons presented a significantly strong biofilm attachment after 24-hrs, as compared to nylon and polycarbonate coupons (P<0.05). Overall nylon had the highest microbial count, followed by wood and polycarbonate (P< 0.05). Material surface characteristics, eg. services, and cracks influenced biofilm structure and formation on the different materials as highlighted by the different images obtained.

Significance: Understanding biofilms attachment properties and growth abilities on different surfaces may enhance sanitation strategies in food processing environments.

T12-05 Effect of Water Activity (aw) and pH on Thermal Tolerance of *Salmonella* in a Low-Moisture Energy Bar

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❖ ***Developing Scientist Entrant***

Introduction: Low moisture foods, especially those manufactured without a lethality step (e.g., energy bars), often rely on low water activity (a_w) and pH to prevent microbial growth and increase shelf life. However, at low a_w conditions, pathogens like *Salmonella* can exhibit increased thermal resistance and enhanced survival, thus creating potential food safety issues.

Purpose: To evaluate the effect of a_w and pH on the thermal resistance of *Salmonella* (D- and z-values) in a low moisture energy bar matrix.

Methods: A prune-based energy bar was inoculated (8.0 log CFU/g) with a five serovar cocktail of rifampicin-resistant *Salmonella*. Samples were prepared at combinations of 0.55 and 0.65 a_w, and 5.5 and 4.8 pH (adjusted using lactic acid). Ten gram samples (n=24) were sealed in aluminum bags and heated in an isothermal water bath (80, 70, 65 and 60°C). At predetermined times, bags were removed and placed in an ice water bath to prevent further thermal inactivation. Samples were then homogenized in buffered peptone water and plated on rifampicin-supplemented tryptic soy agar. Survivor curves were plotted and D- and z- values were calculated. Significance was determined using F-test and non-linear regression analysis.

Results: At the four temperatures tested, samples prepared at 0.65 a_w and 4.8 pH were the most thermally susceptible ($p<0.05$) compared to the other formulations. At 80°C (4.8 and 5.5 pH) and 70°C (4.8 pH), the 0.65 a_w samples had significantly lower thermal resistance compared to the 0.55 a_w samples. All other combinations tested showed no significant difference in thermal resistance. Water activity had a significant effect on z-values with results of 24.4°C and 24.5°C for 0.55 a_w samples, and 13.9°C and 15.7°C for 0.65 a_w at 5.5 and 4.8 pH, respectively.

Significance: Data suggests that decreasing a_w and increasing pH in the energy bar matrix increased the thermal resistance of *Salmonella*.

T12-06 Increased Heat Resistance and Transcriptome Sequencing of *Salmonella enterica* Serovar Enteritidis Seduced by Mild Heat

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Introduction: Microorganisms that subjected to mild heat (at sub-lethal temperatures) would adapt to the environment and perform higher heat resistance afterwards. This adaption process is widely known, but the degree of change (on heat resistance parameters) and molecular mechanisms behind this process were not fully understood.

Purpose: We aimed to understand how much and why the bacterial heat resistance increased at sub-lethal temperature.

Methods: *Salmonella enterica* Enteritidis PT 30 (*S.* Enteritidis) in tryptone soy broth was heated at 42 to 50°C for 5min to 3 h at either logarithmic (6 h after broth transfer) or stationary phase. The heat resistance parameters (D_{60} value) of treated cells was determined by isothermal treatments at 60°C via 2 ml eppendorf tubes. Transcriptome sequencing of *S.* Enteritidis after 42°C treatments was conducted in TruSeq PE Cluster Kit v30cB0t0HS (Illumia) by paired-end methods.

Results: Mild heat of *S.* Enteritidis at 42 to 50°C increased its heat resistance up to ≈ 300 %. For instance, the D_{60} of *S.* Enteritidis in control, treated at stationary 42°C for 2 h, and treated at stationary 50°C for 10 min were 1.1, 1.56, and 3.2 min, respectively. High-temperature-short-time could increase the bacterial heat resistance at same degree as low-temperature-long-time: the D_{60} of *S.* Enteritidis that treated at 45°C for 50 min (2.43 min) was statistically same as that treated at 50°C for 5 min (2.49 min). Large amount of genes were up- and down-regulated in *S.* Enteritidis heated in this study. For instance, 944 up- and 1124 down-regulated genes of *S.* Eneritidis were identified under the 42°C treatment at logarithmic stage than that at stationary stage.

Significance: We have quantified the degree of heat resistance increase of *S.* Enteritidis treated at 42-50°C, and identified the gene expressions under specific treatments. The data would benefit us to understand the effect of sub-lethal heat treatments on bacterial cells.

T12-07 Effect of Oil Exposure on Heat Resistance of *Salmonella enterica* Enteritidis PT 30 in Peanut Flour

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Introduction: In oil-rich low-moisture foods (LMFs) such as peanut-derived products, pathogens like *Salmonella* have been observed with high heat resistance and long-term survival. Oil has shown protective effect of bacteria in desiccated environment, yet how the oil protects bacteria were not clear.

Purpose: We aimed to understand when and how the oil in LMFs enhance bacterial heat resistance.

Methods: In this study, *Salmonella enterica* Enteritidis PT 30 (*S.* Enteritidis) was inoculated with 5 g peanut flour (PF, 49% fat) or defatted peanut flour (DPF) to exposure the cells in or without oil-protection in desiccation stage. The inoculated seeds (5 g) were then mixed with PF or DPF to get the inoculated samples of PF-PF, DPF-PF, and DPF-DPF. Heat resistance parameters (D-values) of *S.* Enteritidis were obtained via thermal-death-time cells and high-temperature water activity cells (that controlled water activity at elevated temperatures). At last, 49% of the peanut oil was added back to heat-treated

DPF-DPF samples prior to serial dilutions for estimating the effect of oil-protection on the recovery of heat injured cells (DPF-DPF-Oil).

Results: At constant moisture content, *S.* Enteritidis showed significant higher D_{85} values in PF-PF (45% fat adjusted, 50.5 ± 3.2 min) than that in DPF-PF (45% fat, 33.4 ± 2.5 min) and DPF-DPF (0% fat, 18.8 ± 1.5 min). At controlled water activity at 85°C (0.32 ± 0.02), the D_{85} values in PF-PF and DPF-PF became close (64.4 ± 1.8 min and 56.2 ± 3.1 min, respectively), but still are higher than that in DPF-DPF (42.1 ± 1.6 min). After the heat treatments, the *S.* Enteritids was observed with slightly higher D_{85} value (20.2 ± 2.2 min) in DPF-DPF-Oil than it was in DPF-DPF (18.8 ± 1.5 min).

Significance: We confirmed that oil protects the *S.* Enteritidis in peanut flour in all three stages: the desiccation process, the heat treatment, and the recovery of bacteria cells in plates.

T12-08 Validation of *E. faecium* as a Surrogate for *Salmonella* in Thermal Processing of Low Water Activity Dough

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Introduction: *Salmonella* outbreaks have been linked to flour, cocoa, and fruit, all of which can be found in a bakery setting. Salmonellae in a low a_w environment can be more heat resistant which means special consideration is required when applying a thermal treatment to a low a_w dough.

Purpose: In a laboratory setting, *Enterococcus faecium* and *Salmonella* were freeze dried on flour to validate *E. faecium* as a surrogate in the thermal processing of low a_w dough.

Methods: To validate *E. faecium* as a surrogate, three types of dough (cocoa, berry, and apple) were coinoculated with *E. faecium* and a 3-strain *Salmonella* cocktail (*S. typhimurium*, *S. mons*, and *S. enteritidis*). The inoculated doughs were formed into 45-gram bars and baked for 12 minutes at 200°C in a laboratory setting. Samples (n=3) were enumerated on tryptic soy agar overlayed with either xylose lysine deoxycholate agar or m-Enterococcus agar.

Results: Overall, *Salmonella* and *E. faecium* shared similar reductions in the low a_w doughs. For the cocoa dough, *Salmonella* and *E. faecium* reduction was 3.69±0.43 and 3.01±0.87, respectively. *Salmonella* and *E. faecium* reduction was 3.87±0.49 and 3.89±0.79, respectively, in the berry dough. In the apple dough, *Salmonella* and *E. faecium* reduction was 4.29±0.10 and 4.56±0.32, respectively. The a_w of the inoculated doughs were 0.50±0.024, 0.45±0.042, 0.49±0.020 for the cocoa, berry, and apple doughs, respectively.

Significance: The thermal treatment applied to the low a_w dough produced a >3 log CFU/g reduction of *Salmonella*. *E. faecium* was found to not be a conservative surrogate for *Salmonella* in low a_w dough.

T12-09 Improvement of Gaseous Chlorine Dioxide Inactivation of *Salmonella* spp. in Chia Seeds Assisted by Mild Heating and Its Effect during Ambient Storage

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❖ ***Developing Scientist Entrant***

Introduction: Chia seed powder has been implicated with a multistate foodborne outbreak in the United States in 2014 due to *Salmonella* contamination; antimicrobial intervention technologies for chia seeds are limited and needs further exploration.

Purpose: To investigate the efficacy of interaction of chlorine dioxide (ClO_2) gas with mild heat in inactivating *Salmonella enterica* in chia seeds

Methods: Chlorine dioxide gas was generated using a Minidox-M (Clordisys) system and the target gas concentration and RH was monitored and maintained the throughout the treatment. Chia seeds were inoculated with a cocktail of five *Salmonella enterica* serovars and equilibrated at a target a_w of 0.53. The gaseous treatment was performed at relative humidity of 80 and 90% and gas concentration of 5 mg/L and 3 mg/L respectively exposed for 2 h. The ClO_2 treated samples were subjected to mild heating at 40 and 60°C for 1 and 2 hours and further analyzed for *Salmonella* survival during storage at ambient conditions for one month.

Results: Gas treatment of chia seeds at 90% RH achieved significantly ($p<0.05$) higher log reduction (2.7 log) of *Salmonella* than 80%RH (1.8 log); additional log reductions of 1.0 and 0.8 log, respectively, were achieved by mild heat treatment at 60° C for 2 hours immediately after treatment. Storage study revealed that *Salmonella* population continued to decrease over time. ClO_2 treatment at 80% and 90% RH without mild heating achieved a cumulative log reduction of 2.4 and 3.6 after 3 days of storage. ClO_2 treatment followed by mild heating at 60°C for 2 h achieved 4.4 log reduction after 3 days of storage.

Significance: The results revealed that combination of antimicrobial ClO_2 gas and mild heat is effective in achieving desired log reduction in chia seeds after 3 days of storage. This treatment could be used by the industries to improve the safety of chia seeds.

T12-10 Metabolomics of Foodborne Pathogenic Fungi and Their Derivatives

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Introduction: High precision detection methods (such as metabolomics) required to profile pathogenic microorganisms and their metabolites in foods and other biological systems are gaining popularity as recent analytical approaches to determining the presence of microorganisms and their metabolic by-products in food systems.

Purpose: This study examined the different metabolomic approaches that are currently used to determine the presence of pathogenic fungi and their metabolites, especially mycotoxins, in foods to understand their characteristics and functionality.

Methods: Published works on the use of different mass spectrometric approaches and their tandem enhancements (such as Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Mass Spectrometry (LCMS) and Matrix Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry (MALDI-TOF/MS)), nuclear magnetic resonance (NMR, 2D-NMR and H-MNR) and other methods such as Fourier Transform Infra-red spectroscopy (FTIR) were reviewed to understand the presence of pathogenic fungi, their toxins and other metabolites in various food systems.

Results: Fungal pathogens and their metabolites have been considerably studied. Potentials for the production of biomarkers from the toxins of pathogenic fungi identified exist and should be explored for a more rapid detection. Also, other important metabolites, apart from mycotoxins were detected and could be linked to the functionality and metabolism of these pathogenic fungal strains.

Significance: The study expands the potentials for rapid detection of pathogenic fungi and their metabolites in foods and food systems.

T12-11 Phage Biocontrol of *Salmonella* and *E. coli* on Wheat Kernels

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Introduction: In the wake of recent outbreaks and recalls due to foodborne pathogens *Salmonella* and Shiga toxin-producing *E. coli* (STEC), the milling industry is grappling with and requires novel antimicrobial intervention strategies.

Purpose: We tested the efficacy of commercial bacteriophage preparations in reducing the STEC and *Salmonella* levels on soft wheat kernels in the laboratory and a commercial mill.

Methods: For laboratory evaluations, wheat kernels were inoculated with ~5.0 log CFU/g of low nutrient acclimated single strains of *Salmonella* Enteritidis or *E. coli* O157:H7 and treated with phages at a rate of 1×10^7 PFU/g or water. For the mill trial, 54,000 pounds of wheat was inoculated with ~5 log CFU/g of a non-STEC, non-pathogenic surrogate strain of *E. coli* and split into two groups of equal size. Untreated soft wheat was tempered with water. A

concentrated stock solution of phages was introduced into the tempering water stream just before water entered the Turbolizer at a set flow rate, yielding an application of 1×10^7 PFU/g. Soft wheat was then tempered for 6 h and samples were collected before and after tempering at multiple steps throughout the milling process.

Results: Phage application reduced *Salmonella* and EHEC levels by up to ~1.0-1.25 log ($p<0.01$) and ~2.0 log ($p<0.01$) after 15 min and 4 h of tempering, respectively in the bench tests. Phage treatment at the tempering step in the commercial mill run reduced the levels of surrogate *E. coli* ~3 log ($p<0.01$) from inoculated dirty wheat to straight grade and patent flour compared to ~1.0 log reduction observed for water tempering.

Significance: Phage preparation is only moderately effective against non-STEC strains used in the mill test; therefore, it is possible that the efficacy against pathogenic STEC strains would have been even more profound. Altogether, the data suggest that phage biocontrol is an effective and scalable intervention for managing *Salmonella* and STEC contamination in the milling industry.

T12-12 Effects of Caco-2 Cells or Indole/Mucin on *Clostridium perfringens* Toxin Production

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Introduction: *C. perfringens* enterotoxin(CPE) toxico-infections are the most frequent foodborne diseases reported in several countries. However, the impact of biotic and abiotic factors on toxin production, as well as pathogen-toxin-host interactions require further studies.

Purpose: To investigate the effect of *C. perfringens* and human intestinal Caco-2 cells proximity on CPE expression, and cytotoxic and mitochondrial effect of formed CPE on targeted Caco-2 cells. Besides, the effect of indole and mucin on CPE production and measurable toxicity was studied by comparing *C. perfringens* supernatant(CPS) and Δ*cpe* supernatant(CMS).

Methods: The CPE expression level with/without cells in different time points was detected by RT-PCR, and supernatants(SNs) were collected after 24h. Next, the cytotoxic effect of CPS was assessed using MTT and SRB; after determining the IC_{50} of CPS, four concentrations below IC_{50} were selected to investigate the impact on mitochondrial (dys)function on Caco-2 cells using Seahorse XF24. Subsequently, 400 mM indole and 240 mg/L mucin were added into mDSM and collected SNs after 24h. Finally, the concentration of CPE in both SNs was evaluated by adding 0.5% anti-natural toxin production serum(ATPS).

Results: The CPE expression level achieved the peak at 8h co-incubation with Caco-2 cells, while the highest was achieved at 12h in control samples. Even low doses of CPS(IC_{10}) influenced the mitochondrial oxidative phosphorylation. Meanwhile, CPE production with indole and mucin was induced 2 and 5.15 times higher than control samples. Compared to CPS, the IC_{50} of the total toxin was reduced 9.71, 19.01, 55.09-fold in CMS with control, indole and mucin. There was no significant difference in cell viability between CPS and CMS with or without ATPS.

Significance: Human intestinal epithelial cells, indole and mucin could promote toxins production of *C. perfringens*, amongst which CPE is the major toxin. It also provides a new direction for further research on how these factors trigger toxin production.

T13-01 Leveraging the Synergistic Effect of Organic Acids with Mild High Pressure Processing to Reduce *Salmonella* spp. in Pork Trim

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Introduction: Pork processors often use organic acid dipping for surface decontamination process, further reductions may be achieved if coupled with mild high pressure processing (HPP) treatment to maintain sensory quality of the meat.

Purpose: To investigate the effect of combining mild HPP treatment with USDA-approved organic acids dipping on the reduction of *Salmonella* spp., pH and color of raw pork trim.

Methods: Pork trim (80% fat) were inoculated with a five-strain cocktail of *Salmonella* spp. and incubated at room temperature for at least 1 h prior to dipping for 30 s in distilled water (Control) or the following organic acid: (a) lactic acid, 4% (w/v); (b) acetic acid, 4% (w/v); (c) citric acid, 4% (w/w); and (d) peroxyacetic acid, 400ppm (w/v). Afterwards, samples were stored at 4 °C for 24 h. Half the samples were treated with HPP at 300MPa for 3 min, and the other half were not treated with HPP. *Salmonella* counts were enumerated using a thin agar layer of xylose lysine deoxycholate topped with tryptic soy agar at the following timepoints: (a) immediately after dipping; (b) 24 h after dipping and prior to HPP; (c) 24 h after HPP treatment; and (d) 7 days after dipping treatment. A second set of non-inoculated samples were prepared and treated in similar manner and timepoint for pH and color analyses

Results: Overall, acid dipping and combination of HPP and acid dipping reduced *Salmonella* populations by 0.4–1.50 log and 2.08–4.50 log respectively. There was a significant ($P<0.05$) increase in *Salmonella* reduction when organic acids were combined with HPP treatment, except for PAA. pH and color of meat from combination treatment of HPP and acid did not significantly ($P>0.05$) different from acid dipping alone.

Significance: The combination of acid dipping with mild HPP treatment can be used to enhance *Salmonella* reduction in raw pork trim without detrimental effects on meat color.

T13-02 Cold Shock Proteins Promote Nisin Tolerance in *Listeria monocytogenes* through Modulation of Cell Envelope Modification Responses

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Introduction: To resist nisin, *Listeria monocytogenes* (*Lm*) deploys several defense mechanisms that are not yet fully understood. *Lm* possesses three genes (*cspA*, *cspB,* and *cspD*) encoding cold shock proteins (Csps) with regulatory roles in various stress responses.

Purpose: Evaluating the contribution of Csps to nisin tolerance in *Lm.*

Methods: *Lm* EGDe and its *csp* gene deletion mutants (n=7) were compared based on survival [7.5 ppm nisin or 10 ppm benzalkonium chloride (BC)], growth in Brain Heart Infusion media under nisin (5 ppm) or BC (1.25 ppm) stress, antibiotic sensitivity, and cell surface charge using the cytochrome c binding assay. Quantitative RT-PCR assays were used to compare gene expression under nisin stress. Data were analysed using t-tests and ANOVA.

Results: Without *csp* genes, a *Lm* Δ*cspABD* mutant displayed significantly ($P<0.05$) compromised growth under nisin stress (growth rate; Δ*cspABD*: 0.0028 vs 0.43 OD_{600}/hr: wild-type). Characterising single (Δ*cspA*, Δ*cspB,* and Δ*cspD*) and double (Δ*cspBD*, Δ*cspAD,* and Δ*cspAB*) *csp* gene deletion mutants revealed a hierarchy (*cspD*>*cspB*>*cspA*) of importance in *csp* gene contributions toward *Lm* nisin tolerance. Individual eliminations of either *cspA* or *cspB* increased nisin tolerance (2.4- and 1.5-fold, respectively), suggesting that their expression has inhibitory effects on CspD mediated nisin resistance. Gene expression analysis revealed that nisin induced *csp* expression upregulation (16.8- to 50.4-fold) and Csp deficiency significantly ($P<0.05$) altered expression (>2-fold) of genes encoding proteins involved in cell envelope modification such as DltA, MprF, RmlT, and penicillin-binding proteins. A Δ*cspABD* mutation induced a more electronegative cell surface charge increasing sensitivity to nisin and other cationic cell-envelope-targeting antimicrobials such as daptomycin and BC.

Significance: Csps contribute to *Lm* stress tolerance of food preservation-related antimicrobial peptides such as nisin through regulatory functions that contribute to modulation of cell envelope protective responses. Such knowledge can be harnessed in the development of better *Lm* control strategies.

T13-03 Antimicrobial Efficacy of the Combination of Organic Acid and Essential Oil in Chitosan Coating Against *Salmonella* and *Listeria* on Tomatoes

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Introduction: Tomato is a versatile vegetable on account of possessing numerous beneficial nutrients. However, multiple outbreaks and recalls associated with fresh tomatoes have been reported. Hence, it is necessary to apply antimicrobial treatments on tomatoes.
Purpose: This study was to investigate the antimicrobial efficacy of chitosan coating combined with organic acid and essential oil against foodborne pathogens on tomatoes.
Methods: Three groups of cherry tomatoes were used. Group 1 was surface-inoculated *Salmonella enterica* and *Listeria monocytogenes* and used for the reduction of the pathogens; Group 2 was used for the reduction of native bacteria (TPC) and yeasts&molds (Y&M), and Group 3 for quality analyses (texture, color, water loss). Tomatoes were surface-coated in two coating solutions: Solution 1 contained 1 % chitosan and 2% acetic, 2% lactic and 2% levulinic acids and labeled as "Chitosan"; Solution 2 contained Solution 1 plus 5% allyl isothiocyanate (AIT), labeled as "Chitosan+AIT". Tomatoes were placed in a container and stored at 10°C for 21 days.
Results: Chitosan and Chitosan+AIT treatments reduced *Salmonella* population from 3.65 to 1.28 and <0.70 log CFU/tomato on day 1, respectively. Both reduced the population to undefective levels (<0.70 log CFU/tomato) on day 2 through 21 days. Similarly, Chitosan+AIT treatments reduced more listeria populations than Chitosan treatment on day 1, but no differences between them after day 2. Chitosan and Chitosan+AIT, reduced TPC to a undefective level after 2 days and reduced the population of native Y&M to a undefective level after 1 day. There were no significant differences in texture and color among all samples for 21 days. Moldy appearance was only observed on control sample after 21 days.
Significance: This study suggests that it may be not necessary using both acids and essential oil for fresh tomatoes or we could reduce both concentration of acids and AIT in the coating solution.

T13-04 Effect of Peracetic Acid, Cultured Dextrose Fermentate, and Buffered Vinegar on *Salmonella* and Aerobic Bacteria in Raw Chicken Livers

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Introduction: Chicken livers are increasingly being used as ingredients in human and animal products. *Salmonella* remains a concern in raw poultry products where undercooking and cross contamination may lead to illness. Therefore, exploring alternatives to minimize risk is critical.
Purpose: This study aimed on evaluating the use of peracetic acid (PAA), cultured dextrose fermentate (CDF), and buffered vinegar (BV) in reducing *Salmonella* and aerobic bacteria in raw chicken livers.
Methods: Samples were inoculated with a five-strain cocktail of poultry-borne *Salmonella* to obtain 10^6 CFU/g. Samples were immersed for 90 s with agitation (40 rpm) in one of the following treatments: distilled water (control), 450 ppm PAA, 1.5 % CDF and 2.0 % BV, prior to storing at 4°C. Three independent replications were performed with two subsamples for each treatment and timepoint. A separate set of uninoculated chicken livers were subjected on the same treatments to measure aerobic plate count (APC). *Salmonella* and APC were monitored for 14 days through enumeration on XLD agar and 3M Petrifilm™, respectively. Data were analyzed using ANCOVA.
Results: PAA resulted in the highest log reduction at 0.65±0.12; yet there were no significant differences in the reductions for any treatments ($p > 0.05$). After 14 days, higher reductions were still observed for PAA, but the difference was only seen when compared to CDF and not to BV or the control. Although similar reductions ($p > 0.05$) were noted after 14 days except for CDF, *Salmonella* population was lowest in all timepoints when PAA was used. PAA and CDF were able to control growth of aerobic bacteria until day 3 while BV had controlled the growth up to 7 days.
Significance: This work provides information on chemical interventions that can reduce pathogens and the microbial load of chicken livers which could be used in the industry.

T13-05 Isolation and Characterization of Effective Bacteriophages Against Multiple Serovars of *Salmonella enterica*

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Introduction: *Salmonella* 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 percentage of antibiotic-resistant *Salmonella* has been growing rapidly and that warns as a serious healthcare threat specifically effectiveness of antibiotic therapy in gastrointestinal infection. Recent studies show that bacteriophage (phage) could be a promising alternative therapy to replace antibiotics use in animal farming as it can only infect a specific bacteria strain instead of animal cells.
Purpose: This study focuses on discovering novel bacteriophages by which infection with multiple serovars of *Salmonella enterica*(SE) can be controlled without interfering host gut health and growth.
Methods: Bacteriophages were isolated and characterized from collected samples from dairy/poultry farms. Lytic ability of isolated phages was tested against various serovars of SE using biphasic culture and spot test. The selected phage genomic DNAs were purified and used for next-generation genome sequencing (NGS). The bioinformatics of phages was compared and analyzed by the BLAST and relative analysis software tools.
Results: A new *Salmonella* bacteriophage SE/1252 was confirmed, which belongs to the genus *Duplodnaviria* in the order *Myoviridae* family. The complete genome of phage SE/1252 consists of dsDNA of 244,421 bp in size, with G+C content 48.51% and is 97% to relate to *Salmonella* bacteriophage SPN3US, Enterobacteria phage SEGD1, and Proteus phage 7. The lytic ability of this phage showed highly effective against several SE serovars, including Typhimurium, Enteritidis, Newport, Pullorum, and Gallinarum.
Significance: These findings indicate that this novel bacteriophage SE/1252 could be a promising antimicrobial for controlling *Salmonella* colonization in animals.

T13-06 Isolation and Characterization *Escherichia coli*-Specific Phages Infecting Indigenous Antibiotic-Resistant *E. coli* Isolates

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Introduction: Antibiotic-resistant (AR) *E. coli* outbreaks associated with fresh produce have increased. Phage has potential for controlling foodborne pathogens due to its excellent specificity. Therefore, searching host strain is necessarily required for isolation of appropriate target-specific phage that can selectively lyse AR *E. coli* in fresh produce and agricultural environment.
Purpose: The purpose of this study was to isolate AR *E. coli* as an indigenous host and AR *E. coli*-specific (EC) phage as a novel biocontrol agent.
Methods: *E. coli* was isolated and confirmed from hundreds of fresh produces and the agricultural environment by Endo and EMB agar, IMViC test, 16S rRNA sequencing, and API 20E identification. Antibiotic resistance of confirmed *E. coli* was investigated by disc diffusion method against sixteen antibiotics. Each AR *E. coli* was incubated with each fresh produce and agricultural environment for phage isolation. After purification, the specificity of each phage

was investigated against forty-five AR and non-AR *E. coli* using dot and plaque assay. The bactericidal effect was determined by incubating host with phage at MOIs of 0.01 to 10 for measuring bacterial number with plate count method.

Results: Thirty-seven *E. coli* was confirmed from fresh produces and the agricultural environment. Eleven *E. coli* were resistant at least one antibiotic and even eight strains had a multidrug resistance property. Two phages infecting AR *E. coli* (CMD05061) were isolated and purified with the final concentration of 4.3×10^{10} and 1.4×10^{11} PFU/mL from wastewater, respectively. EC1 phage infected three non-AR *E. coli* and three AR *E. coli* whereas EC2 phage infected eight non-AR *E. coli* and one AR *E. coli*. EC1 and EC2 phages exhibited 3.9 and 4.5 log reduction of its host at MOI of 0.1 and 0.01, respectively.

Significance: This study demonstrated that two EC phages were successfully isolated and exhibited excellent efficiency in bactericidal effect as a promising biocontrol agent.

T13-07 Morphological, Biological, and Genomic Characterization of a Newly Isolated Phage Infecting *Pectobacterium carotovorum* subsp. *carotovorum*

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Introduction: Agricultural antibiotics are widely used to control *Pectobacterium carotovorum* subsp. *carotovorum* (PCC) causing soft rot in several fresh produces including napa cabbage, lettuce, and tomato. Phage has emerged as an eco-friendly alternative to agricultural antibiotics. Exploring of phage for use in fresh produce has attracted considerable interest.

Purpose: The purpose of this study was to isolate and characterize a PCC-specific phage for the verification of its efficiency and safety.

Methods: Phage isolation was conducted by incubating PCC suspension with each fresh produce and its soil. Isolated phage was purified using CsCl-gradient ultracentrifugation and its final concentration was confirmed. The morphology of PCC-specific phage was observed by TEM. Specificity was investigated against thirteen PCC and eight non-PCC phytopathogens by dot assay. Its lytic efficiency was evaluated by incubating it with PCC suspension at various MOIs (0.01, 0.1, 1.0, and 10) and then monitoring the bacterial growth by LB plate count method every 3 h for 27 h. DNA of PCC-specific phage was extracted for WGS analysis. The open reading frames (ORFs) predicted by BLAST was compared with ResFinder 2.1, virulence factor, and allergen database.

Results: PCC-specific phage was isolated from tomato soil and purified with the final concentration of $6.6\text{x}10^{11}$ PFU/mL. TEM revealed that PCC-specific phage consisted of a head and a contractile tail. It infected specifically seven out of thirteen PCC, and it didn't show any cross-reactivity with non-PCC. Moreover, it inhibited the growth of PCC up to 21 h regardless of MOI with a great efficiency. PCC-specific phage has a genome size of 148,166 bp with GC content of 50.55% containing 299 ORFs and 11 tRNA genes. No lysogeny-related, antibiotic resistance, virulence, or allergen genes were found in the PCC-specific phage.

Significance: PCC-specific phage was successfully isolated and the biological and genomic characterizations were confirmed as an efficient and safe antibiotic alternative.

T13-08 Isolation and Evaluation of Bacteriophages in Lactic Acid Bacteria (LAB) and Acetic Acid Bacteria (AAB) Spoilage of Fermented Beverages

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Introduction: Lactic acid bacteria (LAB) and acetic acid bacteria (AAB) has been associated with beer and wine spoilage. Bacteriophages could be a useful strategy for its control.

Purpose: The objective of this study was to isolate and evaluate the antimicrobial activity of bacteriophages on LAB and AAB with the spoilage potential of beer and wine.

Methods: To obtain strains of LAB, AAB and bacteriophages, three samplings in wine cellars and five in craft beer producers in Querétaro, Mexico were carried out. Bacteria were confirmed as LAB and AAB by growth in selective media and catalase, oxidase and Gram stain tests. The effect of isolated bacteriophages (1×10^5 PFU/ ml) on bacteria ($1\text{x}10^8$ CFU/ml) was evaluated by means of spot test on double layer agar. The most efficient phages of LAB and AAB were tested with three replicates of each pair bacteriophage/bacteria on suspended cells. An ANOVA and Dunnett's test was applied to validate the antimicrobial effect.

Results: Thirteen strains of LAB, thirteen of AAB, and eight bacteriophages were isolated from the production environment of craft beer and wine. Two bacteriophages showed the most significant reduction in suspended cells against one LAB strain and one AAB strain. The statistical analysis showed significant differences ($p<0.05$) in the cells inoculated with bacteriophages with respect to the control without bacteriophage. The reduction of LAB cells was 4±0.82 log CFU/ml and 3±0.47 log CFU/ml for the AAB strain.

Significance: Bacteriophages could be used as a biopreservative in fermented beverages.

Poster Abstracts

P1-01 Killing *Salmonella* in Pet Food

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Introduction: Raw pet food manufacturers are presented with the challenge to produce raw-meat-based products that are free from pathogens. Thus, the raw pet food industry constantly seeks new alternatives that will ensure safer products.
Purpose: To determine if bacteriophage technology can lead to *Salmonella* load reduction when applied on raw ground pet food containing different meats and vegetables.
Methods: Frozen raw ground pet food (BARF®, MN, USA) was thawed before use. Samples of 25 grams were contaminated with a streptomycin-resistant *Salmonella* Enteritidis at a level of 2×10^4 CFU/g using 2 µL/g of inoculum. All treatments were conducted in duplicate per treatment group and time point. The bacteriophage was diluted in tap water at 2.5% (5×10^7 PFU/g) or 5% (10^8 PFU/g) solution and was applied at 10 µL/g (1% pickup) and homogeneously mixed. Control samples were treated with tap water. Post-treatment samples were stored at 4 ºC for 24 hours until *Salmonella* enumeration. The data presented are mean values of three separate individual experiments, analyzed with a two-way ANOVA (Sidak's multiple comparisons test).
Results: After 24 hours, bacterial concentration was considerably reduced when using either of the phage concentrations. Application of 1×10^8 PFU/g resulted in a 0.7 log bacterial reduction ($P < 0.05$), while $5x10^7$ PFU/g gave a 0.6-log total bacterial reduction ($P < 0.05$), when compared to control.
Significance: In conclusion, *Salmonella-specific* bacteriophages have proven successful as a green, friendly and convenient tool in reducing *Salmonella* by 0.7 logs (~79%) in 24 hours in increasingly popular raw-meat-based pet food. Thus, bacteriophages represent an effective and safe intervention that pet food manufacturers can employ to ensure the overall safety of the final products.

P1-02 Whole-Genome Sequencing Supports Pathogen Investigations of Animal Food

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Introduction: The U.S. Food and Drug Administration's Center for Veterinary Medicine (FDA/CVM) monitors the safety of animal food including pet food and animal feed through its animal food compliance program. Whole-genome sequencing (WGS) offers a high-resolution understanding of the relatedness of foodborne pathogens from various sources.
Purpose: This report summarizes the use of WGS to facilitate the investigation of animal food contamination events for select animal food samples collected and analyzed by FDA and state partners between 2018 and 2021.
Methods: WGS was run for all major foodborne pathogens recovered from animal food samples, primarily *Salmonella* and *Listeria monocytogenes*, using the GenomeTrakr protocol and uploaded to the National Center for Biotechnology Information (NCBI). The NCBI cluster consisting of environmental and/or clinical isolates was downloaded, and a pipeline developed by the FDA's Center for Food Safety and Applied Nutrition (CFSAN) was used to perform single nucleotide polymorphism (SNP) analysis and phylogenetic tree building. Interpretation was based on previously established criteria for regulatory applications and outbreak investigations.
Results: Forty-one investigations occurred from August 2018 to December 2021, implicating 237 animal foodborne isolates, dominated by *Salmonella* (*n* = 142) and *L. monocytogenes* (*n* = 65) and followed by *Escherichia coli* (*n* = 24), and *Campylobacter jejuni* (*n* = 6). The animal food samples implicated were almost exclusively from pet food and pet food facilities, predominately raw pet food (ingredients and finished ones). Linkages (i.e., ≤ 20 SNP differences) between these isolates and recent clinical isolates were identified in over half of the cases (*n* = 23).
Significance: These data demonstrate the utility of WGS in supporting animal food investigations. The high resolution of WGS greatly facilitates these animal food contamination event investigations. WGS provides a necessary tool to aid in safeguarding our nation's animal food supply and protect public health and animal health.

P1-03 Effect of a Commercial Bacteriophage Preparation Against Dairy-Relevant *Salmonella enterica* Serovars in Raw and Pasteurized Milk

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Introduction: Non-typhoidal *Salmonella* are contaminants of raw milk and outbreaks of salmonellosis from consumption of contaminated milk and milk products continue to occur. Bacteriophages are a potential control within the Standards of Identity for these products.
Purpose: This study characterized the host range of a commercial bacteriophage preparation against nine dairy-relevant serovars of *Salmonella enterica* and the antimicrobial effect in raw and pasteurized milk.
Methods: Titers were determined using a standard agar overlay assay. Inhibitory phage/pathogen ratios (PFU/CFU) or multiplicities of infection (MOI) were determined by a microtiter plate-based assay with doubling dilutions of phage added to wells containing 2 log CFU/mL of *Salmonella* in tryptic soy broth and incubated at 37°C for 24h. Raw and pasteurized milk (25 mL) was inoculated with 8 log PFU/mL of phage and 2 log CFU/mL of selected serovars (n=3) and incubated at 7°C. Milk without phage served as the control. *Salmonella* were enumerated at 1, 4, and 24h and every 48h up to 168h. All experiments were repeated at least three times.
Results: Phage titers using Dublin were higher than all others ($P<0.025$) except Mbandaka and Typhimurium. Typhimurium had the lowest MOI (1.84 log PFU/CFU) compared to all others ($P<0.001$). Meunster had the highest MOI (5.97 log PFU/CFU), which was similar to Cerro and Mbandaka. Significant reductions in Cerro (0.79), Typhimurium (1.97), and Montevideo (1.29 log CFU/mL) were observed in pasteurized milk at 1h. Counts in treatments remained unchanged throughout storage while controls increased 0.72-1.04 log CFU/mL. Slightly higher reductions occurred in raw milk (0.85, 2.05, and 2.04 log CFU/mL, respectively) but control counts decreased (0.32-0.87 log CFU/mL) so the reduction in Cerro was not significant.
Significance: Results suggest that determination of MOI is a better screening tool than titers. Bacteriophage addition to milk can rapidly reduce *Salmonella* without regrowth during storage, but the effect is serovar dependent.

P1-04 Moved to Technical

P1-05 Thermal Inactivation of *Listeria monocytogenes* in Vegan Dairy Analog Products as a Function of pH and Water Activity

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Introduction: Plant-based dairy analogs is a rapidly expanding market, but little is known about the effect of different matrices on pathogen thermal inactivation rate. Although these products have similar functionality as process or cream cheese, the humectants and acidity are different.

Purpose: To determine D and z-values for *Listeria monocytogenes* in model vegan dairy analog (process cheese or cream cheese equivalent) products.

Methods: Four different treatments (2x2 block design; pH - 4.8 or 5.8; a_w - 0.98 or 0.95) of a model vegan dairy analog were prepared and inoculated with 8-log CFU/g of *L. monocytogenes* (5-strain mixture). One-gram samples were flattened into a thin film (0.5-1.0 mm thickness) in a moisture-impermeable pouch and vacuum-packaged. Samples were submerged in pre-heated water baths to achieve one of five temperatures: 57.2, 60.0, 62.8, 65.6, 68.3°C. At predefined time intervals, duplicate samples were removed from heating, immediately chilled to ≤4°C, and serial dilutions enumerated for surviving *L. monocytogenes* by plating on Modified Oxford agar overlaid with thin layer Tryptic Soy Agar. D-values for *L. monocytogenes* were calculated from survival data using linear regressions of log CFU/g over time at a given temperature (duplicate trials). Log of D-values were plotted for each temperature and a z-value was calculated from the inverse slope of the linear regression of those data points.

Results: As expected, D-values were consistently shorter at both pH 4.8 treatments compared to the pH 5.8 treatments and a_w 0.95 had longer D-values than 0.98. D-values at reference temperature 60°C were 3.12, 6.17, 4.47 and 11.40 minutes for treatments a_w 0.98/pH 4.8, a_w 0.98/pH 5.8, a_w 0.95/pH 4.8, and a_w 0.95/pH 5.8, respectively and z-values were 7.83, 6.33, 8.17 and 8.17°C, respectively.

Significance: These results confirm variability in thermal inactivation rates in plant-based dairy analogs depending on pH/a_w combinations.

P1-06 Viability of *Lacticaseibacillus rhamnosus* and *Bifidobacterium* Species in Synbiotic Yoghurt Incorporating Inulin and Lactulose

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Introduction: As a popular dairy product, yoghurt has proven to be an effective delivery food for beneficial probiotic bacteria. However, the health benefits of such probiotic foods are often limited by the survival and viability of probiotic bacteria during manufacturing and cold storage.

Purpose: To investigate the effect of lactulose and inulin on the viability and survival of *Lacticaseibacillus rhamnosus* and *Bifidobacterium* species in synbiotic yoghurt

Methods: Synbiotic yoghurt samples containing inulin (2%) and lactulose (4%) were prepared using raw milk (2.5% (m/v fat content, 10.7% total solids), and a starch-based stabilizer (2.5% m/v). Probiotic organisms, *L. rhamnosus*, and *B. bifidum* were added to each fermentation together with the starter. Probiotic viability and physicochemical characteristics of the synbiotic yoghurt samples were monitored over a 12-day shelf-life.

Results: There were no significant differences in the physicochemical characteristics of yoghurt samples in the presence of inulin and lactulose. The addition of the prebiotics did not cause a significant effect on the viability of *L. rhamnosus*, but a healthy level of the organism (7.5 - 8.0 Log CFU/g) was sustained in the yoghurt samples over the storage period with a viability proportion index (VPI) of 0.95 - 1.00. A progressive decline in *Bifidobacterium* spp viability was observed in all yoghurt samples incorporating both lactulose and inulin. The counts declined to around 3.0 - 3.5 Log CFU/g representing a VPI of 0.5 - 0.58. Approaches for improving the viability survival ability of *Bifidobacterium* spp are part of ongoing experiments in our research lab. While both inulin and lactulose did not show a direct effect in terms of the ability to stimulate increased levels of *L. rhamnosus* and *Bifidobacterium spp,* in the yoghurt, the influence on the metabolite profile of the yoghurt is a subject of on-going investigation.

Significance: The therapeutic benefit of probiotics depends on the sustained viability of the organisms in the carrier food system.

P1-07 Evaluation of an Alternative Rapid Method as Compared to the GB Method for Enumeration of Lactic Acid Bacteria in Yogurt-Based Drinks

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Introduction: Standards in China require that the concentration of *Lactobacillus casei* in yogurt-based dairy drinks should be higher than 10^7 CFU/mL when the finished products are released. Following the China National Standard (GB) agar method,the growth and enumeration of *Lactobacillus* takes three days and requires special equipment to provide the anaerobic environment .

Purpose: Comparative study on the enumeration of *Lactobacillus* using the 3M™ Petrifilm™ Lactic Acid Bacteria (LAB) Plate and GB 4789.35-2016 method.

Methods: Natural yogurt-based dairy drink samples (n=80) were analyzed with 3M Petrifilm LAB Count Plate and GB 4789.35-2016. The 3M Petrifilm LAB Plates were incubated at 36°C for 48h, and the Man Rogosa Sharpe (MRS) agar plates of the GB method were incubated in an anaerobic box at 36°C for 72h. A paired-t test was used to determine statistical differences (P>0.05).

Results: No statistical differences were observed between the 3M Petrifilm plate method and the GB method when enumerating *Lactobacillus* colonies in yogurt-based dairy drinks. The absolute value of the Mean $\log_{10}$ difference was 0.001, and the P-value obtained was 0.92.

Significance: The alternative method enabled reliable and rapid enumeration of *Lactobacillus* in yogurt-based dairy drink samples as compared to the reference GB method. Moreover, the Petrifilm LAB plate was designed to create an anaerobic environment during incubation, thus simplifying the testing process.

P1-08 Evaluation of a Microbial ATP Bioluminescence-Based Method as a Rapid Detection System for Testing Commercial Sterility in Ultra High Temperature (UHT) Fermented Milk

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Introduction: Fluid milk is commercially sterilized by UHT methods to ensure the product can be safely transported and stored without refrigeration. In China, commercial sterility is commonly evaluated utilizing pH and/or agar which requires a 10-day sample incubation period. The use of rapid methods for commercial sterility testing can provide test results in 2-3 days, enabling faster release of milk products to consumers. In China shelf-stable fermented milk is popular but there are no rapid test methods qualified to assess commercial sterility because of the high background ATP residual and viscosity resulting from the fermentation process.

Purpose: To evaluate a microbial ATP-bioluminescence-based method as a rapid test for commercial sterility in fermented milk.

Methods: Different test strategies were evaluated to decrease the high background ATP values for use with microbial ATP-bioluminescence based method, including ATPase volume and reaction time, samples mixing model, and sample dilution ratio. A total of 18 UHT-fermented milk products from different manufacturing locations were tested to determine threshold ATP values for the ATP-bioluminescence based method. Artificially contaminated with yeast (range of approximately 1000CFU/container) and naturally contaminated fermented products were also tested.

Results: Basal ATP levels in uncontaminated fermented milk were decreased from original 700-1000RLU to 40-50RLU level. The threshold ATP limit value was decreased from 4500RLU to 100-200RL which enabled differentiation between contaminated fermented milk. Detection of natural contamination in fermented milk and in samples artificially contaminated was achieved using an ATP-bioluminescence based test method that required 48h of incubation instead of 10 days.

Significance: The microbial ATP-bioluminescence may be applicable as an alternative method for rapid commercial sterility testing of UHT fermented milk.

P1-09 Matrix Validation of 125 g Nonfat Dry Milk for *Listeria* Using the Hygiena™ BAX® System

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Introduction: Wet processing is frequently used during the production of dry dairy products and powders. Most dairy powders are hydroscopic and will readily take up moisture from the surrounding environment, which can lead to the survival and growth of *Listeria* or other pathogens.
Purpose: The purpose of this study was to validate the performance of a PCR-based method compared to the U.S. FDA BAM reference method for the detection of *Listeria* in nonfat dry milk.
Methods: Nonfat dry milk was inoculated with *Listeria monocytogenes* at levels expected to produce low (0.2-2 CFU/test portion) and high (5 CFU/test portion) spikes after a 2-week equilibration at 20-25°C. Unpaired samples were then prepared for either the test method (125 g, n=30) or the FDA BAM method (25 g, n=30) using different enrichment protocols. Test method samples were analyzed with 2 real-time and 2 standard PCR assays and all samples were confirmed according to the procedures in the FDA BAM Chapter 10.
Results: Test method samples returned fractional positive results for 6/20 low-spiked samples and all positives for 5/5 high-spiked samples analyzed by all PCR assays. All PCR results matched culture with 100% sensitivity and 100% specificity. When compared to the reference method, the difference in the probability of detection (dPOD) indicated no significant differences.
Significance: Overall, the results between the BAX® System method and the FDA BAM reference method were indistinguishable, allowing dairy manufacturers to utilize a rapid and reliable PCR method for screening *Listeria*.

P1-10 Detection of *Salmonella* and *Listeria* in Large Test Portions of Mexican Style Cheeses Using the Hygiena™ BAX® System

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Introduction: Mexican style cheeses continue to be a source of foodborne illness. Their soft texture and high moisture content make them especially vulnerable to contamination by pathogens, including *Salmonella* and *Listeria.*
Purpose: The performance of a PCR-based method was assessed in comparison to the FDA BAM reference method for the detection of *Salmonella* and *Listeria* in separate matrix studies for 2 Mexican style cheeses.
Methods: Two matrix validations for Queso Fresco, and two matrix verifications for Queso Blanco were prepared by inoculating each product separately with *Salmonella* or *L. monocytogenes* at a fractionally low level (0.2-2 CFU/test portion) and a high level (5-10 CFU/test portion). After 48-72 hours of stabilization, unpaired test portions for *Salmonella* (375 g) and *L. monocytogenes* (125 g) were enriched in BPW and 24 LEB Complete, respectively. Test portions for the FDA BAM reference (25 g) were enriched according to their respective chapter methods.
Results: Test method samples were assayed with real-time and standard PCR kits demonstrating no false positives or false negatives for any matrix-organism combination. Using the probability of detection (POD) to compare the test and reference method, a significant difference was observed for *Salmonella* with the test method recovering significantly more positives while there were no significant differences for *Listeria*.
Significance: These results demonstrate that the BAX® System method is specific, sensitive, and accurate for the detection of *Salmonella* and *Listeria* in Mexican style cheeses.

P1-11 Detection of Coliforms in Dairy Starter Cultures Using the Hygiena™ Microsnap™ Coliform Assay

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Introduction: The MicroSnap™ Coliform assay is a rapid bioluminogenic test for detection and enumeration of coliforms from products within 6-8 hrs, based upon the detection of the indicator enzyme, beta-Galactosidase expressed by coliform bacteria.
Purpose: To validate the assay for detection of coliforms from seven commercial dairy starter cultures.
Methods: Detection assay for background beta-Galactosidase was performed by diluting the starter cultures and taking readings from the incubated devices set at 37°C for 0 hrs, 6 hrs, 8 hrs, and 24 hrs. The limit of detection (LoD) of coliforms in the starter cultures were performed by spiking the starter cultures with four overnight cultures of *Escherichia coli*, *Citrobacter fruendii*, *Klebsiella oxytoca* and *Enterobacter cloacae.* For each culture, the detection assay was run on selected dilutions and compared to standard plate counts with readings taken at 24 hrs.
Results: There was no background enzymatic signal from any of the starter cultures. Growth was evident in the 1:10 product dilution and all spiked dilutions showed detectable growth as early as 6 hours of incubation. The LoD study showed that at 6 hours the average LoD was 857 CFU (range of 50 to 1,480 CFU); at 8 hours, the LoD was 48 CFU (range of 10 to 90 CFU); and at 24 hours or less, LoD was 17 CFU (range of presence/absence to 50 CFU). The fractionality shows that the CFU added may go through attrition and be lower when growth and detection occurs; hence, the true LoD will be even lower.
Significance: The technology demonstrates that coliform detection from dairy starter cultures is feasible from the product within 8 hrs. These results demonstrates that the technology can be used as a simple and accurate method to measure coliforms from starter cultures within the 8 hr time frame when compared to traditional methods which take 24-48 hrs.

P1-12 Detection of Coliforms and *E. coli* in String Cheese and Cream Cheese Using Hygiena™ Microsnap™

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Introduction: MicroSnap™ Coliform and *E. coli* are rapid bioluminogenic tests for detection and enumeration of coliforms and *E. coli* in products that reduces time to results significantly compared to standard plating methods.
Purpose: To validate the assays for detection of coliforms and *E. coli* from commercial cream cheese and string cheese.
Methods: The cheese products were inoculated with a cocktail of coliform species diluted in buffered peptone water (BPW). The cocktail included *Escherichia coli*, *Citrobacter fruendii*, *Klebsiella oxytoca* and *Enterobacter*. Samples were analyzed via MicroSnap™ at 0 hrs, 6 hrs, 8 hrs, and 24 hrs to determine if the matrix and starter culture would interfere with the assay, background signal, and to determine the level of detection at each timepoint. Samples were plated on selective agar to confirm growth of the target organisms.
Results: There was no background enzymatic signal from cream cheese products whereas string cheese products had a weak background signal when tested with MicroSnap™ Coliform and *E. coli* detection devices. Background signal remained constant over time indicating that the signal was enzymatic. Limit of detection (LoD) was calculated by subtracting the background signal from positive detection values. For cream cheese, the LoD was 4,450 CFU for coliforms and 7,500 CFU for *E. coli* at 6 hrs, and it was less than 100 CFU for both coliform and *E. coli* at 8 hrs. For string cheese, the LoD was 4,300 CFU for coliforms and 1,500 CFU for *E. coli* at 6 hrs, and 35 CFU for coliforms and 155 CFU for *E. coli* at 8 hrs, respectively. LoD improved further at 24 hr timepoints.
Significance: MicroSnap™ technology can be used as a simple, rapid, accurate method to measure coliforms and *E. coli* from cheese products within an 8 hr time frame as compared to traditional methods which take 24-48 hrs.

P1-13 Prevalence and Antibiotic Resistance Pattern of *Campylobacter* in the Dairy Farms of Maryland-D.C. Area

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Introduction: *Campylobacter* is one of the prominent causative agents of diarrheal diseases in the US. Dairy cattle are considered one of the major sources of *Campylobacter* along with poultry. Thus, dairy cattle and farm environment play a significant role in transmission of this bacterial pathogens to the environment and ultimately to human.

Purpose: The objective of this study was to determine the prevalence of *Campylobacter* in dairy cattle and farm ecosystems in Maryland-DC area and determine their drug resistance pattern.

Methods: A total 882 samples, including water, feces, bedding, soil, feed, grass, compost, and lagoon water were collected from three different dairy farms. Samples were inoculated in enrichment media and cultured on Karmali agar for isolation of presumptively positive colonies. Isolates were confirmed by PCR and antibiotic resistance pattern were performed using agar dilution method following the Clinical and Laboratory Standard Institute (CLSI) recommendation.

Results: Among the total samples, 19.16% (169) were confirmed as *Campylobacter* by PCR. The confirmed isolates were distributed among water (10.65%), feces (5.92%), bedding (14.2%), soil (14.2%), feed (5.91%), grass (3.55%), compost (28.4%), and lagoon water (17.16%) samples. All isolates were resistant to at least one of the antibiotics tested. Among the antibiotics tested, streptomycin was recognized as the most effective, 48.67% isolates were sensitive. On the other hand, 94.67% isolates were resistant to tetracycline which was recognized as the least effective antibiotic.

Significance: Prevalence of *Campylobacter* and the antibiotic-resistant pattern shows that better farming practices are needed to control *Campylobacter* transmission and growing antibiotic resistant pattern in dairy farms of Maryland-DC area.

P1-14 Understanding Dairy Goat Farmer Food Safety Perceptions While Assessing the Microbial Profile of Raw Goat Milk on Small Mississippi Farms

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❖ ***Developing Scientist Entrant***

Introduction: Currently, 30 states allow the sale of raw milk, including Mississippi, which imposes strict regulations that limit sales to very small farmers. While this poses a potential threat to consumers, the direct ban of raw products would be detrimental for local farmers who lack the resources needed to pasteurize.

Purpose: The objectives of the study were to assess the microbial profile of raw goat milk and to survey farmers' practices to identify deficiencies and opportunities for improvement in safety.

Methods: Raw milk samples (n=30) were collected by farmers using their everyday milking techniques and enumerated on Petrifilm™ for various pathogenic and indicator bacterium. For collecting data on farm management and milk handling practices, a knowledge-based survey was designed through Qualtrics and distributed through social media groups targeting Mississippi dairy goat producers. Statistical analysis was conducted through SPSS.

Results: *Staphylococcus aureus* had a greater presence but aligned with the drying-off period for many goat producers. No farms displayed any *Salmonella* spp. or *Listeria monocytogenes* presence. *E. coli*, *Enterobacteriaceae,* and coliforms were observed in 4, 10, and 12 samples, respectively. The survey portion of the study displayed our farmers' needs for more educational opportunities on safe milk handling, with emphasis on the effectiveness of handwashing, as only 44.8% of surveyed producers wash their hands between animals during the milking process. Results showed that 80.8% of producers would invest more in their farms if they had more freedom to advertise and sell their products thus creating a strategic opportunity for extension outreach to help with market expansion and sustainability.

Significance: This data is significant to Mississippi dairy goat producers and other producers as further research will help improve their market conditions and overall food safety. Additional research into lactation stage and milk handling techniques could help develop statewide extension training programs to promote safe milk handling practices.

P1-15 *Bacillus mosaicus* Contamination in Milk Processed with Microfiltration

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Introduction: *Bacillus mosaicus* is a recently described genomospecies that includes emetic *B. cereus*. While Bacillus contamination of fluid milk is typically assumed to originate from raw materials, there is increasing evidence that Bacillus and other sporeformers may also be introduced into fluid milk from environmental sources in processing plants and processing equipment.

Purpose: The purpose of this case study was to test the hypothesis that *Bacillus mosaicus* identified in microfiltered fluid milk originated from the processing environment.

Methods: Raw milk was subjected to microfiltration with a 1.2 µm membrane before HTST pasteurization at 75°C for 20 seconds. Isolates were obtained from samples stored at 3, 6.5, and 10°C at the end of tested shelf-life (63 days) from 7 different batches of milk. Identification of isolates was performed by PCR amplification and subsequent sequencing of a 632-nucleotide fragment of the *rpoB* gene. Following the identification of *B. mosaicus* as the most frequently isolated species from the milk described above, to test the microfiltration unit for Bacillus persisting after sanitation, UHT milk was circulated in the microfiltration unit for 30-minutes before samples were collected from the permeate and retentate valves.

Results: Of the isolates gathered from the extended shelf-life milk, 32 of 38 (84%) isolates were characterized into 4 different *rpoB* allelic types belonging to the *B. mosaicus* group. *B. mosaicus* also represented 6 of 11 isolates (55%) obtained from the UHT milk that was circulated through the microfiltration unit after sanitation. Allelic types 59 and 194 were isolated from both the extended shelf-life milk and UHT milk.

Significance: Our findings further support that processing plant environment and equipment must be considered as sources of sporeforming spoilage organisms and pathogens.

P1-16 Matrix Extension of M-a-98 Listed Phosphatase Methods to Testing Eggnog

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Introduction: Milk pasteurization is a process control in microbiological risk management. Alkaline Phosphatase (AP) is an enzyme in raw milk with heat-time destruction that mirrors pasteurization specifications. AP absence retrospectively confirms dairy products' pasteurization effectiveness, containing <350 mU/L. The FDA reviews and NCIMS approves published AP methods. Memorandum of interpretation M-a-98 is a living document that lists approved laboratory methods including AP and their validated matrices. Eggnog is seasonally AP tested but has no validated method in M-a-98.

Purpose: Generate data demonstrating eggnog performance with phosphatase methods.

Methods: PasLite and F-AP tests with novaLUMII-X (Charm Sciences, Inc.) flavored product protocols were used to test two different purchased eggnog products in triplicate after adding trace levels of local raw bovine milk: 0.01, 0.025, 0.05, 0.075 and 0.1%. Eggnog 1 (EN1) was pasteurized and Eggnog 2 (EN2) was HTST pasteurized by different manufacturers. Both products were evaluated against the calibration performed with EN1.

Results: Both products by both test methods had triplicate AP levels < 15 mU/L consistent with pasteurization. At 0.025% raw milk in eggnog, the triplicate measured AP was less than the NCIMS 350 mU/L action level: FAP EN1=248±6 mU/L and EN2=332±18 mU/L; PasLite EN1=291±4 mU/L and EN2=245±8 mU/L. At 0.05% raw milk in eggnog, the triplicate measured AP was greater than the 350 mU/L threshold: FAP EN1=526±19 and EN2=659±4 mU/L; PasLite

EN1=515±15 mU/L and EN2=500±22 mU/L. Enzyme Units, standard deviations, and sensitivity are consistent between the two spiked samples and the two methods. Results and SD are similar to prior publications using other dairy matrices spiked with raw milk. Reactivated AP could be observed with room temperature abuse of EN2, explained by the shorter hold time at higher temperature of HTST.

Significance: Testing matrices not listed with validated methods in M-a-98 can create state regulatory confusion that can be avoided by updating the memoranda.

P1-17 High Milk Protein Chocolate Chip Cookies Baking Validation to Control *Salmonella*

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Introduction: *Salmonella* can survive in low water activity ingredients such as wheat flour and milk protein powders for long periods of time and could multiply when hydrated to produce cookie dough. Therefore, effective inactivation of *Salmonella* during the baking step is vital to ensure the safety of finished food products.

Purpose: Validate the effectiveness of a simulated baking as a pathogen kill step for *Salmonella* during high milk protein chocolate chip cookies manufacturing.

Methods: This study was designed as a completely randomized block design with seven treatments and three replications. Flour was initially mist inoculated with *Salmonella* Typhimurium and dried back to pre-inoculation water activity (a_w). *Salmonella* inoculated flour was weighed and mixed with high milk protein concentrate (MPC 80), brown sugar, butter, egg, skim milk powder, vanilla extract, salt, sodium bicarbonate and water in a kitchen mixer for two minutes to prepare the cookie dough to achieve a 6.21 log CFU/g Salmonella population. Cookie doughs (30 g) were baked for 18 minutes at 350°F, followed by 15 minutes of ambient-air cooling. Cookie samples during baking were drawn at 3 min intervals and after 15 min cooling periods for *Salmonella* enumeration and a_w analyses. *Salmonella* populations in pre- and post-baking cookie samples were enumerated on injury-recovery media.

Results: The *Salmonella* population in high milk protein chocolate chip cookies decreased by ≥ 4.5 logs (6.21±0.26 log CFU/g in cookie dough to 1.55±0.18 CFU/g) after 18 minutes of baking. There was a significant decrease in water activity during the cooking process.

Significance: This study validates that baking high milk protein chocolate chip cookies at 350°F for at least 18 minutes will ensure in reduction of at least 4.5 log (CFU/g) *Salmonella* Typhimurium population.

P1-18 Inhibition of *Listeria monocytogenes* by Combination of Nisin and Organic Acids in Refrigerated Ready-to-Eat Egg Products

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Introduction: USDA lists nisin as "safe and suitable" for use in egg products at ≤6.25ppm. An initial inoculation experiment of egg products formulated with 0 or 6.25ppm nisin and stored at 4.4°C showed *Listeria* growth by 2.7 and 2.5-log CFU/g in three-weeks, respectively.

Purpose: Evaluate the efficacy of combination of nisin and organic-acids.

Methods: Five egg treatments- a, b, c, d, e, consisted liquid-whole-eggs formulated with 6.25ppm nisin combined with either acetate, propionate, acetate-diacetate, acetate-diacetate, and lactate based antimicrobials at 1.0, 0.3, 1.0, 0.6, 2.0%, respectively. Products were cooked to ≥74°C, and diced into pieces.

The individual sample unit consisted 30±2g diced eggs placed and packed in a plastic pouch with modified head-space gas composition (MAP) of N2:CO2 80:20 and sealed. A 0.5ml *Listeria* cocktail of 5-strains was syringed and stuck through sanitized adhesive septum previously placed over individual units to surface-inoculate eggs.

MAP-composition, organoleptic, aerobics, anaerobes, yeast and molds counts, and pH of eggs were assessed from duplicate un-inoculated units. *Listeria* was enumerated from three units.

Samples were stored at 4.4°C and evaluated at day-0, weeks-4, 6, 8, 10, 12, and weekly thereafter until week-20.

Two independent replicate trials were performed.

Results: Treatments had pH, moisture and salt of 5.97±0.21, 72.4±0.9%, 0.7±0.0%, respectively.

In un-inoculated controls, MAP-composition and product pH showed no changes (p>0.05). The total aerobic plate counts, anaerobic and yeasts and molds in all samples were not detected (<1-log CFU/g) for 20 weeks. Subjective evaluation of product after the opening of packages showed minimal changes in product color and odor.

On day 0, treatments had 2.5±0.3-log CFU/g of *Listeria*. Treatments a and c, although not intended, demonstrated ≥1-log kill over 20 weeks (p<0.05). Treatments b and e supported ≥2-log growth by week 15 and 6, respectively (p<0.05), while d supported one-log growth over 20 weeks (p>0.05).

Significance: This study demonstrates that egg products can be formulated to control *Listeria* growth during refrigerated distribution and extended storage.

P1-19 Development of Standardized Metadata for Machine-Readable Swab Site Descriptions That Support Digitization of Environmental Monitoring Data

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Introduction: The analysis of large open-source DNA sequence data generated through environmental monitoring is limited by the variability, incompleteness, and lack of international standards available for metadata describing the swab sites.

Purpose: Our goal was to develop a framework for collecting standardized, machine-readable metadata that document swab site descriptions in environmental monitoring.

Methods: We assessed 1,498 historical swab site descriptions to evaluate the lexicon used in unstructured environmental monitoring metadata. The types of information and diversity of terms contained in unstructured metadata was assessed. Comparisons were made using a chi-squared test of association. Subsequently, we queried OBO Foundry library ontologies to identify IDs for each of the unique terms found in the unstructured metadata. Where none existed, we proposed new terms.

Results: Five minimum metadata categories were identified in this analysis: (1) the structure and (2) subpart of the structure that was swabbed, the material the structure was made from (3), the condition of the material (4), and the orientation of the swab site on the structure (5). A total of 334 unique terms were found: 253 described structures, 25 described materials, 10 described conditions, and 46 described orientation. While nearly all swab site descriptions described the structure, only 14%, 27%, 9%, and 8% of descriptions included information for material, orientation, condition, and sub-parts, respectively. We determined that some terms ("wheel" and "broom") had significantly higher proportions *L. monocytogenes* positives compared to other structure terms (p<0.05).

Significance: Collectively, this study illustrates the utility of a possible framework for collecting standardized, machine-readable metadata that describe swab site descriptions in environmental monitoring. Collective utilization of such a scheme increases the interoperability of DNA sequence databases, enabling large-scale approaches to data sharing, artificial intelligence, and big-data approaches to food safety.

Poster

P1-20 The Value of the National Center for Biotechnology Information's Pathogen Detection Website in Identifying Geographic Clues to Aide Outbreak Investigations

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Introduction: Some bacterial pathogens have unique genetic patterns that link them to a specific geographic region; foodborne outbreak isolates may share a similar, if not the same, genetic profile as pathogens endemic to geographic-specific regions.
Purpose: The purpose of this study was to highlight several recent outbreak examples where isolates outside the immediate outbreak cluster provided geographic clues and helped identify the outbreak vehicle and possible causal source.
Methods: Once a foodborne outbreak is identified, the U.S. Food and Drug Administration (FDA) collaborates with Centers for Disease Control and Prevention (CDC) and state partners to identify the outbreak source and implement public health actions. One tool FDA uses to perform whole genome sequencing (WGS) analysis is the U.S. National Institutes of Health, National Center for Biotechnology Information (NCBI)'s online pathogen detection browser, which displays genomic data and metadata about isolates. This information along with other CDC, FDA, and state collected data are used to develop and evaluate food vehicle hypotheses.
Results: FDA investigators identified that the single nucleotide polymorphisms (SNP) trees of 3 outbreaks of *Listeria monocytogenes* (2019, 2020) and *Salmonella* Weltevreden (2021) had isolates from foods sampled upon import or by foreign health agencies. The sample and geographic metadata aided epidemiological and traceback investigations which linked imported avocado from Mexico, enoki mushrooms from South Korea, and frozen pre-cooked shrimp from India to the outbreaks, respectively. The SNP genetic trees of 5 outbreaks of *L. monocytogenes* (2021(2)), S. Enteritidis (2020), and *S.* Newport (2020) all contained both food and environmental isolates from California. These clues aided investigations, which linked domestic leafy greens, peaches, and red onions to illnesses.
Significance: Geographic trends observed from isolate data in NCBI provide an early signal for potential contamination sources. This information assists investigators by narrowing down foods to those known to be sourced from similar areas of interest.

P1-21 Enhancing the Foodborne Diseases Active Surveillance Network (FoodNet) Trends Model Using Bayesian Approaches

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Introduction: The Foodborne Diseases Active Surveillance Network (FoodNet) monitors illnesses caused by enteric and foodborne pathogens at 10 US sites. The model used to assess incidence trends does not account for site-specific trends and treats year a categorical variable. As a result, the model is sensitive to outbreaks and single-year aberrations, and is shaped primarily by trends at populous sites in the southeastern US.
Purpose: To enhance the model to better model trends overall and by site using Bayesian approaches and by re-parameterizing year.
Methods: Separate Bayesian regression models were implemented using FoodNet data collected during 1996–2018 for *Campylobacter, Salmonella, Shigella, Vibrio, Yersinia*, and 15 *Salmonella* serotypes. Different distributions (e.g., negative binomial, Poisson, geometric) and approaches to parameterizing the model (e.g., including year-site interaction, using splines to model the effect of year) were assessed. Model performance was compared with the current model visually and using adjusted R^2 and difference in expected log pointwise predictive density (ELPD).
Results: Bayesian spline models that incorporated a year-site interaction were the top performing models across pathogens regardless of distribution, and best captured site-specific trends. For *Yersinia,* the top-performing model (R^2=0.69) fit the data better than the current model (R^2=0.17; ELPD0.44)). After *Yersinia,* the improvement in R^2 ranged between 0.08 and 0.20 for the other four pathogens. and between 0.06 and 0.65 for the 15 serotypes with improvements in R^2 ≥0.50 for 8 serotypes.
Significance: The top-performing Bayesian model better captured spatiotemporal trends, was more flexible, and better fit trends across pathogens, serotypes, and FoodNet sites than the current model. The top-performing model reduced the effect of outbreaks and single-year aberrations by accounting for site-specific trends using a year-site interaction, and by allowing trends to change in slope and direction using splines and through treating year as continuous.

P1-22 Changes in the Incidence of Diagnosed Foodborne Illnesses in a Pediatric Patient Population during the COVID-19 Pandemic

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Introduction: At the onset of the COVID-19 pandemic, changes in the incidence of infectious diseases, such as foodborne diseases (FBD), were observed. It is unclear if these changes reflect changes in healthcare seeking behaviors or true changes in incidence.
Purpose: The objective of this study was to examine changes in the incidence of diagnosed FBD during the COVID-19 pandemic.
Methods: A retrospective cohort study of 1,263 pediatric patients with a diagnosed FBD was undertaken using electronic medical record data from a large tertiary pediatric hospital. Data, including age, gender, race, ethnicity, zip code, medical history, discharge diagnosis codes, and stool sample laboratory tests, were obtained for all patients diagnosed with *Salmonella*, *Campylobacter*, shiga toxin-producing *E. coli* (STEC), *Shigella* and norovirus from 2017 through 2020. The annual incidence of each FBD was calculated by dividing the number of diagnosed cases by the total number of patients seen at the hospital that year. Regression models were used to compare the 2020 incidence and proportion of positive tests (positivity rates) to an average from the previous three years.
Results: The 2020 incidence was not significantly different from the 3-year baseline for the majority of FBD. The incidence (per 100,000 person-years) of norovirus and shigella significantly decreased from 2017-2019 to 2020 for norovirus (8.5 to 2.4, p<0.001) and shigella (3.5 to 1.0, p=0.001). Positivity rates decreased for norovirus (8.3% to 5.0%, p<0.001), *Shigella* (1.3% to 0.3%, p<0.001), and STEC (1.1% to 0.8%, p=0.031), and increased for *Campylobacter* (2.2% to 2.9%, p=0.020).
Significance: The results suggest that the pandemic significantly affected the incidence of diagnosed cases of two FBD. The significant decreases in positivity rates suggest that incidence reductions were due to reduced care-seeking, but rather true reductions in incidence.

P1-23 Prevalence of Subclinical Mastitis, Its Associated Bacterial Isolates and Risk Factors among Cattle in Africa: A Systematic Review and Meta-Analysis

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❖ ***Developing Scientist Entrant***

Introduction: Subclinical mastitis (SCM) is one of the most economically important diseases affecting the dairy industry. The SCM does not cause a visible changes in the udder or physical changes of the milk as compared to clinical mastitis, and a clear overview of the prevalence and risk factors in the different regions of Africa is still lacking.

Poster

Purpose: The purpose of this study was to investigate the prevalence of SCM and assess the associated risk factors and dominant bacterial pathogens among cattle in Africa.

Methods: Using a systematic review and meta-analysis approach, we gathered literature concerning SCM among cattle in Africa, published from 2020 through 2021in five databases.

Results: A total of 1,560 records were retrieved and at the end of the screening, 38 studies were eligible for inclusion in the meta-analysis. The random-effects meta-analysis model showed that the weighted pooled prevalence of SCM in cattle in Africa was 51.5% (95% CI: 44.0-58.9%). Heterogeneity was high and significant as I^2 (proportion of observed variation) was 98.4% (95% CI: 97.6-99.0%), τ2 was 0.0533 (95% CI: 0.035-0.089), and the Cochran Q statistic was 2244.97 (p<0.0001). Sub-group and meta-regression analyses showed that West Africa had the highest point estimate (85.3%), followed by East Africa (64.1%), and the least was for southern Africa (16.3%) (p=0.007). Other significant moderators for SCM were age (p<0.0001), breed (p=0.015) and parity (p=0.0001) of cattle. *Staphylococcus* species were the most predominant pathogens, followed by *Streptococcus* and *Escherichia* spp.

Significance: The present study showed a high variation of subclinical mastitis prevalence in various parts of Africa. There is a need for more data on subclinical mastitis in some regions on the continent. The information gathered in this paper may be used to guide a multidisciplinary approach to the management, control and reduction of this infection.

P1-24 Antiviral Potential of Products Issued from Cranberry and Blueberry Against Murine Norovirus, Hepatitis A Virus and Herpes Simplex Virus Type 1

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne viruses are a worldwide cause of infections affecting millions of humans every year. The conventional methods used in viral inactivation process do not provide complete food protection against these viruses. Alternative control strategies need to be explored. Among these new methods, several of them rely on natural compounds found in berries, such as polyphenols, antioxidants, etc. These compounds have previously shown control efficiency on bacteria and are safe, used for centuries, and inexpensive. However, little is known on their antiviral properties.

Purpose: The aim of this study is to evaluate the antiviral potential and the mechanism of action of products issued from cranberry and blueberry against naked foodborne viruses [murine norovirus 1 (MNV-1) and hepatitis A virus (HAV)] and a model of an enveloped virus [herpes simplex virus type 1(HSV-1)].

Methods: Seven cranberry and blueberry products were tested (between 0,125 and 10 mg/ml for powders and 25 and 100% for liquids) against the three viruses using plaque assays. Plaque assays were performed after contact of the products with respective host cells and viruses to determine viral reduction.

Results: Cranberry and blueberry products have shown antiviral activities against the tested viruses. Complete inactivation was obtained against MNV-1 and HSV-1 with cranberry and blueberry juices at 50 % after a contact time with the cells and viruses respectively as low as 5 minutes at 37°C. Partial reduction of 3.7 ± 0.536 log (PFU/ml) was obtained with cranberry juice against HAV after a contact time of 90 minutes at 37°C. Potential mechanisms of action were narrowed whether they were related to the virus, the host cell or the process of replication.

Significance: This study provides new findings on the antiviral properties of cranberries and blueberries against MNV, HAV and HSV-1 and open to specific and interesting applications to reduce viral transmission.

P1-25 Rapid Detection of Fermented Maize (*Ogi*) Adulterated with Sorghum Leaf Sheath Using Near Infrared Spectroscopy

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Introduction: Food adulteration is an old and common practice in the low-and middle-income countries. Fermented maize gruel *ogi* undergo adulteration with sorghum leaf sheath to seem like the expensive sorghum-based-*ogi* (*ogi baba*) which physical inspection is insufficient to detect.

Purpose: Maize based gruel especially *ogi* is a high-value commodity and common indigenous complementary food that is a target for adulteration, leading to loss of quality and encroachment on the rights and interests of consumers.

Methods: This study investigated the characterization of the microorganisms found in *ogi* fermented for 0 to 120 hours and the feasibility of using visible - near Infrared (VIS-NIR) spectroscopy combined with multivariate analysis for detection and quantification of *ogi* adulterated with sorghum leaf sheath extract at different concentrations. Near Infrared Spectroscopy (NIR) spectra of the adulterated and pure *ogi* were measured between the regions 400 – 2498 nm. The multivariate methods included Principal component analysis (PCA), multiplicative scatter correction (MSC), Savitzky-Golay derivatization, and partial least square – discriminant analysis (PLS-DA).

Results: Principal Component Analysis gave visible cluster trends for authentic samples and the adulterated ones. The PLS-DA was used to detect the discrimination between the pure and adulterated *ogi* samples. The PLS-DA model with MSC and fist derivative Savitzky-Golay normalization with five smoothing points was able to cross validate adulteration better at 5% adulteration level successfully compared to other adulteration levels. Standard microbiological characterization of the isolates conducted, and results revealed that lactic acid bacteria (LAB) and yeasts were the main microorganisms found in the fermentation medium within 96 hours of fermentation. There was an increase in the population of the bacteria and yeasts ($CFUg^{-1}$) as the fermentation progressed.

Significance: The results suggested that the predominant microorganisms during the fermentation period was the LAB and NIR spectroscopy associated with multivariate analysis has the great potential for a rapid and non-destructive detection of adulteration in maize gruel (*ogi*).

P1-26 Using Ultrafine Ozone Bubble (UO3B) Treatment to Improve Current Fresh Produce Washing Methods

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❖ ***Developing Scientist Entrant***

Introduction: There is a need for an efficient and more environmentally friendly alternative to chlorine as a disinfectant in fresh produce washing because consumption of fresh produce is increasing, and they are usually contaminated by foodborne pathogens.

Purpose: The purpose of this study is to focus on the efficacy of ultrafine ozone bubble treatment to control foodborne pathogens on romaine lettuce and compare that to chlorine and ozone without ultrafine bubbles.

Methods: To compare the effectiveness as a disinfectant for romaine lettuce, *E. coli* O157:H7 and *Listeria monocytogenes* were inoculated into romaine lettuce and measured log reductions of the pathogens before and after treatments. The experiments were conducted in distilled water, chlorinated water (50ppm), ozonated water without ultrafine bubbles and ozonated water with ultrafine bubbles at different temperatures (5, 15°C). We also conducted the experiment with increasing concentration and time of ozonated water treatment with ultrafine bubbles and without ultrafine bubbles. All data were analyzed by the ANOVA procedure.

Results: We compared the effectiveness of disinfectants such as chlorine, ozonated water, and ultrafine ozone bubble treatment based on log reduction of foodborne pathogens on romaine lettuce at different temperatures. We found that low temperature caused increasing concentration of bubbles and stability of ozone in the water. There was a critical concentration and time of ultrafine ozone bubble treatment that show a significant ($P < 0.05$) log reduction than that of chlorine wash and ozonated water.

Significance: These results provide the potential alternative disinfectant for the fresh produce washing and show the possibility of UO_3B applications in fresh produce industry.

P1-27 Edibles: Are We Prepared? A Critical and Comparative Review of the Cannabis Legislation in Reference to Food Safety in Trinidad, Jamaica and Canada

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Introduction: Over 147 million people worldwide consume cannabis in some form. Cannabis in food has therefore become more common. In the Caribbean, Trinidad and Jamaica have amended Cannabis legislation and evidence indicates that edibles have become more accessible to the public. Without regulations or controls consumers essentially need to rely on those producing these products, trusting that they are safe.

Purpose: The purpose of this study was to evaluate the food safety impact of amended Cannabis legislation in Trinidad and Tobago, Jamaica, and Canada and to investigate how the Trinidadian and Jamaican public perceive Cannabis legislation, Cannabis and Cannabis infused food products (edibles).

Methods: Food safety legislation and the statutory changes under the Trinidad Dangerous Drugs Amendment Act 2019, Jamaica Dangerous Drugs (Amendment) Act of 2015, and Canada's Cannabis Act and Cannabis Regulations, were reviewed and comparatively analyzed.

Results: Present legislation in Jamaica fully supports the medical, scientific, and religious use of cannabis. Trinidad continues to adapt its regulations to the marketplace, the primary focus being decriminalization. Both countries allow possession of small quantities and home cultivation of (5) and (4) cannabis plants respectively. No clear definition or mention of edibles or measures to regulate such products is present in food and/or drug legislation in either country. In Canada, medical, recreational, and edible cannabis industries are supported. Legislation covers preparation, packaging, and sale of edibles. Testing, labelling and cannabinoid (CBD & THC) limits are fully regulated.

Significance: Views on cannabis and edibles are changing, the present legislation in Trinidad and Jamaica do not adequately provide protections for the public. If left unchecked this issue could result in serious burdens on the public health system.

P1-28 Food Safety Education and Intervention in Poultry Value Chain in Kenya and Developing Countries

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✦ ***Undergraduate Student Award Entrant***

Introduction: In many developing countries, poultry production is one of the most important livelihood strategies for many low-income households, but traditional rural households in Kenya raise chicken under an extensive system with little inputs, thus introducing numerous food-safety concerns along the poultry processing stages from farm to fork.

Purpose: This study presents a synthesis of published food safety interventions in Kenya and developing countries and outlines the poultry production process, with the goal of identifying the risk factors for implanting viable, culturally appropriate interventions considering existing challenges and limitations.

Methods: A literature review was conducted between May 2021 through August 2021. A search of electronic databases (Google Scholar, PubMed, CABI, WebMD) was performed using a search algorithm constructed from relevant terms and Boolean connectors. Identified relevant articles were imported and managed in Zotero (5.0.96.3 Corporation for Digital Scholarship, 2021). The intervention information was extracted from peer-reviewed research articles and reports describing interventions in the poultry value chain in Kenya and other developing countries.

Results: Risk factors along the poultry chain include cross-contamination during slaughtering and transportation to local markets. Lack of refrigeration system in transportation from the village to local markets puts poultry products in a temperature danger zone leading to the growth of *Salmonella* and *Campylobacter*. As the farmers are the independent sellers of their final product without any prior evaluation of quality with standardized measures upon selling in the local markets, many low-grade quality poultry products are being sold into the market.

Interventions implemented by the government include risk assessment, CCP analysis, risk management strategies, and product certification for food safety.

Significance: There is a significant gap in knowledge and awareness in food safety among the poultry value chain actors. This study addresses some of the food safety risk factors and investigates appropriate intervention methods to mitigate the occurrence of food-borne diseases related to *Salmonella* spp. and *Campylobacter* spp and increase food safety awareness.

P1-29 *Salmonella* Serotypes from Retail Chicken and Human Infections in the United States, 2002–2018

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Introduction: *Salmonella* causes ~1.3 million U.S. illnesses annually and is transmitted commonly through food. A source attribution model estimates that chicken is the most important source.

Purpose: We compared *Salmonella* serotypes isolated from retail chicken with those that caused human infections in the United States.

Methods: During 2002–2018, investigators in the National Antimicrobial Resistance Monitoring System (NARMS) Retail Food Surveillance Program in 20 states, including 10 in the Foodborne Diseases Active Surveillance Network (FoodNet), purchased chicken (mostly parts) from stores and cultured samples for *Salmonella*. We obtained incidence data from FoodNet; chicken and human comparison data were not included for serotype Kentucky because it is rarely isolated from humans.

Results: During 2002–2013, *Salmonella* was isolated from 13% (1,944/14,492) of chicken samples; this declined to 4% (1,216/28,024) during 2014–2018. The five most common serotypes were, in order, Kentucky, Typhimurium, Enteritidis, Heidelberg, and Infantis. Comparing 2014–2018 with 2002–2013, the average annual percentage of chicken samples that yielded the pathogen increased for Infantis (0.29% vs. 0.18%) and decreased for Typhimurium (1.1% vs. 4.2%), Heidelberg (0.51% vs. 1.9%), and Enteritidis (1.1% vs. 1.4%). Comparing 2014–2018 with 2002–2013, the average annual incidence per 100,000 persons increased for Infantis (0.39 vs. 0.23) and decreased for Typhimurium (1.6 vs. 2.4) and Heidelberg (0.25 vs. 0.56); it was similar for Enteritidis (2.8 vs. 2.6).

Significance: Isolation of *Salmonella* from chicken decreased in recent years, which reflects decreased isolation of serotypes Typhimurium, Heidelberg, and Enteritidis. The parallel increases in isolations from chicken and incidence of Infantis infections, and the parallel decreases in isolations from chicken and incidences of Typhimurium and Heidelberg infections, support data indicating that chicken is a major source of salmonellosis. Data from the NARMS Retail Food Surveillance Program could be used to monitor the effectiveness of measures to reduce contamination of chicken.

P1-30 Challenging SPF Chickens with *Salmonella infantis* and *Salmonella* Enteritidis to Establish Parameters for Efficacy of an SRP *Salmonella* Vaccine

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❖ ***Developing Scientist Entrant***

Introduction: Vaccination of poultry against two important serovars of *Salmonella enterica* will reduce the risk of foodborne illness from these organisms, improving food safety associated for both poultry and eggs.

Purpose: Assess infectivity, pathogenicity, and recovery potential of *Salmonella* Enteritidis (SE) and *Salmonella* Infantis (SI) in Specific Pathogen Free (SPF) chickens to establish parameters of efficacy for a Siderophore Receptor and Porin (SRP) vaccine.

Methods: Cultures of SI and SE were prepared at concentrations 10^8, 10^7, 10^6, 10^5, and 10^4 CFU/0.2 mL in TSB, centrifuged (3000rpm for 5 minutes), and reconstituted in HBSS. Six-week-old SPF chickens are divided between two colony houses (35 test chickens, 15 uninoculated controls) and inoculated intravenously with 0.2mL of the cultures (*n*=50). At two days post-inoculation (dpi), five chickens from the 10^8, 10^7, and unchallenged groups (*n*=5 per group), are euthanized, spleens and fecal samples collected, adjusted to 20% weight:volume in sterile PBS, and homogenized. At 7dpi all remaining birds are euthanized, and samples collected. *Salmonella* is enumerated by serially diluting each sample and plating each onto XLT4 + 50 ug/mL nalidixic acid plates, incubated for 24h at 37°C.

Results: Uninhibited, a TSB culture of SI achieved its peak concentration (approximately 1.5×10^9 CFU/mL) after 5.5 hours of incubation at 37°C. In the same time span, an SE culture achieved a concentration of 2×10^9 CFU/mL. The limit of detection for the recovery of both *Salmonella* species, post-inoculation, is fewer than 10 cultivable cells per mL. Post inoculation, survivability and recovery between *Salmonellae* are compared. The efficacy of the vaccine is measured in comparison to the performance of uninhibited, in-vivo bacteria survival.

Significance: Understanding the performance of uninhibited *Salmonella* species in live poultry will allow us to determine metrics by which the performance of a *Salmonella* vaccine may be measured.

P1-31 Persistence of *Salmonella enterica* Inside Biofilms on Food-Contact Surfaces with Chicken Skin Residues and Presence of Native Bacteria

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Introduction: Persistence of foodborne pathogens inside biofilms formed on surfaces contaminated with food residues and native bacteria has been poorly studied.

Purpose: To evaluate the persistence of *Salmonella* inside biofilms formed in presence of skin chicken residues and their native bacteria on food-contact surfaces.

Methods: Stainless steel, plastic, and glass chips were immersed for four h at 25°C in skin chicken extract (SCE; 1:10) with native bacteria (~ 5 log CFU/mL) and three *S. enterica* isolates (~ 4 log CFU/mL), individually. After incubation, not attached cells were removed and chips were rinsed with SCE (1:100). Chips were incubated at 25°C /97% HR for 72 h. Every 24 h, total plate count (TPC), total coliforms (TC), *Salmonella*, and biopolymers were quantified. Inoculated chips with native bacteria without *Salmonella* were used as a control.

Results: Around 2.28 log CFU/chip of *Salmonella* attached to the surfaces. The colonization of the pathogen was affected by the storage time ($p<0.05$), increasing during 24 h and then remained constant the rest of the time (2.8 to 3.1 log CFU/chip). The colonization of TPC and TC on the surfaces was affected by surface type and storage time ($p<0.05$), but not for the presence of *Salmonella*. In both cases, the concentration increased during 48 h and then remained constant. The highest concentrations of TPC were on plastic followed by stainless steel and glass with a mean across the time of 6.36, 5.87, and 5.23 log CFU/chip, respectively; TC populations have similar trend with lower content (1 log CFU less). Regardless the treatment, the biopolymer production was the same in absent or present of the pathogen; however, higher concentration were obtained in plastic (0.58 OD_{595nm}) followed by stainless steel (0.37 OD_{595nm}), and glass (0.20 OD_{595nm}).

Significance: *Salmonella* persist and can coexist inside biofilms with skin chicken native bacteria.

P1-32 Moved to Technical

P1-33 Species Distribution and Genes Encoding Antimicrobial Resistance in *Staphylococcus* spp. Isolated from Chickens in Saudi Arabia

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Introduction: Antimicrobial resistance is a cause of concern for human and animal health worldwide. Several staphylococcal species cause various diseases and are increasingly reported to be resistant to antimicrobials agents such as methicillin. Methicillin-resistant staphylococci have been emerged as important zoonotic pathogens.

Purpose: The purpose of this study was to detect and to characterize staphylococci isolates from cloacal samples from chickens in an industrial farm in Saudi Arabia

Methods: 60 samples of cloacal samples from chickens were collected from an industrial farm in Saudi Arabia (January 2021). Isolates were selected on Chapman agar and one colony was selected randomly from each sample and identified using the Api Staph system. All isolates were tested by the standard disc diffusion method of Kirby-Bauer on Mueller Hinton Agar (Bio-Rad) according to the recommendation of Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). Genes encoding resistance to methicillin (*mecA*/*mecC*), gentamicin/Kanamycin/tombramycin (*aac*(6')-Ia-*aph*(2")-Ie), streptomycin (*ant*(6)-Ia), erythromycin (*erm*(B), *erm*(A), *erm* (C), *msr*(A), and *mef*(A/E), tetracycline (*tet*(M), *tet*(L), *tet*(O), *tet*(K), *tet*(S)) were analyzed by PCR

Results: Among the 60 samples, we collected 26 isolates that were identified as: 12 *S. lentus*, 5 *S. xylosus*, 3 *S. sciuri*, 2 *S. aureus*, 2 *S. capitis*, 1 *S. hominus*, 1 *S. auriculars*). Resistance was observed against erythromycin, tobramycin, tetracycline, cefoxitin, and penicillin. Three isolates were methicillin-resistant (2 *S. xylosus*, 1 *S. lentus*) and harbored the *mec*A gene. The *tetM* gene was detected in the six tetracycline resistant isolates. Among the 20 erythromycin-resistant isolates the *erm*(B) and the *msr*(A) genes were detected in 15 and 6 isolates, respectively.

Significance: High diversity of staphylococci species was observed in the feces of analysed chickens; in addition, isolates were highly resistant to antimicrobial agents. These findings highlight the role of poultry as reservoir of antimicrobial-resistant staphylococci that might be acquired by humans via direct contact or by the food chain

P1-34 Cold Chain Assessment of Poultry at Retail Selling Points

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Introduction: For all meat types keeping cold chain from slaughter to retail sale prevents foodborne diseases and maximizes shelf-life of perishable foods.

Purpose: The research aimed to evaluate temperature of poultry in retail selling points and if temperature recorded were within the range defined by Mexican official standards.

Methods: Surface temperatures were recorded for poultry located at the front, middle and rear of the rack within open refrigerated displays in four supermarkets located in the State of Mexico. Weekly measurements were made from January to November 2021 with an infrared thermometer between 12:00 and 14:00 h. A mixed model was used for the statistical analysis under a completely randomized design with repeated measurements over time. Data was grouped by season of the year and analysis was done by position of poultry within the rack. Means were compared using the t-test.

Results: Deviations from the Mexican official standard temperature (Max. 4 °C for meat products) were found in all four supermarkets specially at the front location. At front and middle locations, two supermarkets kept the highest poultry surface temperatures throughout the study and above the official standard. Only one supermarket had a deficit in cold chain control at the rear position for summer and autumn. No significant differences ($p>0.05$) were found in the interaction between year season and supermarket.

Significance: The data obtained show that it is essential for supermarkets to improve their cold chain management protocols, to harmonize with national standards and reduce risks for consumers and food wastage.

P1-35 Novel Approach for Pathogen Control and Food Safety Management in Poultry Processing: Biomapping Indicator and Pathogen Loads in High- and Low-Level Antimicrobial Intervention Schemes

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❖ ***Developing Scientist Entrant***

Introduction: The poultry processing industry in the US has traditionally implemented antimicrobial interventions against *Salmonella* spp. and *Campylobacter* spp. on the basis of experiences shared among poultry processors; however, the effects of individual interventions using pathogen quantification methodologies has not been conducted. Intervention efficacy is difficult to assess due to plants always running with chemical interventions to comply with regulatory performance standards

Purpose: To develop biomaps of *Salmonella* and *Campylobacter* loads at different stages during processing in a commercial poultry facility operating under conventional chemical intervention and reduced chemical levels to identify critical steps where interventions could result in better pathogen reductions

Methods: A total of 470 samples were collected for conventional processing and for low-chemical interventions respectively in 10 different locations throughout processing. Aerobic counts (AC), *Enterobacteriaceae* counts (EB) and *Campylobacter* counts were conducted on carcass and part rinsates using the TEMPO™ system based on standard methodologies. *Salmonella* spp., enumeration was conducted using BAX® System SalQuant™ and BAX® System RT-Salmonella Assay for detection in samples not suitable for quantification

Results: *Salmonella* spp. counts on conventional processing shows significantly lower levels when compared to the low chemical regime; however, this difference is not evident when comparing prevalence data between the treatments. For *Campylobacter* spp. counts, the rehanger, IOBW #2 and pre-chiller locations were not significantly different between treatments

Significance: Poultry processors, through comprehensive biomapping collecting not only indicators but also pathogen loads and actual reductions can develop statistical process control regimes to better understand the overall process and contributions of individual steps to contamination. From this study, we determined that not all chemical interventions have an overall impact on *Salmonella* spp. or *Campylobacter* spp. and certain interventions can be turned off to achieve better microbial performance, reduce costs and chemical utilization

P1-36 Bio-Mapping of Microbiological Indicators in a Commercial Beef Processing Facility

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Introduction: Bio-mapping is a tool available to beef processors to evaluate their microbial status throughout different processing points in the facility to determine the efficacy of their antimicrobial interventions, used to reduce microorganisms to target concentrations in their final product.

Purpose: The objective of this study was to quantify the microbial levels throughout the whole process in a commercial beef facility to estimate parameters that can be used for food safety management and establishment of baselines.

Methods: Carcasses went through a series of lactic acid interventions at 2-5%. Samples were collected over 8 days: 3 samples at each of the 7 different sampling points on each 4 carcass locations, for a total of 84 samples per day. Samples were taken using 25ml buffered peptone water swabs and quantified using TEMPO® system for aerobic counts (AC), Enterobacteriaceae (EB), and *Escherichia coli* (EC). Statistical comparisons were done using a one-way ANOVA and Kruskal-Wallis.

Results: Overall, indicator counts for each carcass location were reduced from pre-evisceration to after-intervention for all three microorganisms ($P<0.001$). EC counts were significantly reduced, from pre-evisceration to after-intervention, in average by at least 1.43, 1.27, 0.41, and 1.06 LogCFU/Sample in the shank, inside round, midline, and brisket, respectively. EC counts were lowest for each carcass location after-interventions, which is the last step in the slaughter process, implying there was a significant log reduction ($P<0.001$) on the slaughter floor due to antimicrobial interventions in place. However, after-fabrication counts for all 3 microorganisms at all carcass locations increase ($P<0.001$) to numbers greater than on the final carcass, indicating additional interventions need to be added to the fabrication area.

Significance: Quantifying microbial loads throughout beef processing facilities, will allow the facilities to identify their problem-areas as well as evaluate the efficacy of their interventions to help develop baselines and adjust management practices in the future, if needed.

P1-37 Impact of Temperature and Salt Concentrations for Thermal Inactivation of *Salmonella* in Moisture Enhanced Reconstructed Chicken Patties

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Introduction: Chicken products moisture enhanced (MH) with salt and tripolyphosphate can be contaminated with *Salmonella*, which is responsible for approximately 35% of the foodborne illnesses associated with poultry products.

Purpose: To determine the thermal kinetic parameters of *Salmonella* in MH-reconstructed ground chicken patties as affected by temperatures and salt concentrations.

Methods: Fresh 500 chicken breast was grounded, inoculated with Nalidixic-acid (NaL-200 ppm) resistant *Salmonella* Typhimurium followed by adding NaCl (1.0 and 3.0%) + Na-tripolyphosphate (0.5%) solutions to achieve pump rates of 8%. Samples were than weighted for 10-gram and added into filtered food sample bags vacuum packaged and followed by storing at 4°C for 42-h before heating in a circulated water bath set at 62, 66, 70, and 74°C for 0, 15, 25, 30, to 180 s, respectively. Counts of the pathogen cells were analyzed on tryptic soy agars plus NaL-200 ppm. Microbial survival populations and thermal inactivation kinetics (USDA-Global-Fit software) were analyzed by the Mixed Model Procedure (SAS, n=8, 2-repeats, P=0.05).

Results: Initial *Salmonella* counts were 5.4 to 6.0 $\log_{10}$CFU/g. Heating at 62-74°C for 100-180 s reduced (P<0.05) the pathogen populations by 2.8-5.5 $\log_{10}$CFU/g and 2.4-4.8 $\log_{10}$CFU/g for chicken samples containing 1% and 3% salt, respectively. Thermal dynamic data fit Weibull Model (RMSE=0.5163 to 0.7381 and AIC values=-44.026 to -8.868), but not the linear model (RMSE >1.000). Calculated D-values of chickens with 1.0% and 3% salt decreased (P<0.05) from 126.4±14.2, 44.6±6.9, 32.3±3.6, to 30.4±4.9 sec, and decreased (P<0.05) from 171.0±15.2, 56.4±5.7, 29.3±6.5, and to 24.8±4.9 sec, when

heating temperatures increased from 62, 66, 70, to 74°C, respectively. D_{62} and D_{66} values of 3% salt samples were greater (P<0.05) than 1% salt samples, whereas their D_{70} and D_{74} values are similar (P>0.05).

Significance: Results indicate that thermal resistance of *Salmonella* was significantly affected by temperature, and salt concentrations, which will be useful by the poultry meat industry to develop proper thermal processes to eliminate Salmonella in MH chicken products.

P1-38 Impact of Process Humidity and Fat Content on the Inactivation of *Salmonella* on the Surfaces of Beef and Pork Patties Cooked in an Impingement Oven

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Introduction: Recent USDA-FSIS Appendix A revisions have addressed process humidity as a critical parameter for achieving *Salmonella* inactivation on meat and poultry product surfaces. However, the impact of process humidity in high-temperature, short-time (HTST) processes remains a scientific gap.

Purpose: This study aimed to quantify the impact of humidity on *Salmonella* lethality during impingement cooking of beef/pork patties and to develop tools that assist processors in achieving Appendix A compliance.

Methods: Beef patties (10, 30% fat) and pork patties (30, 50% fat) were inoculated with an eight-serovar cocktail of *Salmonella* and cooked (in triplicate with duplicate subsamples) in pilot-scale moist-air impingement ovens at 218.3°C and different humidities (54.4, 62.8, 73.9, or 82.2°C wet bulb temperature (T_{wb})). Cooked samples were immediately cooled in liquid nitrogen, surface portions isolated, and plated on a differential/non-selective medium. Sample surface temperatures and moisture content were also measured.

Results: The efficacy of HTST cooking for inactivating *Salmonella* on the patties depended on the interaction between process humidity, product type, and fat content. For beef patties (10% fat) cooked at process humidities ≤62.8°C T_{wb}, *Salmonella* decreased ~5.3 log with no further reduction seen from 360 to 420 s (p > 0.05); however, >6.5 log reductions were achieved for the same product processed at 82.2°C Twb for 270 s. Increasing the fat to 30% yielded average reductions of ~6.5 log for both beef and pork and at 360s and 54.4°C T_{wb} and ~5.9 log for beef (30% fat) cooked at 82.2°C T_{wb} for 270s. Therefore, average lethality decreased compared to lower fat beef patties cooked under identical conditions (p < 0.05).

Significance: This research clarifying the impact of product type, fat, and humidity on *Salmonella* inactivation will assist USDA-FSIS and processors in achieving Appendix A compliance.

P1-39 Effect of Fat Content on *Salmonella* Lethality on the Surface of Impingement-Cooked Pork Patties

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Introduction: Revisions to USDA FSIS cooking guideline (Appendix A) state relative humidity in high-temperature, short-time cooking processes is necessary for *Salmonella* inactivation on product surfaces. Current research suggests low-fat products may promote desiccation resulting in less surface lethality; however, this phenomenon is not well understood.

Purpose: The objective was to quantify the effect fat content had on *Salmonella* lethality on the surface of impingement-cooked pork patties in processing conditions with and without added humidity.

Methods: Pork patties (5, 15, 30, 50% fat) were surface-inoculated with an eight-strain *Salmonella* cocktail and cooked at 218.3°C dry-bulb under processing conditions either with added humidity (71.1°C wet-bulb) or without added humidity (ambient wet-bulb) in a pilot-scale, two-zone impingement oven. Duplicate samples from three replications were collected at pre-cook and after each zone, immediately placed in chilled BPW, and plated on selective/differential media. Oven and product temperatures were recorded in duplicate for each treatment. Significance for fat content and processing condition effects were determined by ANOVA.

Results: At ambient wet-bulb conditions, fat content had a significant effect on total *Salmonella* log reductions (p = 0.03), showing low-fat products were prone to surface dehydration leading to desiccated, heat-tolerant *Salmonella.* In comparison, no fat content effect was found at 71.1°C wet-bulb conditions on *Salmonella* lethality (p > 0.05). Analyzing all the treatments together showed no fat content or process condition effect (p > 0.05). The average log reduction at 71.1°C wet-bulb conditions was higher than ambient conditions for all fat contents (6.35 vs 5.60 log, respectively).

Significance: These findings provide useful information to USDA FSIS and the meat industry to ensure sufficient surface lethality for products cooked in high-temperature, short-time processes, and they show lower fat content results in lower surface *Salmonella* lethality in ambient desiccation-supporting conditions but minimal effect of fat when surfaces are exposed to hydrated conditions at lethal temperatures.

P1-40 *Salmonella* Inactivation in Bacon Using Microwave or Moist-Air Impingement Oven Cooking

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❖ ***Developing Scientist Entrant***

Introduction: Commercial cooking of bacon in an impingement or microwave oven must achieve both a 40% yield and a >6.5 log reduction in *Salmonella* to meet the ready-to-eat (RTE) product requirement in USDA-FSIS Appendix A. However, the efficacy of both cooking strategies for *Salmonella* inactivation in bacon has yet to be sufficiently validated.

Purpose: Therefore, this study assessed *Salmonella* inactivation during bacon processing in an impingement or microwave oven, mimicking industrial practices.

Methods: Bacon slices (30 g, 3.0±0.5 mm thick) were inoculated with an 8-strain *Salmonella* cocktail (9.2 ± 0.2 log CFU/g) and cooked in a pilot-scale impingement oven (20% fan speed, 60% v/v humidity) or in a microwave oven (2450 MHz, 960 W) (≥6 replications). Microwave-cooked samples were treated up to 150 s, whereas impingement-cooked samples were treated up to 10 min at 177°C, and up to 6 min at 232°C. Survivors were enumerated on a differential/non-selective medium (37°C, 48 h). Sample temperature, moisture content, and yield were also measured. T-tests were used to compare survival data.

Results: After 90 s of microwave cooking, *Salmonella* populations decreased >6.5 logs (P < 0.05) with a cooking yield of 54%. After 1 min of impingement oven cooking, *Salmonella* decreased > 9 logs at both temperatures, with average cooking yields of 84 and 80% at 177 and 232°C, respectively. Using the impingement oven, bacon achieved maximum surface temperatures of 92.8 ± 2.1 and 99.8 ± 4.9°C, respectively, after 1 min of cooking at 177 and 232°C, with the 40% target yield achieved after 6-10 min.

Significance: Based on these findings, *Salmonella* decreased > 6.5 logs in microwave- and oven-cooked bacon before the 40% target yield for RTE bacon was achieved, thereby validating these processes with respect to Appendix A.

P1-41 Validating Inactivation of *Salmonella* spp. in Poultry Feed Mills

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Introduction: Due to the nature of ingredients used in their manufacture, poultry feeds are prone to contamination with salmonellae.

New guidance published by the Agricultural Industries Confederation (AIC), has introduced the requirement that kill steps used to control *Salmonella* in poultry feeds and feed ingredients via heat or chemical treatments should be validated to confirm that a suitable log reduction is achieved.

Purpose: This project aims to provide a validated protocol allowing determination of the lethality achieved towards *Salmonella* in poultry feeds by heat and chemical processes, including inoculation of large quantities of material and qualification of surrogate organisms for use in feed mill challenge tests.

Methods: D- and z-values of target (*Salmonella*) and surrogate (*Enterococcus faecium* ATCC 8459) organisms to steam heating were determined in 3 poultry feeds and data was used to validate the surrogate.

Assessments of 3 organic acid treatments were conducted to provide comparative data assessing the suitability of the potential *Salmonella* surrogate *Enterococcus faecium.*

Results: 1. Heat treatment results:

The following z-values were calculated in each feed assessed for *Enterococcus faecium* and *Salmonella,* respectively.

- **Layer feed** 12.21C° and 12.22C°
- **Broiler breeder** 11.43C° and 11.79C°
- **Sunflower seed feed** 11.61C° and 10.33C°

In all feeds, *Enterococcus faecium* showed slightly higher thermal resistance than *Salmonella* throughout the temperature range typically applied during steam heating of poultry feed, and similar z-values, confirming its suitability as a surrogate organism for use in challenge testing of these processes.

2. Bactericidal study results:

Log reductions of both *Salmonella* and *Enterococcus faecium* of between 1 & 2-log were observed for feeds treated with 0.1% powdered bacteriocides over a 7-day period. Both liquid treatments assessed yielded either very small reductions (<0.5 log) or no reduction across the 7-day test period.

Significance: Data supporting the use of *E. faecium* as a surrogate organism for use in assessment of Poultry Feed Mill steam treatments

P1-42 SMART Multi-Receptor Phage Cocktail to Control the Growth of *Salmonella In Vitro* and on Chicken Skin

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is one of the most prevalent food-borne bacterial pathogens around the world causing gastroenteritis in humans. Several salmonellosis outbreaks have been associated with the consumption of contaminated poultry products. This has been linked to contamination during processing and urges improvement in the prevention and control programs. Lytic bacteriophages (phages) are viruses that specifically infect and kill bacteria. They have been proposed as natural antimicrobials in different settings including food supply chain.

Purpose: To evaluate the efficacy of a multi-receptor phage cocktail to control the growth of *Salmonella in vitro* and in chicken skin.

Methods: Five fully characterized phages were selected for cocktail formulation. This cocktail targets four receptors: O-antigen, BtuB, OmpC, and rough *Salmonella* strains. A total of 66 *Salmonella* strains (comprising 22 serovars) were challenged with different cocktail concentrations for 24 hours in liquid culture at 25°C to determine cocktail host range. *Salmonella* Enteritidis was used to study growth inhibition at different environmental conditions and to investigate the development of phage resistant mutants. Furthermore, the phage cocktail biocontrol efficicacy was evaluated on chicken skin. Chicken skin pieces of 1 cm^2 were dipped into cocktail suspension containing either 5 or 7 $\log_{10}$ PFU/mL and incubated at 25, 15, and 4°C. The bacterial reduction was evaluated overtime for 48 hours by platting on XLT-4 plates.

Results: Treatment with the phage cocktail *in vitro* at MOI of 10^3 significantly inhibited the growth of all *Salmonella* strains tested. Bacteria challenge experiments using *S.* Enteritidis treated with different phage concentrations (MOIs 10^{-1}-10^3) showed complete growth inhibition at 25°C and 15°C for 48 and 96 hours, respectively. Biocontrol experiments showed a 3.5 $\log_{10}$ CFU/cm^2 reduction after 48 hours with treatments of 7 $\log_{10}$ PFU/mL at 25 and 15°C, and 2.5 $\log_{10}$ CFU/cm^2 at 4°C.

Significance: These results suggest that bacteriophage cocktail developed in this study is a promising biocontrol tool against *Salmonella* that can be applied during poultry processing to enhance food safety.

P1-43 Antibacterial Effects of Lactic Acid Bacteria Isolated from Piglet Feces on *Escherichia coli* and *Salmonella*

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Introduction: Lactating piglets may have useful lactic acid bacteria (LAB) which are from a sow through lactating. In recent use of LAB is recommended to control pathogens rather than using antimicrobials because the antimicrobials may increase the resistance.

Purpose: The purpose of this study was to isolate lactic acid bacteria from piglet feces to control *Escherichia coli* and *Salmonella*.

Methods: One hundred eighteen fecal samples from 118 piglets were obtained, and the fecal samples were diluted in 1% buffered peptone water and plated on De Man, Rogosa and Sharpe (MRS) agar, and single colonies were then isolated for 16s rRNA analysis. The LAB isolates were examined for hemolysis, gelatinase activity, and acid, bile and pancreas tolerances. The selected LAB isolates from these analyzes on MRS agar, the brain heart infusion agar mixed with *E. coli* (KVCC-BA2000145, BA2000146, BA2000147, BA2000148, BA2000149, BA2000150, BA2000151, BA2000152, BA2000153, and BA2000154) or *Salmonella* (KVCC-BA2000155, BA2000156, BA2000157, BA2000160, BA2000161, and BA2000159) were overlaid on the MRS agar. After incubation at 37℃ for 24 h, the size of the inhibition zone for *E. coli* or *Salmonella* were measured.

Results: One hundred sixty-four isolates were obtained from 118 piglets. Thirteen LAB isolates were selected after hemolysis, gelatinase activity, and acid, bile and pancreas tolerances tests. The antibacterial effect of the 13 isolates were examined against *E. coli* and *Salmonella*, and *Lactobacillus reuteri* SMFM2021-PF30 had the highest antibacterial effect against 17 strains of *E. coli* (KVCC-BA2000145, BA2000146, BA2000147, BA2000148, BA2000149, BA2000150, BA2000151, BA2000152, BA2000153, and BA2000154) and *Salmonella* (KVCC-BA2000155, BA2000156, BA2000157, BA2000160, BA2000161, and BA2000159).

Significance: *L. reuteri* SMFM2021-PF30 isolated from piglet feces could be used to control *E. coli* and *Salmonella*.

P1-44 Bacterio-Phage as Post-Lethality Intervention to Reduce *Listeria* on Hard Boiled Eggs

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Introduction: FSIS "Zero tolerance" policy towards *Listeria* in RTE products obliges processors to proactively seek post lethality interventions to eliminate *Listeria* in RTE products.

Purpose: To assess if a commercially available, *Listeria*-specific, bacteriophage product (PhageGuard Listex™) can kill *Listeria* on the surface of hard boiled eggs.

Methods: Raw eggs were boiled, cooled to room temperature, peeled, and subsequently contaminated with 2×10^4 CFU/g *L. monocytogenes* by applying 0.2 µL/g of culture. The bacteriophage (phage) product was diluted in tap water to either a high (0.2%, 10^7 PFU/cm²) or low (0.1% and 5×10^6 PFU/cm²) concentration, after which 1.5 mL of either solution was evenly dropped on the surface of the inoculated eggs. The run-off phage solution was collected in the plastic bag used to store the eggs after treatment. Control samples were treated with tap water. Treated samples were stored at 4°C until *Listeria* enumeration at 0, 3, 6, and 24 hours post-treatment. The data presented are mean values of three individual experiments, analysed with a two-way ANOVA (Sidak's multiple comparisons test).

Results: *Listeria* was reduced significantly on the surface of hard-boiled eggs treated with bacteriophages, when compared to water treated controls. Treatment with a 0.2% phage solution resulted in a 1.3 log ($P<0.0001$) reduction of *Listeria* after a dwell time of 3 hours, and a maximum kill of 2.2 logs ($P<0.0001$) after 24 hours. The 0.1% phage solution provided a 1.0 log ($P<0.0001$) kill after 6 hours and a maximum *Listeria* reduction of 1.9 log ($P<0.0001$) after 24 hours.

Significance: Recent recalls (2019-2020) show that the hard-boiled egg production environment poses hazards after peeling. We show here that treating the peeled eggs before packaging with an anti-*Listeria* bacteriophage solution, through a dip or spray application, provides an effective way of killing *Listeria* on hard-boiled eggs.

P1-45 Application of *Debaryomyces hansenii* Isolated from Fermented Food to Improve the Quality of Fermented Dry Sausage

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Introduction: Microorganisms play a role in improving the quality and creating a unique flavor of fermented dry sausage. Thus, microbial strains need to be developed for such purpose.

Purpose: The purpose of this study was to isolate *Debaryomyces hansenii* from fermented foods and to examine the effect of the isolates on fermented dry sausage.

Methods: *D. hansenii* were isolated from soybean paste, cheese, fermented sausage, and dry-aged meat. After examining the isolates for hemolysis, biogenic amine production, β-glucuronidase and β-glucosidase activities, lipid degradation, and salt resistances, selected *D. hansenii* SMFM2021-C14, SMFM2021-S8, and SMFM2021-SL3 were inoculated into meat batches with a commercial starter. Fermented dry sausages were fermented at 20°C and 85% humidity for 2 days and 15°C and 80% for 19 days. During fermentation, microbial counts, color, texture, reducing sugar, volatile basic nitrogen (VBN), thiobarbituric acid reactive substance (TBARS), and fatty acid composition were analyzed every week.

Results: Of 22 *D. hansenii* isolates from 43 food samples, 3 isolates strains (*D. hansenii* strains SMFM2021-C14, SMFM2021-S8, and SMFM2021-SL3) were selected. During the fermentation, no foodborne pathogens were detected, and the lactic acid bacteria, and *D. hansenii* cell counts increased by 1-2 Log CFU/g. Hardness, chewiness, and gumminess values were increased ($p<0.05$) compared to week 0. At week 3, the reducing sugar contents were lower ($p<0.05$) in the groups treated with *D. hansenii* strains SMFM2021-S8 and SMFM2021-SL3 then other groups. *D. hansenii* SMFM2021-S8-treated group showed lower ($p<0.05$) VBN and TBARS values, and the linoleic acid and α-linolenic acid contents were increased ($p<0.05$) in the *D. hansenii* SMFM2021-SL3-treated group.

Significance: *D. hansenii* SMFM2021-C14, SMFM2021-S8, and SMFM2021-SL3 isolated from various fermented foods could be used to improve the quality of fermented dry sausage.

P1-46 Use of Food Additives to Increase the Growth of *Thamnidium elegans* during Dry-Aging to Improve the Dry-Aging Effect

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Introduction: Dry-aged beef has a unique flavor produced by decomposing proteins and lipids with some microbial, like *Thamnidium elegans,* during aging, but temperature in the dry-aging may be too low for the microbial growth. Thus, a technique is necessary to improve the growth.

Purpose: The purpose of this study was to find food additives that enhance the growth of *T. elegans* related to dry-aging and evaluate changes in the quality of dry-aged beef.

Methods: Glucono-delta-lactone (GDL,10%), acidulant, and xylose (10%), sweetener, with different concentrations were applied on beef (sirloin) surfaces by 300 µL. *T. elegans* were inoculated to the surface of the beef by spraying. The beef samples were dry-aged at 4 ℃ for 21 days. Microbial counts, malonaldehyde content, volatile base nitrogen, and texture in the dry aged beef were analyzed.

Results: After dry-aging for 21day, growth rates of *T. elegans* was slightly faster in food additives treatment group than of control group and the total aerobic bacterial population decreased by 1 log CFU/g. Lipid rancidity decreased to 0.48 mg MDA/kg in food additives treatment group compared to control group and 3.1 kg of hardness and 761.8 of chewability, which was lower ($p<0.05$) than that of control. Volatile basic nitrogen concentrations different among the treatment group.

Significance: This result suggests that the treatment of 10% xylose and 10% GDL in beef can help the growth of *T. elegans* during dry-aging and improve their hardness lipid rancidity to improve quality.

P1-47 Comparison of Biological Food Safety Hazards and Risk in Cellular-Based and Conventional Beef Production

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❖ ***Developing Scientist Entrant***

Introduction: Interest in and potential for cellular-based meats for human consumption is increasing. While the potential benefits of cellular agriculture have been widely discussed, potential food safety considerations are limited in peer-reviewed literature.

Purpose: The purpose of this qualitative desktop study was to evaluate the potential food safety hazards of discrete and continuous cellular-based beef processes in comparison to conventional beef slaughter and processing.

Methods: Peer-reviewed literature, expert consultation, trade association resources, and USDA-FSIS guidance were used to define flow diagrams and aggregate potential hazards and risk for conventional beef processing. A similar approach was used for discrete and continuous cellular-based processes; data were more dependent on private-sector discussions and web-based resources. Differences and similarities in potential hazards, the likelihood of a hazard occurring, and potential mitigation strategies were identified for each flow diagram step.

Results: Conventional and cellular-based beef production systems had discrete and overlapping biological food safety challenges. Biological hazards in conventional beef production (e.g., Salmonella, Shiga toxin-producing E. coli, Staphylococcus aureus) are known and controlled at critical control points during processing such as trimming, organic acid wash, and chilling steps, among others. Potential biological hazards in cellular-based beef production such as Mycoplasma, viral (e.g., retrovirus), and bacterial contamination (e.g., S. aureus) during cell culture in bioreactors are controlled through Good Cell Culture Practices, Sanitation Standard Operating Procedures, Good Manufacturing Practices, and cross-contamination prevention are essential to safe production in both systems.

Significance: This qualitative study demonstrates that conventional and cell-based beef systems have overlapping and discrete food safety challenges. Our results highlight opportunities to intently design food safety into cellular agriculture processes as capacity, scalability, and affordability are realized.

P1-48 A Multiplex PCR Workflow for Quantification of *Salmonella* in Diverse Meats

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Introduction: Quantitative analysis enables food producers to monitor contamination trends and employ effective HACCP strategies within production facilities in support of the USDA's FSIS initiative to reduce final product contamination with *Salmonella* and minimize outbreaks in the USA.
Purpose: Following a 7-hour enrichment, ground meat matrices may be analyzed with the Thermo Scientific™ RapidFinder™ Quantitative Salmonella Species, Typhimurium and Enteritidis Multiplex PCR Kit to accurately predict the contamination level of *Salmonella* spp., *S*. Enteritidis and *S*. Typhimurium.
Methods: A total of 1,607 meat samples comprising ground turkey (n=546), chicken parts (n=519), chicken carcass rinse (n=109), ground pork (n=203) and ground beef (n=230) were evaluated with the PCR workflow. Where required, artificial contamination of 1 to 5,000 CFU per sample was conducted prior to enrichment with pre-warmed Buffered Peptone Water plus novobiocin (poultry) or pre-warmed Modified Tryptone Soya Broth (red meats) for 7 hours. Post-enrichment, the purpose-built software for the PCR workflow used predictive modelling to estimate the starting CFU per sample. Estimates were compared to the known artificial contamination level and relative accuracy reported. To enable accurate prediction, each matrix was tested against various matrix-specific parameters within the kit files of the software. The performance of the PCR workflow was also evaluated against the USDA FSIS MPN method.
Results: The contaminating CFU per sample was accurately estimated for all targets for all matrices using the PCR workflow, achieving results within 0.5 $\log_{10}$ for 81.5% of samples and within 1 log for 99.5% of samples of the known contamination level for the *Salmonella* species target. The correlation coefficients for red meats were 0.84, 0.99 and 0.96 for the *Salmonella* species, *S.* Enteritidis and *S.* Typhimurium targets respectively, similarly the correlation coefficients for poultry matrices were 0.95, 0.78 and 0.80, respectively.
Significance: Utilization of matrix-specific predictive modelling during quantitative analysis demonstrated that the PCR workflow accurately correlated predicted and known contamination levels in an 8-hour working shift from a single PCR tube.

P1-49 Quantification of *Salmonella* at Various Stages of Poultry Processing

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Introduction: The USDA's FSIS is driving initiatives to lower the risk of illness caused by *Salmonella* in poultry products by reviewing and improving the practices at slaughter and processing sites. Control and measurement strategies, including enhancement of strategies used during pre-harvest in the primary production setting, are critical for developing optimized strategies for control of *Salmonella* in the poultry food chain.
Purpose: This study evaluated performance of the Thermo Scientific™ RapidFinder™ Quantitative Salmonella Species, Typhimurium, and Enteritidis Multiplex PCR Kit for the quantification of *Salmonella* from poultry primary production samples, production rinses, and finished products.
Methods: 712 samples were evaluated comprising; poultry house boot socks (n=40), 30 mL production environment rinses (n=55), and poultry meat samples (n=617). Testing included both naturally and artificially contaminated samples enriched with pre-warmed Buffered Peptone Water (BPW) plus novobiocin, prior to testing with PCR. The relative accuracy of the PCR workflow was determined by comparing the test method estimated original contaminating CFU per sample to the results using the USDA FSIS MPN method and the known artificial contamination level.
Results: A total of 90.0%, 98.2%, and 99.8% of samples returned quantitative results for *Salmonella* species within 1.0 $\log_{10}$ of the MPN or known contamination level when *Salmonella* was detected for boot socks, in-production rinses and finished products respectively.
Significance: The data demonstrates that the PCR workflow accurately estimates *Salmonella* contamination level at multiple points in the slaughter and processing procedure; enabling poultry producers to respond to the USDA's FSIS initiative with this rapid and effective solution.

P1-50 AOAC-RI Validation of Hygiena™ BAX® System Salquant™ Methods for Poultry Rinsates, Ground Beef, Ground Pork, Beef Trim, Pork Trim, and Microtally™ Manual Sampling Devices on Beef and Pork Trim

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Introduction: Process control and final product decisions based only on prevalence have shown limitations in reducing consumer risk, therefore, adoption of validated quantification methodologies with low error and wide enumerable ranges should be utilized to make data-driven food safety decisions.
Purpose: The aim of this certification study was to compare the performance of the candidate method, BAX® System Real-Time PCR Assay for *Salmonella* (KIT2006) utilizing the SalQuant™ methodology, to the USDA-FSIS MLG Most Probable Number (MPN) quantitative reference method.
Methods: Twenty samples per matrix were divided into 4 inoculation levels with *Salmonella* ATCC strains at Low: 0.0–1.0 Log CFU/g(mL), Medium: 1.5–2.5 Log CFU/g(mL), High: 2.5–3.5 Log CFU/g(mL), and negative controls, then cold-stressed for 48-72-hours. After the stress period, sample processing followed the Hygiena™ Pathogen Quantification Procedures Manual with incubation at 42˚C for 6 or 8 hours for SalQuant™ with reference method performed in parallel. Candidate results were compared to reference results with statistical equivalence within 90% confidence intervals and mean difference of ± 0.50 Log_{10} CFU/g(mL) utilizing the AOAC-RI quantitative worksheet.
Results: The BAX® SalQuant™ candidate method was comparable to the USDA-FSIS MLG MPN reference method at all inoculation levels for poultry rinses, ground beef, ground pork, beef trim, pork trim, and MicroTally™ for beef and pork trim.
Significance: This certification provides the poultry, beef, and pork industries with an accurate, reliable, and validated quantification tool to reduce product hold-times, verify corrective actions, monitor process control, and promote faster data-driven diversion decisions which ultimately reduces consumer risk in animal protein products.

P1-51 Risks of *Salmonella* Foodborne Illness by Egg Consumption Patterns in Korea

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is the most commonly reported bacteria responsible for foodborne illness and is usually associated with eggs and egg products. Although *Salmonella* is heat-sensitive and easily destroyed with the mild-heat treatments for cooking, it can be found in cooked eggs due to inadequate cooking.
Purpose: This study estimated the risk of *Salmonella* outbreaks by consumption of eggs cooked with various methods.
Methods: Prevalence of *Salmonella* in 201 eggs was investigated by plating the samples on Xylose Lysine Deoxycholate agar. Data for storage temperature and time were collected, and probabilistic distributions for the data were determined, using @RISK program. Predictive models describing fates of *Salmonella* in eggs were developed, using the Baranyi model (primary) and polynomial model (secondary). Amounts and frequency of consumption were surveyed. Reductions in *Salmonella* cell counts by cooking methods (raw consumption, moist-heating, and dry-heating) were examined. A dose response model for *Salmonella* infection was searched. Subsequently, these data were used in a simulation model in @RISK to estimate the risks of *Salmonella* foodborne illness according to the egg consumption patterns.
Results: *Salmonella* was not detected in all samples. Thus, Beta distribution (1,202) estimated that the initial contamination level was to be -4.0 Log CFU/g. Temperature during transportation and market display were fitted with Pert distribution (0,4,15) and Uniform distribution (0,10), respectively. The average chicken consumption amount fitted by Gamma distribution [(1.0103,49.278,RiskShift(-0.00012681),RiskTruncate(0,400))] was 49.8 g at 60.1% of

Poster

frequency, and at the consumption phage, eggs were consumed raw (1.5%), and after dry-heating (57.5%) and moist-heating (41%). Beta-Poisson model [1-(1+Dose/4.4×10^5)$^{-0.89}$] was selected for dose response of *Salmonella*. The simulation with the data showed that the probability of *Salmonella* foodborne illness by the egg consumption was 6.8×10^{-10} per person per day in Korea.

Significance: This result could be used to estimate that the risk of *Salmonella* foodborne illness by egg consumption in Korea.

P1-52 Detection of *Salmonella* Typhi and *E. coli* O157:H7 within Liquid Whole Egg during Refrigerated Storage by Organoleptic Sensing

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✦ ***Undergraduate Student Award Entrant***

Introduction: Presence of bacterial pathogens in liquid whole eggs (LWE) is an ongoing concern, especially because pasteurization does not equate to sterilization or prevent post-pasteurization contamination. Product sampling may detect pathogen contamination, but there are economic incentives for non-destructive detection methodologies. Modern electronic-nose (E-nose) sensors may be able to differentiate between organoleptic profiles of LWE containing pathogenic and non-pathogenic microflora; however, this application needs investigation.

Purpose: Detect the presence of *Salmonella* Typhi and *E. coli* O157:H7 in LWE using organoleptic analysis and estimate population kinetics during refrigerated storage.

Methods: Triplicate batches of LWE were individually inoculated with attenuated *Salmonella* Typhi and a four-strain cocktail of *E. coli* O157:H7 (8.0 log(CFU/g)), then stored at 0 and 4°C for up to 28 d. Periodic samples were taken to quantify organoleptic profiles, via Principal Component Analysis (PCA), using a Sensigent M160 E-Nose, and to enumerate *E. coli* (plated on SMAC), *Salmonella*, and background flora (both plated on differential TSA).

Results: *Salmonella* populations decreased at a rate of 0.05±0.01 and 0.02±0.01 log(CFU/g)/day in LWE at 0 and 4°C, respectively. *E. coli* concentrations decreased at a rate of 0.02±0.01 and 0.03±0.01 log(CFU/g)/day in LWE at 0 and 4°C, respectively. Background flora, initially below the limit-of-detection, increased up to 6 log CFU/g at both temperatures by 28 d. The PCA of the organoleptic sensor profiles were significantly different ($p < 0.05$) between the LWE inoculated with *Salmonella*, *E. coli*, and control samples at 0.5 and 24 h post-inoculation. However, samples stored past 24 h could not differentiate between inoculated and control samples ($p > 0.05$) due to LWE spoilage during storage.

Significance: This project demonstrated a limited capacity of E-nose technology for differentiating between pathogen and non-pathogenic contamination of LWEs. However, the ability to detect organoleptic profiles of microflora in a post-pasteurized product may have other value-added applications.

P1-53 Efficacy of Organic Acid Treatments for the Reduction of *Listeria monocytogenes* on Hard-Boiled Eggs

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❖ ***Developing Scientist Entrant***

Introduction: Ready-to-eat hard-boiled eggs (HBE) are a popular and convenient choice for consumers and food servicers. Recent recalls of HBEs have highlighted the susceptibility of contamination with *Listeria monocytogenes*. HBEs are generally treated with antibacterials to ensure the safety and quality of the product. While citric acid is often used, research has determined it is not effective in some situations; therefore, the assessment of additional organic acids is necessary.

Purpose: To evaluate the efficacy of acetic, lactic, and malic acid treatment of HBEs to reduce the population of *L. monocytogenes*.

Methods: Fresh eggs were cooked in boiling water, peeled and stored at 4°C for 24 h before use. HBEs were dip-inoculated with a 4-strain cocktail of rifampicin-resistant *L. monocytogenes*, resulting in 8 log CFU/egg. Following air drying, HBEs were treated at 5 or 25°C with 2% acetic, malic, or lactic acid. *L. monocytogenes* populations were enumerated at intervals up to 24 h by homogenization of HBEs with BLEB and cultivation on BHIA^{rif}. Triplicate eggs were assessed at each timepoint and three independent trials were conducted. Data were analyzed by Student's t-test, $p \leq 0.05$.

Results: The initial inoculation level of *L. monocytogenes* on HBEs was 8.27± 0.37 log CFU/egg. After 24 h treatment, all *L. monocytogenes* populations were significantly reduced on HBEs. At 5°C, populations were reduced by 3.15±0.70, 3.46±0.02, and 4.78±0.23 log CFU/egg for acetic, lactic, and malic acid treatment, respectively. At 25°C, *L. monocytogenes* was reduced by 5.15±0.15, 4.47±0.23, and 4.76±0.26 CFU/egg. Compared to 5°C, a significantly higher population reduction occurred with acetic and lactic acids when treatment occurred at 25°C.

Significance: The results of this study aid in understanding the efficacy of organic acids against *L. monocytogenes* on HBEs. Results are useful in the development of preventive controls and guidelines to ensure the safety of HBEs.

P1-54 Validation of in-Shell Whole Egg Pasteurization to Achieve a 5-Log Reduction in *Salmonella* and Study Its Outgrowth at 4°C and 20°C during 10 Weeks of Storage

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* spp. can invade through the shell from fecal matter causing foodborne illness. In-shell whole egg pasteurization can eliminate *Salmonella* inside the egg while reducing food safety risk.

Purpose: To validate pasteurization parameters to eliminate 5 logs of *Salmonella* per egg and study the pathogen's outgrowth during refrigerated (4 ºC) and moderate temperature abuse (20 ºC) storage for 10 weeks.

Methods: A cocktail mixture of Salmonella (*Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Heidelberg) were inoculated into *Salmonella*-negative eggs (n=297/trial x 2 trials). Eggs were inoculated in close proximity to the vitelline membrane with 0.1 mL of the mixed inoculum using a sterile syringe (target inoculum: 5 log CFU/egg). Eggs (n = 30 eggs/rack) were placed onto three trolleys onto top, middle, and bottom racks of the pasteurizer and pasteurized for 1h with steam at 56.1 ºC (133.0 ºF). Post-pasteurized eggs from each rack and trolley evenly distributed and stored at 4 ºC and 20 ºC for 10 weeks. Inoculated but unpasteurized eggs (n=27) and post-pasteurized eggs (n=27/temperature/trial) were analyzed using a spread plating on trypticase soy agar with a xylose lysine deoxycholate agar overlay and incubating the plates at 37 ºC for 24h. Viable colonies were counted a reported as log CFU/egg. Statistical analysis was conducted using ANOVA with Tukey's HSD to determine significant differences in the means at $p<0.05$.

Results: Pasteurization at 56.1 ºC (133.0 ºF) eliminated 5 log CFU/egg of *Salmonella* inoculated in the egg. The pathogen did not show any outgrowth during refrigerated (4 ºC) and temperature abuse (20 ºC) storage for 10 weeks.

Significance: The study validates the in-shell whole egg pasteurization process to eliminate 5-logs of *Salmonella* in the eggs.

P1-55 Influence of Beef Carcass Exudate on Peroxyacetic Acid Tolerance in Shiga-Toxin Producing *E. coli*

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❖ ***Developing Scientist Entrant***

Introduction: Environmental conditions and strain fitness could play an important role in sanitizer tolerance among foodborne pathogens.

Poster

Purpose: The influences of beef carcass exudates on the bactericidal concentration of peroxyacetic acid (PAA) against Shiga-Toxin Producing *E. coli* (STEC) serogroups was evaluated.

Methods: STEC serogroups – *E. coli* O157:H7 H1730, *E. coli* O157:H7 43895, *E. coli* O121 and *E. coli* O26 were suspended in beef exudate, tryptic soy broth (TSB), sterile deionized water (SDW), and phosphate buffered saline (PBS) at a concentration of 5 log CFU/ml and exposed to PAA concentrations ranging from 15 ppm to 350 ppm for 5 min. The cells were then spun down and washed to remove residual antimicrobial and then grown in TSB for 24 h at 37°C during which the recovery of cells was monitored through turbidimetric measurements at 600 nm. The lowest concentration of PAA that resulted in the absence of bacterial growth was considered to be the minimum bactericidal exposure time after 5 min of exposure. Significant differences were analyzed using a one-way ANOVA.

Results: The average bactericidal concentrations of PAA in beef exudates and TSB were 211.11 ± 31.78 ppm and 210.00 ± 44.72 ppm respectively and were significantly higher than the average bactericidal concentrations in SDW (60.3 ± 12.58 ppm) and PBS (59.3 ± 11.51 ppm) ($P < 0.05$). *E. coli* O26 was the most PAA tolerant serogroup in media with high nutrient content/organic load, requiring the highest bactericidal concentration of 200.00 ± 0.00 ppm to inhibit its growth in beef exudate and TSB. *E. coli* O121was the most PAA tolerant serogroup in media with low nutrient content/organic load and required 60.00 ± 0.00 ppm in PBS and SDW.

Significance: Differences in PAA tolerance among STEC could depend on serogroup and environmental conditions such as presence of organic matter and nutrients.

P1-56 *Salmonella* Serotypes Vary in Ability to Use Dust as a Vehicle for Produce Contamination

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Introduction: Dust is ubiquitous in the pre-harvest field environment. Wind driven dust dispersal after processes such as manure application could result in the transfer of foodborne pathogens on to produce surfaces.

Purpose: The potential for dust to serve as a vehicle of produce cross contamination was tested. Differences in the ability to survive on dust and transfer to produce surfaces among Salmonella serotypes Newport, Typhimurium and Oranienburg was also determined.

Methods: Three particulate sizes of soil, (a) ≥3,360 µm, (b) >100 µm and (c) <100 µm were seeded by placing 5g of dust on a 24h culture of *S*. Newport, *S*. Typhimurium and mixed using a sterile glass rod. The inoculated dust was removed using a cell scraper and then mixed with 45 g of dust by hand massaging to obtain a final concentration of 8-9 log CFU/g. The populations from the dust samples were enumerated at 0, 4 and 24 h. Dust was dispersed on to the surfaces of bell peppers and tomatoes using pressurized air, following which, the population of pathogen from the fruit surfaces was enumerated.

Results: After 24h, there was a 2.59±0.91 log CFU/g difference in population between *S*. Typhimurium and *S*. Newport and in dust ($p<0.05$). The size of the dust particle did not influence bacterial survival. Tomatoes exposed to <100 µm-*S*. Typhimurium contaminated dust were the most contaminated (3.44±0.91 log CFU/g). Bell peppers were most contaminated (3.3 log CFU/g) after contact with both >100 µm-S. Typhimurium and S. Newport contaminated dust.

Significance: Dust could serve as a vehicle for *Salmonella* transfer on to produce surfaces.

P1-57 *Salmonella* Quantification (SalQuant™) with the Hygiena™ BAX® System for Turkey Carcass Swabs

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Introduction: Prevalence testing in poultry for *Salmonella* provides important data but consumer illness caused by this pathogen still represents a significant portion of foodborne illnesses each year. The poultry industry has therefore been seeking analytical methods to quantify *Salmonella* pre- and post-harvest.

Purpose: 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 turkey carcass swabs.

Methods: Fifteen turkey carcass swabs provided by an industry partner were inoculated with a cold-stressed *Salmonella* culture creating 3 replicates across 5 levels (1, 10, 100, 1,000, and 10,000 CFU/mL). One additional sample was reserved for a negative control. Immediately following inoculation, swabs were enriched in 50 mL of pre-warmed (42°C) BPW, incubated for 6-7 hours and then tested in quintuplet using real-time PCR. At the same time, a 3-tube x 5-dilution MPN was conducted for each inoculation level following the USDA FSIS Appendix 2.05. At each timepoint, total positives and cycle threshold values (CT) for the real-time PCR were compared to known inoculation levels in order to create a best-fit equation with the goal of achieving an $R^2 \geq 0.75$ and $\log_{10}$ RMSE ≤ 0.60 utilizing JMP® v. 15. Estimations from the SalQuant™ linear-fit model were compared to MPN based on a 95% confidence interval.

Results: The 6-hour enrichment produced the best linear fit equation with a R^2 of 0.896 and $\log_{10}$ RMSE of 0.478. When comparing PCR results to traditional MPN, there were no statistical differences.

Significance: These results demonstrate that complete quantification of *Salmonella* from 0.0 to 4.0 log CFU/mL can be achieved using the BAX® System Real-Time PCR assay.

P1-58 *Salmonella* Quantification (SalQuantTM) with the HygienaTM BAX(R) System for Poultry Crops and Lungs

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Introduction: *Salmonella* is capable of colonizing and spreading throughout many internal organs in poultry. To prevent further dissemination during slaughter and processing, the pathogen status of the flock should be determined prior to transport. Quantitative data for on-farm sampling can provide the most accurate and reliable information for decision-making.

Purpose: The purpose of this study was to develop and verify the BAX® System Real-Time PCR assay for *Salmonella* quantification (SalQuant™) in poultry crops and lungs.

Methods: Poultry crops and lungs provided by an industry partner were pre-screened for naturally occurring *Salmonella*. Reserved homogenates that produced negative results were combined together to create a bulk negative slurry. Fifteen test portions (30 mL) from each slurry were inoculated with a cold-stressed *Salmonella* Typhimurium across five inoculation levels (1, 10, 100, 1,000, and 10,000 CFU/mL). Samples were combined with equal volumes of pre-warmed MP media with Quant™ Solution, incubated at 42°C for 4-24 hours, and tested by real-time PCR in quintuplet. The best linear fit equation was determined through R^2 and $\log_{10}$ RMSE using JMP® v. 15. Estimations from the linear fit equation and MPN were statistically compared using a 95% confidence interval.

Results: The 6-hour enrichment produced the best linear fit equation for both matrices. For crops, R^2 was 0.91 and $\log_{10}$ RMSE was 0.35. For lungs, R^2 was 0.94 and $\log_{10}$ RMSE was 0.28. When compared to MPN, PCR results displayed no statistical difference.

Significance: These studies demonstrate accurate and rapid quantification of *Salmonella* with an enumerable range of 1 – 10,000 CFU/g from poultry crops and lungs using the BAX® System. Processors that utilize these protocols for organs can apply SalQuant™ as an analytical tool to identify positive flocks and improve interventions during grow out to reduce incoming levels of *Salmonella* to the plant.

P1-59 Detection of *Campylobacter* from Boot Swabs Using the Hygiena™ BAX® System Real-Time PCR Assay

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Introduction: Distribution studies have demonstrated a strong association between pre- and post-harvest *Campylobacter* loads in poultry. Regulatory testing of *Campylobacter* in poultry has largely focused on processing plants, however if an integrated approach was used to analyze both on the farm and processing samples, strategies to control and reduce *Campylobacter* can be improved.
Purpose: The purpose of this study was to evaluate a real-time PCR method for the detection of *Campylobacter* from boot swabs compared to the culture.
Methods: Eighteen boot swabs were provided by an industry partner and evaluated for naturally occurring *Campylobacter*. Samples were enriched within 48 hours of collection using 100 mL of pre-warmed (37°C) BPW. After briefly homogenizing, 27.5 mL was transferred to a 50 mL conical tube and 27.5 mL of pre-warmed (42°C) double-strength Bolton broth with 2X supplement was added. Tubes were tightly capped and incubated aerobically at 42°C for 48 hours before analysis by real-time PCR and confirmation using modified Charcoal Cefoperazone Deoycholate Agar (mCCDA) and Campy Cefex Agar.
Results: *Campylobacter* was detected by real-time PCR in 9/18 samples at 48 hours. All PCR results matched culture with 100% agreement. Statistical significance using the probability of detection (POD) determined there were no differences between PCR and culture.
Significance: Overall, these results demonstrate the ability of the BAX® System to accurately detect *Campylobacter* from boot swabs providing the industry with way to assess prevalence and implement effective farm management practices.

P1-60 Detection of *E. coli* O157:H7 and *Salmonella* in Baby Carrots Using the Hygiena™ BAX® System

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Introduction: Fresh fruits and vegetables are a staple in healthy lifestyles; however, reports of food-borne disease outbreaks have been substantially increasing in recent years. Since this produce is sold and consumed raw and unprocessed, microbial hazards need to be identified.
Purpose: This study was designed to evaluate the performance of a rapid PCR method for the detection of *E. coli* O157:H7 and *Salmonella* in organic cut baby carrots.
Methods: An unpaired matrix study for organic cut baby carrots was performed following the technical guidelines in AOAC INTERNATIONAL Official Methods of Analysis Appendix J to compare two real-time PCR assays for *E. coli* O157:H7 and one real-time PCR assay for *Salmonella* to the appropriate US FDA BAM reference method. Twenty-five-gram test portions were co-inoculated with *E. coli* O157:H7 and *Salmonella* at low and high levels; one level was left uncontaminated as a control. Half of the samples were enriched and assayed by the test method and half by the reference method.
Results: Fractional recovery was met for the low-level inoculated test portions and all real-time PCR results were determined correctly by culture. There was no significant difference between the test method and the reference method for the detection of either *E. coli* O157:H7 or *Salmonella*.
Significance: This study shows that the BAX® System Real-Time PCR assays for *E. coli* O157:H7, *E. coli* O157:H7 Exact and *Salmonella* are indistinguishable from the FDA BAM reference methods and are fit-for-use to rapidly screen organic baby carrots for the detection of *E. coli* O157:H7 and *Salmonella* using a single enrichment.

P1-61 Associations between Soil Nutrient Levels with *Escherichia coli* and Total Coliform Concentrations and *Listeria* and *Salmonella* Prevalence

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❖ ***Developing Scientist Entrant***

Introduction: Prior research has shown foodborne pathogens and microbial indicator organisms can survive and persist in agricultural soils from produce production environments.
Purpose: The purpose of this study was to (i) determine the prevalence of *Listeria* and *Salmonella*, and concentration of generic *Escherichia coli* (gEC)and total coliforms (TC), and (ii) investigate associations between each microbial target and soil macro- and micronutrients in soil from Virginia produce farms.
Methods: Five soil samples were collected from 20 plots ($25m^2$) that were selected from three produce farms in different VA regions (western, northern, and eastern VA). The five soil samples from each plot were pooled in equal quantities to form one sample per plot and tested for each microbial target. Samples (25g) were processed for *Listeria* and *Salmonella* using a modified FDA BAM method, while samples (5g) were enumerated for gEC and TC using Petrifilm. Presumptive positive samples were confirmed using a single gene target by PCR. Factors (e.g., macro – and micronutrients) were examined for their association with each microbial target using univariate logistic (*Listeria, Salmonella*) or linear (gEC, TC) regression.
Results: A total of 60 samples were analyzed for *Listeria, Salmonella*, gEC and TC. The prevalence of *Salmonella* and *Listeria* spp. was 10.0% (6/60) and 8.3% (5/60), respectively. Interestingly, all *Salmonella*-positive samples were from one farm in eastern VA. The average gEC and TC concentration across all samples was ca. 1.0 with range 0.95 to 4.0, and 4.0 with range 1.2 to 5.7 log CFU/1 mL (n=60), respectively. Organic matter (%) influenced the likelihood of detecting *Listeria,* while phosphorus (mg/kg), pH, and manganese (mg/kg) influenced the likelihood of detecting *Salmonella* ($p \leq 0.10$). For example, plots with more organic matter increased the likelihood of detecting a *Listeria* positive sample.
Significance: Preliminary findings suggest soil nutrient data may be associated with *Listeria* and *Salmonella* prevalence, and other microbial targets.

P1-62 Evaluating Antimicrobial Efficacy of Cover Crops to Reduce Pathogen Load in Contaminated Soil

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Introduction: The contamination of fresh produce can occur at any time during the produce supply chain. Beyond agricultural water runoff events (flooding), farm soil management can constitute a high risk for pathogen transmission due to the popularity of practices like mixed crop-livestock farming and application of animal wastes (i.e., manure) for fertilization. The proximity of produce to contaminated soil and the concentration of pathogens within the soil are two risk factors contributing to the probability of produce contamination during field production.
Purpose: This study aims to determine the microbial reduction of generic *Escherichia coli* using three different cover crops.
Methods: Three cover crops including mustard greens (*Brassica juncea*, 'Kodiak'), sunn hemp (*Crotalaria juncea*), and buckwheat (*Fagopyrum esculentum*) were used for this study. Autoclaved farm soil was inoculated with rifampicin resistant generic *E. coli* (ATCC 25922) and experiment was setup in the food microbiology lab at K-State Olathe . Sterile DI water was added weekly to maintain the soil moisture. The data on pot weight, room temperature, humidity, and generic *E. coli* survivor population were recorded on day 0, 4, 10, 15, 20, 30, 40. Agar based methodology (Limit of Detection 25CFU/mL) was used to enumerate bacterial population.
Results: From day 0 to day 40, all three cover crops reduced generic *E. coli* population as compared to control (buckwheat > mustard greens > sunn hemp). The sampling day ($p<0.0001$), cover crop type ($p<0.0001$) and the interaction between sampling day*cover crop type ($p<0.0001$) were all statistically significant.
Significance: The study demonstrates the antimicrobial efficacy of cover crops and contributes to increased understanding of human pathogen survival in contaminated soils. Future studies should include identifying the best performing cover crops in regard to their antimicrobial effects, and help develop a targeted bio-mitigation strategy to improve food safety on farm.

P1-63 Spatial Versus Non-Spatial Variance in *E. coli* Levels Differs by Scale of Analysis in Virginia Ponds

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Introduction: While produce safety programs monitor fecal indicator bacteria (FIB) levels to determine surface water quality, recent studies reported variations at different scales of analysis (SOA).

Purpose: To examine how SOA affects the association between environmental factors and FIB levels, and the variance attributable to spatial versus temporal sources.

Methods: Over 20 sampling events, grab samples were collected from 30 sites representing nine Virginia ponds (N=600). *E. coli* (EC) levels were enumerated, and physicochemical, weather, and environmental data were collected for each sampling event. Bayesian regressions quantified the variance ratio (variance in EC levels attributable to spatial versus temporal source), and associations between environmental factors and EC levels. Analyses were performed separately for each pond with ≥3 sampling sites (intra-pond, N=5) and using all available data (inter-pond).

Results: More variance in EC levels was attributable to spatial than non-spatial sources in the inter-pond model (ratio=1.62), while the variance ratio was <1.0 for all five intra-pond models. Similarly, inter-pond EC levels became spatially independent for sampling sites >1,512m apart [Interquartile Range (IQR)=1290-2377], while distances varied between 55m and 90m for intra-pond models. Rainfall had a substantial positive effect on EC levels in the inter-pond models (0.45-0.79 log) and four of the five intra-pond models (0.40-1.23 log). The presence and strength of associations between EC levels and other factors varied between models. For instance, the presence of waterfowl was associated with EC levels in one intra-pond model, while the presence of submerged or emergent vegetation was associated with EC levels in two intra-pond models.

Significance: This study suggests that (i) more variance in EC levels is attributable to temporal factors within individual ponds and spatial factors when multiple ponds are considered, and (ii) EC levels are spatially independent for sites >100m apart within individual ponds and these sites should be treated as separate sources for monitoring purposes.

P1-64 Characterization of *Escherichia coli* Isolates from Produce Irrigation Water in Kansas and Missouri by Whole-Genome Sequencing

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Introduction: Irrigation water has been found to be a major source of pathogens that can grow and persist on produce. Contaminated water has been the reported source of pathogenic *Escherichia coli* in recent produce-related outbreaks. It can subsist for weeks depending on temperature and other environmental conditions, posing an increased risk for produce safety.

Purpose: The purpose of this study was to characterize *E. coli* isolates from produce irrigation water sources using Whole-Genome Sequencing (WGS).

Methods: Ground and surface water samples were collected quarterly from five farms in Missouri and Kansas over a one-year period. Each sample was filtered, and *E. coli* was isolated using EPA Method 1603. Samples were then confirmed by Polymerase Chain Reaction (PCR). WGS was carried out using an Illumina MiSeq system. *De novo* genome assemblies were obtained with the Shovill pipeline version 0.9. NCBI Pathogen Detection was used to detect antimicrobial resistant (AMR) genes and single nucleotide polymorphism (SNP) clusters.

Results: A total of 238 PCR confirmed *E. coli* samples were collected in this study. *E. coli* levels in irrigation water were affected by ambient temperatures. Confirmed *E. coli* numbers were higher during spring and summer compared to winter ($P< 0.05$). An ample amount of diverse serotypes were observed from the collected samples. Isolates were divided across SNP clusters and using the NCBI database, strains were matched with other environmental and clinical isolates. AMR analysis showed that 40% of the strains carried at least one antimicrobial resistant gene.

Significance: Agricultural water source and climate conditions should be considered by growers when assessing produce safety. Recognizing the diversity of *E. coli* serotypes may be employed in developing guidance on agricultural water assessments as proposed in the new agricultural water rule.

P1-65 Not Dead Yet: Generic *E. coli* Die-Off Rates That are Faster Than Expected and the Importance of Accounting for Stress-Resistant Bacterial Populations

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Introduction: Through the "Food Safety and Modernization Act" agricultural water has been identified as a major source of microbial contamination. Current regulations require surface water that comes in contact with edible portions of produce to meet mean concentrations of < 126 generic *E. coli* CFU/100ml. This regulation assumes a 0.5 log/day die-off rate under natural field conditions.

Purpose: Die-off rates are highly variable by growing region and time of year. Here, we evaluated generic *E. coli* inactivation under field conditions using artificially contaminated tomatoes with non-linear biphasic models that account for the effect of site and season.

Methods: Field trials were conducted at two sites in Virginia during 2015 and 2016. Mature tomatoes were inoculated with 4 $\log_{10}$ CFU of generic *E. coli* per tomato. Following, tomatoes were collected and processed in the laboratory for enumeration using both CFU and MPN. We used R package 'nlme' to fit biphasic models to log transformed counts to estimate inactivation rates of both stress-susceptible (*ks*) and -resistant (*kr*) populations.

Results: We found that combined site and seasonal effects yielded the most parsimonious model, but growing season contributed more significantly to model fit than site. Overall, sensitive *E.coli* populations were decimated within 1-2 days, with reduction rates ranging from -1.55 $\log_{10}$/day (site1/summer) to -2.40 $\log_{10}$/day (site1/fall). Resistant *E. coli* populations, making up 20% of the total population decreased marginally or not at all over a 4-6 day period.

Significance: Our results suggest that current FSMA regulation (1) underestimate inactivation rates of generic *E. coli* in the field and (2) lack considerations of biologically significant differences between stress-susceptible and resistant bacterial population. Stress-resistant *E. coli* that endure beyond the initial 1-2 day die-off of the susceptible population may be driving the risk of foodborne illness, and warrant a closer consideration in U.S. regulations for consumer safety of produce.

P1-66 Cross-Contamination from Environmental Matrices: A Vehicle for Transfer of Foodborne Pathogens to Melons Grown in Various Regions of the United States

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Introduction: Melons are one of the fruits commonly purchased in the US since consumers like these healthy fruits. The high consumption of melons along with their rough surface make these fruits risky for foodborne outbreaks.

Purpose: Investigate the risks of cross-contamination of *Salmonella* Newport and *Listeria monocytogenes* from soil and dust onto melons. Determine melon varieties/hybrids that are more or less prone for cross-contamination.

Methods: Experimental melon hybrids/varieties grown in four states (AZ, CA, IN and TX) were evaluated. Soil was obtained by sieving melon-field dirt collected from Yuma, AZ and further sieved to create dust. Rinds were placed on soil inoculated with *S.* Newport or *L. monocytogenes* and allowed to

attach for 1 h. Inoculated dust was sprayed onto the melon rind in a specially designed chamber. Melon rinds were removed and sonicated in Phosphate Buffered Saline (PBS), while soil and dust samples were vortexed in PBS. *S.* Newport and *L. monocytogenes* were recovered from soil, dust and melon rind by plating on Xylose Lysine Desoxycholate and Modified Oxford agars with antibiotics. Transfer (%) was calculated using the formula: (Population on Destination×100) ÷ (Population on Destination+Population on Source).

Results: Transfer (%) from soil ranged 0.00188%-3.36186% and 0.00132%-0.48142% for *Salmonella* Newport and *L. monocytogenes*, respectively. Dust transfer (%) had a range from 0.000009%-0.009421% and 0.000024%-0.000606% for *Salmonella* Newport and *L. monocytogenes*, respectively. Transfer (%) was higher from soil than dust. Hybrid melon TH5 (IN) had the highest transfer (%) for *Salmonella* (3.36186±0.07636%) and hybrid melon TH1 (IN) for *L. monocytogenes* (0.48142%±0.04161%), from soil. From dust, highest transfer (%) for *Salmonella* (0.009421%±0.000006%) was on hybrid melon TH1 (IN) and for *L. monocytogenes* it was on hybrid melon TH14 (IN) (0.000606%±0.000227%).

Significance: Results will help understand risk of cross-contamination of melons from environmental matrices due to foodborne pathogens. Data can be used for a science-based risk analysis.

P1-67 Investigation of the Effect of Growth Media on the Survival of *E. coli* in Agricultural Soil

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Introduction: Many studies have examined the survival of bacterial foodborne pathogens in agricultural soils to determine the likelihood to transfer to produce. Results of these studies may be influenced by conditions used to prepare bacterial cultures.

Purpose: (i) To determine the growth rate of *E. coli* in three different growth media; tryptic soy agar (TSA), tryptic soy broth (TSB), and poultry pellet extract (PPE); (ii) to evaluate influence of growth media on the subsequent survival of *E. coli* in agricultural soil.

Methods: Poultry pellet extract (PPE) was prepared by filter-sterilizing a 1:5 suspension of heat-treated poultry pellets in sterile water. Rifampicin-resistant *E. coli* TVS 353 was grown in TSA-R, TSB-R, and PPE-R, supplemented with 80 µg/ml rifampin (R), at 35°C. Growth curves were determined by quantifying *E. coli* at 0, 4, 8, 16, 24 and 36 h. Sandy loam soil was inoculated with a culture grown from growth media and stored at 25°C, and *E. coli* levels were determined at 0, 1, 3, 7, 14, 21, 28, and 42 d. Growth curves and survival models were generated using DMFit and GinaFit respectively.

Results: *E. coli* growth rates were 0.88, 0.77, and 0.69 log CFU/ml/hr in TSA-R, TSB-R, and PPE-R respectively. *E. coli* populations in stationary phase were greater when grown in TSA-R (9.4 log CFU/ml) and TSB-R and (9.1 log CFU/ml) compared to PPE-R (7.9 log CFU/ml). *E. coli* populations were ca. 6.0 log CFU/g soil immediately following inoculation and declined by 2 log CFU/g in soils for all growth media from 3-7d. *E. coli* die-off rates slowed from 7 to 42d. Growth media type had no significant (*P>0.05*) impact on the die-off rate of *E. coli* in soil.

Significance: Media significantly affected E. coli growth levels, but soil-specific factors minimized any effect that growth media had when *E. coli* was introduced in soils.

P1-68 Survival of *Escherichia coli* and Changes in Physicochemical Parameters in Aquaponic Systems during Basil and Lettuce Production

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Introduction: Aquaponics, a soilless, circular economy production system, integrates aquaculture and hydroponics to provide local fresh produce while conserving natural resources (land, soil, water, air). Plant nutrients are supplied through bacterial transformation and metabolism of fish excreta, but limited data are available to assess food safety risks from aquaponic systems (APs).

Purpose: To evaluate the survival of *Escherichia coli TVS 354* populations and associated physicochemical parameters in simulated raft APs.

Methods: A 32-day experiment was conducted using 12 recirculating APs, with two *E.coli* inoculated treatments and an uninoculated control (n=4 replicates/AP). Each system had four (~1g each) goldfish (*Carassius auratus*)/37L tank; a 3-step trickling biofilter; two basil (*Ocimum basilicum*), and two lettuce (*Lactuca sativa var. Truchas)*/15L plants/hydroponic tank. Treatments were inoculated with *E.coli*^{Rifr} TVS 354 at 2 Log CFU/mL (low dose) and 4 Log CFU/mL (high dose). *E.coli*^{Rifr} populations, mesophilic aerobic counts (APC), and physicochemical parameters were measured in fish tank water samples, and biofilters until plant harvest. *E.coli*^{Rifr} (MacConkey Agar-Rifampicin spread plates) and APC (Petrifilm®) colony counts from basil and lettuce shoots and roots were determined at harvest. Biofilter *E.coli*^{Rifr} were assayed by MPN-TSB^{Rif} and MacConkey Agar-Rifampicin spread plates.

Results: *E.coli*^{Rifr} populations in fish tanks declined significantly (P <0.05) within 24h post-inoculation and were undetectable 24h and 72h after inoculation; declines were similar for high and low-dose treatments. However, *E.coli* populations were detected in biofilters throughout the study. Also, *E.coli*^{Rifr} was recovered from basil roots, lettuce leaves and roots at harvest, although undetected in bulk water samples. Water acidity increased over time, but other physicochemical parameters remained similar (P>0.05) in all treatments.

Significance: Results provide new insights into *E.coli* survival across APs compartments (fish tank, biofilter, and hydroponic), harvested plants, and their associated physicochemical conditions, which indicate potential harborage risks to mitigate.

P1-69 Aggregative Swab Sampling Performs No Worse Than Composite Tissue Sampling in Recovering Quality and Safety Indicator Bacteria from Commercial Romaine Lettuce Fields

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Introduction: Aggregative swab sampling over product instead of collecting physical samples is a new sampling technique used in the meat industry. The adoption of this sampling technology in the fresh produce industry presents an opportunity for a potentially more powerful pre-harvest sampling alternative for pathogen detection.

Purpose: This study aims to compare current pre-harvest composite tissue sampling strategies to aggregative swab sampling, by evaluating recovery of quality and safety indicator bacteria from commercial fields, to justify future, more expensive, work on pathogen recovery.

Methods: Two field trials were performed in commercial fields with beds planted with Romaine lettuce in Salinas, California. Each trial consisted of collecting: (1) Aggregative Swab samples (AS) (n=30), (2) Composite Tissue samples (n= 30. Each of 60 tissue grabs for a total of 150g per sample) (CT), and (3) High Resolution composite tissue samples (n=72. Each of 25 g per sample) (HR). Samples were tested for quality metrics: aerobic plate counts (APC), and safety indicators: Coliform and generic *Escherichia coli* counts. Means and variances of the counts were analyzed in ANOVA and F-tests, respectively.

Results: AS showed significantly (p<0.05) higher mean APC 5.58±0.54 Log(CFU/g), compared to both CT and HR, which showed 4.48±0.27 Log(CFU/g), and 4.27±0.32 Log(CFU/g), respectively. Variance was significantly higher for AS than both tissue samples(p<0.05). Coliform detection was significantly higher in tissue samples (p<0.05), where CT recovered 2.45±1.29 Log(CFU/g) and HR recovered 1.81± 0.93 Log(CFU/g), compared to 1.23±0.36 Log(CFU/g) recovered in AS. But, critically, variance in coliform count was significantly (p<0.05) lower in aggregative swabs. No generic *E. coli* was detected in any of the samples tested.

Significance: This study suggests that aggregative swab sampling is at least not worse recovering quality and safety indicator bacteria compared to current tissue grab sampling. It justifies future work on pathogen detection.

Poster

P1-70 Presence and Persistence of Generic *E. coli*, STEC, and *Listeria monocytogenes* in Certified Organic Integrated-Crop Livestock Farm Spinach Fields in California and Minnesota

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❖ ***Developing Scientist Entrant***

Introduction: Integrated crop-livestock farming (ICLF) enhances soil health and quality of organic fields. However, untreated animal manure may mediate transfer of foodborne pathogens to fresh produce through soil contact.

Purpose: Assess the presence/persistence and prevalence of generic *E. coli* (gEc) and foodborne pathogens in spinach field soils where sheep graze cover-crop.

Methods: In 2021, a randomized complete block experiment (n=4 replicates), with winter cover-crop, grazed with sheep (WG), tilled (WT), and fallow (WF) treatments was conducted on certified organic spinach fields in CA and MN. Thirty-six soil samples were collected on 0-, 7-, 30-, 60-, 90-, and 120-days post-grazing (DPG). Soil, pre- and post-grazing feces, and spinach samples were assayed for gEc (Most Probable Number (MPN)), Non-O157 Shiga toxin-producing *E. coli* (STEC), and *Listeria monocytogenes* (*Lm*).

Results: Descriptive statistics and ANOVA were used to summarize pathogen prevalence and compare mean MPNs of gEc (log(MPN/g)) recovered from soil and spinach sample treatments collected over time at each sampling period from the treatments. In CA and MN, the mean gEc MPNs were highest at 30-DPG in WG, but constant low levels in WF or WT. A significant difference in gEc MPNs among treatments was observed at 30-DPG in CA, but 7-60-DPG in MN. No STEC or *Lm* were detected in any soil or spinach samples. In feces, the prevalence of STEC at 0-DPG was 30% (6/20) in CA and 5% (1/20) in MN. No *Lm* were detected in CA, nor in MN.

Significance: Results show that gEc populations in WG soil peaked at 30 DPG and declined significantly thereafter, resulting in no STEC or *Lm* contamination detected in organic plots by 90-120-DPG. Data indicate that ICLF complies with NOP 90-120 day wait-period between manuring and harvesting. Additional studies are needed to assess the effect of different climate, animals, and soil conditions on pathogen persistence.

P1-71 Prevalence of *Escherichia coli* and Coliform Bacteria in Lettuce and Soil Samples as a Result of the Use of Organic Fertilizers in Cambodia

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❖ ***Developing Scientist Entrant***

Introduction: Fresh manure as well as animal waste "compost" are commonly used for vegetable cultivation by Cambodian farmers (33% and 11%, respectively). While organic fertilizers such as manure and compost offer many advantages, they can also be sources of bacterial contamination such as coliforms and *E. coli*.

Purpose: This study evaluated the level of contamination of coliforms and *E. coli* in soil and lettuce samples when using manure or compost as organic fertilizers in Cambodia.

Methods: Research plots were randomly assigned treatments of organic fertilizers (manure and compost), and inorganic fertilizer (control). Organic fertilizer samples were collected on day 0 prior to placement on research plots, soil samples were collected from research plots on day 0 and day 30 and lettuce samples were collected on day 30. Samples were processed and methods for microbial enumeration of coliforms and *E. coli* were conducted using ECC petrifilms™. Results were analyzed using the GLM model in a randomized complete block design in Statistical Analysis System (SAS®) Studio with a level of significance of $P < 0.05$

Results: On day 0 there were higher counts of coliforms (5.78 log CFU/g) and *E. coli* (2.44 log CFU/g) in manure than in compost (4.31 and 0.12 log CFU/g, respectively). Soil samples treated with manure and compost showed statistically significant ($P < 0.05$) growth of *E. coli* after 30 days. Additionally, soil samples treated with organic and inorganic fertilizers showed statistically significant differences in coliforms and *E. coli* load after 30 days. Lettuces samples showed no statistical difference between organic and inorganic fertilizers on the load of coliforms and *E. coli*.

Significance: More research is needed to understand the transmission of bacteria between organic fertilizers, soil and produce, the present study represents an initial baseline for the impact of using organic vs. inorganic fertilizer in coliforms and *E. coli* loads in Cambodia.

P1-72 Efficacy of Nanoemulsified Benzyl Isothiocyanate for Controlling *Escherichia coli* O157 on Spinach at the Pre-Harvest Level

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Introduction: Recent outbreaks linked to the contaminated spinach highlight the need for identifying effective natural approaches to improve produce safety at pre-harvest level.

Purpose: Efficacy of nanoemulsified benzyl isothiocyanate (nano BIT) as a pre-harvest antimicrobial treatment against *Escherichia coli* O157 on spinach grown in high tunnel was investigated.

Methods: Spinach cultivars "Lakeside", "Matador", "Butterfly", and "Regiment" grown in high tunnel were spray-inoculated with *E. coli* O157:H12 (5.5 log CFU/g) as non-pathogenic surrogate for *E. coli* O157:H7 five days prior to harvest. Inoculated spinach plants were spray-treated with 0.5% pure or nano BIT. On 0, 3, and 5 days, four samples from each group (20 g/sample; N=144) were collected and surviving *E. coli* O157:H12 populations were determined by plating on selective agar. The droplet size of nano BIT was determined by using Zetasizer. Data were analyzed by SAS and the differences were detected at $P<0.05$ using Fisher's least significance test.

Results: Both pure and nano BIT immediately reduced *E. coli* O157:H12 by 1.0 log CFU/g on leaves of all spinach cultivars compared to control spinach leaves on day 0 ($P<0.05$). On day 3, nano BIT exerted significantly greater antimicrobial effect than the pure BIT. Pure BIT significantly reduced *E. coli* O157:H12 populations by 1.4 and 2 log CFU/g on "Lakeside" and "Matador" cultivars as compared to control, whereas an additional 1 log reduction was observed from nano BIT-treated samples. On day 5, nano BIT treatment significantly reduced *E. coli* O157:H12 populations on "Butterfly", "Matador", and "Lakeside" cultivars by 1.3, 1.8, and 2.5 log CFU/g, respectively, as compared to the control. The color of nano BIT-treated spinach leaves was comparable to control leaves on 0-3 days. The droplet size of freshly prepared nano BIT was 15.7±0.3 nm.

Significance: Nanoemulsified BIT can be potentially used as a pre-harvest antimicrobial treatment against *E. coli* O157 on spinach.

P1-73 Aggregative Swab Sampling Method for Romaine Lettuce Show Similar Quality Indicators and Microbial Profiles Compared to Composite Tissue Samples in a Pilot Study

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❖ ***Developing Scientist Entrant***

Introduction: Leafy greens have been the source of high-impact recalls. Current practice of collecting composite tissue samples produce few positive pathogen results. Aggregate sampling is potentially more representative.

Purpose: The purpose of this pilot study was to determine the aggregative swabs' ability to pick up quality indicators, determine if microbial profiles of swabs and tissues were similar, and investigate the capability of swabs to detect *Escherichia coli*.

Methods: Aggregative swabs (n=12) and composite tissues (n=14) were collected from a one-acre commercial Romaine field in Salinas, California with some portions being inoculated with a rifampicin resistant *E. coli* cocktail. Aerobic plate counts (APC), coliform counts, and generic *E. coli* presence were determined using culture-based methods. Further identification of bacteria, their abundances, and microbial diversity of the samples were determined from microbial community profiling using PacBio 16S rRNA sequencing.

Results: APC and coliform counts revealed swabs could pick up similar, if not higher, concentrations of bacteria compared to tissues. Recovery of APC were 9.17±0.43 and 9.21±0.42 log(CFU/g) for tissues and swabs, respectively. Means and variance differences between sample types were insignificant (p=0.38 and p=0.92, respectively). Coliform recoveries were 3.80±0.76 and 4.19±1.15 log(CFU/g) for tissues and swabs, respectively. Means and variance differences between sample types were insignificant (p=0.30 and p=0.16, respectively). Presence of generic *E. coli* showed that swabs could detect *E. coli* more often than tissues with 8/12 swabs detecting *E. coli* compared to 3/14 tissues. Microbial profiling revealed that swabs and tissues have similar alpha diversities (p=0.75) and the most prevalent bacterial taxa in similar abundances. Inoculation with r*E. coli* did not have a significant effect on plate counts or microbial diversities.

Significance: This data suggests aggregative swabs perform similar, if not better, than tissue samples for quality and food safety indicators. Future work will determine their potential as a food safety tool for leafy greens.

P1-74 Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* on Daikon Microgreens Grown on Different Cultivation Matrixes

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Introduction: Consumption of microgreens has increased lately due to availability of bioactive compounds. However, they may present food safety risk similar to sprouts. It is important to study the potential contamination of foodborne pathogens on microgreens.

Purpose: Effect of cultivation matrixes on the survival of *Escherichia coli* O157:H7 (EHEC) and *Salmonella* on daikon microgreen was investigated.

Methods: Daikon seed (50 g) were dip-inoculated with 100 ml of a five-strain cocktail of nalidixic acid-resistant EHEC or rifampicin-resistant *Salmonella* to obtain 5 or 3 log CFU/g. After overnight air-drying, inoculated seeds were cultivated on three matrixes including (1) Composted cattle manure with soil (CG), (2) Vermiculite perlite (VP) and (3) Biostrate pads (BP) for 14 days in a growth chamber. On day 7 and day 14, surviving populations of pathogens on microgreens (5 g/sample; N=144) were determined by plating on selective agars with antibiotics.

Results: At the low inoculum level, *Salmonella* populations significantly increased to 5.6 log CFU/g on BP-grown microgreens compared to other two matrixes (~4.5 log CFU/g) on day 14. At the high inoculum level, *Salmonella* recovered from CG-grown microgreens were significantly lower (4.7 log CFU/g) as compared to VP- (5.6 log CFU/g) and BP- (6.4 log CFU/g) grown microgreens on day 14. Similarly, EHEC populations recovered from CG-grown microgreens on day 7 and 14 (5.6 and 4.9 log CFU/g) were significantly lower than EHEC recovered from microgreens grown on VP (6.2 and 6.0 log CFU/g) when inoculated at the higher level. Overall, CG had the highest microgreen yield (265±20 g), followed by VP (69±3 g) and BP (36±3 g).

Significance: Contaminated seeds pose potential food safety risk as bacterial pathogens could survive on microgreens for 14 days. Survival pattern of pathogens on microgreen varies with cultivation matrixes used; composted manure is superior to other matrixes in controlling pathogen contamination on microgreen.

P1-75 Presence of Indicator and Foodborne Pathogens from Pre- and Post-Harvest Integrated Crop-Livestock Farm Environments and Fresh Produce on the Eastern Shore of Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Integrated crop-livestock farm (ICLF) systems, for sustainable, organic food production, are increasingly popular. However concerns remain about contamination risks from pathogens associated with on-farm animal reservoirs, their proximity to crop fields, and use of biological soil amendments (BSAs).

Purpose: This study assessed the bacterial quality of three ICLFs and compared them to similar samples obtained from two crop-only farms (COFs) and three farmers-markets (FMs).

Methods: A total of 990 samples were collected and analyzed using standard methods. Composite soil samples were evaluated before and after BSA application, every thirty days, and at produce harvest. Animal reservoirs were evaluated every 30 days, while untreated produce was examined at harvest or when available at FMs. All samples were analyzed for mesophilic aerobic bacterial (MAB), *Escherichia coli* (*E. coli*), *Salmonella*, and *Listeria monocytogenes* (*LM*). Presumptive *Salmonella* and *LM* were each confirmed using BAX PCR and PCR (*hlyA* gene), respectively.

Results: Results indicated incorporation of BSAs enhanced ICLF-soil health, but also significantly increased MAB and *E. coli* populations. Furthermore, *LM* and *Salmonella* were detected in soil samples after BSA application. At harvest, COF-soils had significantly ($p<0.05$) higher MAB populations than ICLF-soils, but 22.4% of ICLF-soils were positive for *E. coli* compared to 8.9% of COF-soils. FMs-produce had significantly ($p<0.05$) less MAB than corresponding ICLF- and COF-produce, but also tested positive for *E. coli*. *Salmonella* was absent on all produce samples, but present in COF-soil (2.2%), ICLF-soil (2.60%), and animal pen (3.85%) samples. *LM* was detected in ICLF-soils (3.75%), animal-pens (0.96%), and one ICLF-squash and one FM-kale sample.

Significance: Presence of fecal indicator *E. coli* and pathogenic organisms indicates contamination risks in pre- and post-harvest ICLF and COF environments and FM produce. Further research is needed to evaluate survival/transmission mechanics of foodborne pathogens in ICLF and COF farm and retail environments, and mitigation practices.

P1-76 Application of Bacteriophages to Prevent Attachment of Non-O157 Shigatoxigenic *Escherichia coli* to Caco-2 Cells

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Introduction: Non-O157 Shigatoxigenic *Escherichia coli* (STEC) are important foodborne pathogens that have emerged in the last two decades and pose serious public health concerns. Ruminant animals are their primary reservoirs, with about 30% feedlot cattle shedding them in their feces, leading to contamination of food-products. Very few studies have examined mitigation strategies against this group of pathogens, especially in cattle intestinal tract. In recent years, bacteriophages have garnered significant attention as therapeutic agents against human pathogens. Targeted use of bacteriophages could be used effectively to control this problem.

Purpose: Examine the efficacy of bacteriophages to prevent attachment of non-O157 STEC to Caco-2 cell-lines.

Methods: Three of the six major non-O157 STEC serotypes, O103, O45 and O26, were individually used to infect intestinal cell-lines. Bacteriophages (9 logs PFU/mL) specific to each serotype (P19-O103, P9-O45 and P13-O26) were used as treatments. Caco-2 monolayers were prepared ($1x10^5$ viable-cells/mL) in complete growth medium (CGM). Phage-treatments were added to the cells before (preventive) pathogen attachment. Overnight pathogen culture (1 ml; 6 logs CFU/cm^2) was centrifuged (12,000xg; 5 mins) and pellet re-suspended in CGM. Re-suspended culture was added to the cells and centrifuged (164xg; 5 mins) before incubation (2h; 37°C; 5% CO_2), to facilitate attachment. Sterile distilled water was used as control. After incubation, CGM was removed and cells lysed with 0.25% trypsin (3 ml;10 mins). Attached STEC population was determined by plating on MacConkey agar. Data were analyzed using one-way ANOVA ($P<0.05$).

Results: Phage-treatments effectively prevented the attachment of STEC to intestinal cells. Compared to the control (5.1 logs CFU/cm^2), phages P9-O103 and P13-O26 reduced the pathogen populations by 3.3 and 4.0 logs CFU/cm^2, respectively. Phage P19-O45 prevented the attachment of STEC O45 completely, with no attached pathogens recovered on the cells.

Significance: Bacteriophages could be used as effective treatments to prevent the attachment of non-O157 STEC to intestinal cells.

P1-77 Evaluation of Lactic Acid Bacteria for Acid and Bile Tolerance and Inhibition of Shiga-Toxigenic *Escherichia coli*

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Introduction: Shiga-toxigenic *Escherichia coli* (STEC) are responsible for numerous foodborne outbreaks and recalls, with beef as the implicated vehicle. Cattle are the known reservoirs, with primary sites being rumen and colon. Appropriate measures to reduce STEC in cattle is warranted, and utilization of lactic acid bacteria (LAB) may be an effective strategy. However, to produce the desired effects in cattle, LAB must be carefully selected and screened to maximize their inhibitory activity.

Purpose: To evaluate LAB for tolerance to acid and bile and inhibition towards STEC

Methods: A total of 240 LAB strains (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, or *Streptococcus*) were evaluated for inhibition against STEC, using the agar-spot-test. Cocktails ($5×10^7$ CFU/ml) of 4 STEC strains per serotype (O157, O111, O103, O26, O121, O45, O145) were used. LAB that inhibited STEC significantly (inhibition zones >10.0 mm) were further tested for acid/bile tolerance. Each strain ($1×10^8$ CFU/ml) was added to MRS broth adjusted to pH (2, 4, 5) and bile (0.1, 0.3, 0.5%), along with controls, and incubated for 0, 1, 3, and 6h at 37°C. Bacterial growth was determined by measuring absorbance at 620nm and 660nm for acid and bile tolerance, respectively.

Results: Of the 240 strains tested, 15% showed excellent (>15 mm), 32% showed very good (>10 mm), and 29% showed good (>5 mm) inhibition against STEC. LAB (*n*=112) showing >10mm inhibition were further screened for acid/bile tolerance. Compared to 0h (A_{620}=0.222-0.736), all isolates showed stable growth up to 6h (A_{620}=0.243-0.986) at all pH values. For bile tolerance, 80% isolates at 0.1%, 40% at 0.3% and 30% at 0.5% showed increased growth over 6h, indicating that they were better able to tolerate 0.1% compared to the 0.3 and 0.5% bile concentrations.

Significance: Selected LAB isolates showed good survival capabilities for the GI-tract environment and significantly inhibited STEC. They show promise for use as competitive-exclusion microorganisms in cattle.

P1-78 Survival of Shiga Toxin-Producing *Escherichia coli* on In-Shell Pecans Contaminated with Soil

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Introduction: Presently, small-scale pecan growers do not fall under the rules of the Food Modernization Act (FSMA). However, pecan growers should expect future legislation and be prepared to enact preventative controls.

Purpose: The aim of this study was to determine the survival of Shiga toxin-producing *Escherichia coli* (STEC) on directly inoculated in-shell pecans, soil-inoculated in-shell pecans, and directly inoculated soil over a twenty-eight-day period.

Methods: A cocktail of STEC (0157:H7, 0157: NM, 0121, and 026) was prepared from lawns grown on tryptic soy agar. One batch of pecans (200 g) and two batches of autoclaved soil (200 g each) were misted with the STEC cocktail (ca. 10.0 log CFU/mL) and dried for 30 min in a biosafety cabinet at 22°C. One of the soil batches was combined with uninoculated pecans and hand massaged to indirectly inoculate the pecans with the STEC-contaminated soil. Soil, pecans, and soil-inoculated pecans were stored in bags at room temperature for 28 days. Samples from each were taken on days 0, 1, 7, and 28 and enumerated on *E. coli*/Coliform Petrifilm™.

Results: STEC survived on soil for 28 days and only decreased by 0.9 ± 0.4 log CFU/g. On directly inoculated pecans, STEC decreased 1.5 ± 0.6 log CFU/g over 28 days. On soil inoculated pecans, STEC decreased 0.5 ± 0.2 log CFU/g over 28 days. The survival of soil-inoculated pecans was significantly higher than that of directly inoculated pecans during the 28 days ($p=0.0016$).

Significance: Data indicates that STEC can transfer to pecans via inoculated soil and successfully survive on in–shell pecans for more than 28 days. Moreover, the presence of soil may prolong or enable STEC survivability under certain conditions. Small pecan growers will find value in the presented data if they are required to develop small-scale interventions to comply with FSMA regulations.

P1-79 A Continuous Flow-Through System Utilizing White-Rot Fungi, *Pleurotus ostreatus*, and Its Effects on the Inhibition on *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: Lignin-degrading white-rot fungi may reduce zoonotic bacteria in biological soil amendments of animal origin when included in a continuous flow-through system.

Purpose: To analyze and confirm effects of *Pleurotus ostreatus* (PO) grown on varying support matrices on the inhibition of *Escherichia coli* TVS355.

Methods: Controlled continuous-flow tests with PO-treated woodchips (WC), spent mushroom compost (SMC) and reticulated polyurethane foam (RPF) were performed. Bioreactors (*n*=12) were maintained within a biosafety cabinet. For all, matrices (300g) were inoculated with PO millet-spawn (at 60%) and incubated at 22°C for 2 weeks. Inoculated matrices were then added to sterile 1L bioreactors and treated with a $MnSO_4$ tracer solution for the first 40hrs and a nitrogen limiting solution for 1 week. Inoculated PBS (5.91 log-CFU/mL) was added to each reactor at a flow rate of 0.5 mL/min. PBS effluent (3mL) was sampled on 0, 5, 10, 15, and 20 d post-inoculation (dpi) and matrices (30g) on 0, 10, 20dpi. *E. coli* TVS355 was enumerated on MacConkey agar with ri-

fampicin. Controls included bioreactors without fungi and/or bacteria. Chemical analysis included pH, moisture, and ergosterol which quantifies the fungal presence within each bioreactor. Data were analyzed using one-way ANOVA and student T-test across 3 trials, with n=12 per treatment.

Results: Between 5 and 20dpi all bioreactors showed similar decline of *E. coli* in the absence of PO (P=0.0435). There was a significant bacterial reduction across all live PO-treated matrices (P<0.0001). A greater reduction was observed in WC (P=0.0044) compared to SMC and RPF (p<0.0001). Bacteria remained undetectable in appropriate controls. *E. coli* detection was corroborated with metrics used for ligninolytic activity, as determined through ergosterol analysis, indicative of fungal integrity and concentration.

Significance: *Pleurotus ostreatus* inhibited *E. coli* across all matrices. Greater reduction was witnessed in woodchips, indicative of the ligninolytic activity and overall fungal activity.

P1-80 Cecal Metabolome Profiles of Turkey Poults in Response to *Salmonella* Heidelberg Challenge with or without Turkey-Derived *Lactobacillus* Probiotic and *Trans*-Cinnamaldehyde

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Introduction: *Lactobacillus*-based probiotics and a plant-derived compound, *trans*-cinnamaldehyde (TC), were effective in reducing multidrug-resistant (MDR) *Salmonella* Heidelberg (SH) in turkey poults. Determining the effects of these treatments on the cecal metabolome could assist in devising *Salmonella* control measures.

Purpose: The objective was to characterize the cecal metabolome of poults following SH challenge and treatment with turkey-derived *Lactobacillus* strains (LB; *L. salivarius* UMNPBX2 and *L. ingluviei* UMNPBX19) and TC.

Methods: Forty cecal samples were collected from poults from five groups (n = 8). The groups were treated with either LB (~10 logs CFU/mL), TC (0.08% vol/vol), or both (CO) through drinking water. Negative (NC; no SH, no treatment) and positive (PC; SH inoculated, no treatment) controls were included. Poults in the treatment and PC groups were challenged with ~4.5 log CFU SH by crop gavage on day 7. Cecal contents were collected on day 14. Gas chromatography mass spectrometry was used to analyze samples in quadruplets. Data analysis was performed using R and MetaboAnalyst.

Results: There were 895 metabolites detected, with 720 different across the groups (P <0.05, Tukey HSD). The greatest dissimilarity was observed between PC and other groups based on principal component analysis. PC differed the greatest with NC (P = 0.001), indicative of SH challenge effects. Higher abundances of cholic acid, cholesterol, putrescine, and chenodeoxycholic acid were detected in PC compared to NC (P <0.05). The greatest similarity was observed between TC and CO groups, with TC more similar to NC, potentially implying a beneficial effect of TC on the cecal response to SH.

Significance: The results identified alterations to the cecal metabolome caused by the SH challenge that differs when treatments are applied. It also provides insight into the metabolite shifts observed with supplementation of LB and TC. This information will serve as a foundation to devise *Salmonella* control strategies in poultry.

P1-81 Reduction of *Salmonella* and *E. coli* O157:H7 in Fecal Samples Collected from Beef Cattle Treated with Commercial Direct-Fed Microbials

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Introduction: Cattle are identified reservoirs for foodborne pathogens, like *Escherichia coli* O157:H7 and *Salmonella*; therefore, controlling pathogens in the feedlot by using pre-harvest interventions to target pathogens will reduce carriage rates, improving food safety.

Purpose: The objective of this study was to compare the prevalence of *E. coli* O157:H7 and *Salmonella* in the feces of beef cattle who were fed a standard diet and those supplemented with a direct-fed microbial (DFM).

Methods: This study took place in eastern Nebraska at 3 different feedlots, with a total of 6 pens (3 pens/feedlot). Cattle on the same feeding program were given different DFMs throughout the feeding period: (i) Control: no added DFMs; (ii) Bovamine Defend®(*Lactobacillus acidophilus* and *Propionibacterium freudenreichii;* BD): 50 mg/hd/day and (iii) Probicon® (*Lactobacillus salivarius;* PC): 50 mg/hd/day. Composite fecal samples were collected monthly from each pen, over a 5-month period, from May to September. A total of 300 fecal samples were collected, 110 from control treatment, 75 from BD treatment, and 115 from PC treatment. The presence of *Salmonella* and *E. coli* O157:H7 was determined using IMS protocol combined with molecular confirmation in a third party, independent laboratory.

Results: When referring to overall numbers, both treatments resulted in significantly lower *Salmonella* prevalence compared to control (Control: 26/110, BD: 2/75, PC: 2/115) and only PC resulted in significant reduction in *E. coli* O157:H7 prevalence (Control: 22/110, PC: 12/115; BD: 8/75). The effect of PC treatment on *E. coli* O157:H7 prevalence was statistically significant (OR=0.42, p=0.039), while the effect of BD was not (OR=0.43, p=0.099). The effects of PC (OR=0.05) and BD (OR=0.07) on *Salmonella* prevalence were statistically significant (p<0.001).

Significance: Data indicates that using Probicon as a pre-harvest intervention in a feedlot setting will decrease, but not eliminate, fecal shedding of *E. coli* and *Salmonella* meaning it may be an effective pre-harvest intervention strategy.

P1-82 Effects of *Bacillus* and *Lactobacillus* Supplementation in Milk Replacer Diets of Angus×Holstein Calves on the Prevalence and Concentration of *Salmonella* spp. and *Escherichia coli* O157 in Mesenteric Lymph Nodes, Spleen, Cecal Fluid, Rumen Fluid, and Feces

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❖ ***Developing Scientist Entrant***

Introduction: Recent developments in direct-fed microbial (DFM) research have indicated that administering these products may have an influence on the intestinal microbiota and health of an animal.

Purpose: This study utilized a DFM product containing *Bacillus* and *Lactobacillus* strains in milk replacer diets of calves from birth to weaning to determine impact on the prevalence and concentration of *Escherichia coli* O157 and *Salmonella* spp. in the mesenteric lymph nodes (MLNs), spleen, cecal fluid, rumen fluid, and feces of calves.

Methods: Newborn Angus×Holstein calves obtained from a commercial dairy were fed milk replacer diets containing *Bacillus* and *Lactobacillus* at $1.25x10^9$ CFU/head/day in the treatment group (N=30), while control group animals received milk replacer diets without treatment (N=30). Control and treatment calves were sacrificed on days 30, 60, and 90 and samples obtained during a necropsy. Fecal samples were obtained throughout life. Samples were analyzed for *E. coli* O157 and *Salmonella* spp. enumeration and detection.

Results: For samples collected at necropsy, *E. coli* O157 was detected in 20.8% and 11.9% of calves fed the control and DFM diets, respectively. Feeding the DFM reduced the odds of *E. coli* O157 prevalence by 49% (P=0.045) in comparison to control calves. *Salmonella* prevalence was lower in samples collected at necropsy for calves fed the DFM (17.4%) in comparison to control calves (25.3%); however, the odds ratio was not significant (P=0.142). While *Salmonella* concentration in the spleen was significantly lower in DFM calves (P=0.009), diet did not significantly impact pathogen concentration (P>0.05) in other samples. Diet did not impact E. coli O157 (P=0.655) or *Salmonella* prevalence (P=0.886) in feces.

Significance: While this DFM may impact the prevalence of *E. coli* O157, future research should aim to utilize larger numbers of animals with higher likelihood of pathogen harborage to better identify treatment effects.

P1-83 The Effects of the Administration of a *Saccharomyces cerevisiae* Direct-Fed Microbial on the Prevalence of *Salmonella* in Bovine Mesenteric Lymph Nodes

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Introduction: Contamination of ground beef products with *Salmonella* harbored within lymph nodes of cattle presents a potential risk to public health and pre-harvest interventions aimed at reducing *Salmonella* in cattle prior to entering the abattoir may improve public health.

Purpose: This study was designed to determine if *Saccharomyces cerevisiae* (SC) can reduce the prevalence and/or concentration of *Salmonella* in mesenteric lymph nodes (MLNs) of feedlot cattle at harvest when fed at $3.0x10^{10}$ CFU/head/day in finishing diets.

Methods: Cattle at a commercial feedlot were fed finishing diets, with treatment cattle receiving diets supplemented with SC and control animals receiving no supplemental SC. A total of 427 MLNs were collected by abattoir personnel at harvest from control (N=217) and treated (N=210) cattle. MLNs were trimmed, boiled, smashed, and homogenized with tryptic soy broth (TSB) to prepare a lymph node homogenate (LNH). The LNH was enumerated on Enterobacteriaceae petrifilm®, which were replica-plated to xylose lysine desoxycholate (XLD) agar. The LNH was then enriched, subjected to immunomagnetic separation with a secondary enrichment in rappaport-vassiliadis (RV) broth, and streaked for isolation on XLD. Colonies were confirmed as *Salmonella* via PCR.

Results: Overall *Salmonella* prevalence of the 427 MLNs was 13.8% and the model-adjusted *Salmonella* prevalence was 12.3% and 10.6% for MLNs from control and treated cattle, respectively (P=0.7763). The odds ratio comparing prevalence from control and treated cattle MLNs was 0.85, suggesting that the treatment diet reduced the odds of *Salmonella* prevalence by 15%. However, because of the wide confidence interval, efficacy could not be determined. Diet did not impact *Salmonella* concentration (*P*>0.05).

Significance: While the data suggest that SC may impact *Salmonella* prevalence in MLNs, future research should include a larger sample size from cattle that likely have a higher *Salmonella* prevalence to increase power and the likelihood of detecting a treatment effect.

P1-84 Characterization and Comparison of *Salmonella* spp. Isolated from the Mesenteric Lymph Nodes of Cattle and the Feedlot Environment

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Introduction: Cattle lymph nodes (LNs) harbor *Salmonella* and understanding if a relationship exists between environmental and LN contamination is important for identifying mitigation strategies to protect public health.

Purpose: This study aimed to characterize *Salmonella* spp. isolated from the mesenteric lymph nodes (MLNs) and feedlot environment of cattle to probe at a possible relationship between environmental and MLN contamination.

Methods: Samples were collected from water troughs (WT) and pen surfaces (PS; dirt floor) at the feedlot of a convenience sample (n=2,160) of cattle enrolled in a commercial feeding trial, and MLNs (N=427) were collected at harvest. Environmental samples were enumerated on xylose lysine desoxycholate (XLD) agar+antibiotics, while MLNs were enumerated on Enterbacteriaceae petrifilm® replica plated to XLD. Samples underwent immunomagnetic separation, secondary enrichment in Rappaport-Vassiliadis broth, and isolation plating on XLD. Isolates were serotyped and subjected to multiplex PCR to identify and quantify up to 7 virulence genes, indicating Highly Pathogenic *Salmonella* (HPS) serotype designations (HPS1-7).

Results: Salmonella was detected in 98.6%, 50%, and 13.8% of PS, WT, and MLN samples, respectively. *Salmonella* in PS ranged from 200-5,200 CFU/g, with 29.2% ≥200 CFU/g. Anatum HPS3, Lubbock HPS4, and Montevideo HPS4 were most frequently detected from PS and WT, while MLNs most frequently harbored Lubbock HPS4, Montevideo HPS1, and HPS4, with Anatum HPS3 also commonly recovered. Pens with enumerable levels of *Salmonella* in PS were more likely to have *Salmonella* isolated from corresponding WTs, than those with lower levels of *Salmonella.* Some *Salmonella*subtypes were recovered from MLNs but not from the environment.

Significance: These data raise additional questions about point of cattle LN contamination, duration/persistence of *Salmonella* in LNs, and suggest that *Salmonella* in LNs may not reflect prevalence and subtypes detected in the most proximal environment prior to harvest. These questions must be addressed to effectively mitigate LN contamination.

P1-85 Detection of *Salmonella* spp. and *Escherichia coli* O157:H7 on Beef Cattle Hides

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Introduction: The presence of *Salmonella* and *E. coli* O157:H7 on hides of cattle may lead to their transfer to carcasses during hide removal.

Purpose: The objective of this study was to investigate the occurrence of both *Salmonella* and *E. coli* O157 on beef cattle hides in a commercial beef packing plant located in the USA.

Methods: In July 2021, 76 beef cattle lots were sampled in the following way. After the exsanguination, 5 animals from the same lot were sampled with the same swap on the inguinal region to form a composite sample and the sponge was put into a bag with BPW. The composites were refrigerated and shipped to the Texas Tech Microbiology laboratory and processed in the following way: 1ml from each composite bag was added to 9 ml of modified tryptic soy broth (mTSB) and incubated at 42°C for 15-24 h. After the incubation, the detection of *Salmonella* and *E. coli* O157:H7 was performed using BAX® System PCR following the manufacturer's instructions.

Results: From the 76 lots sampled, a total of 46 were found presumptive positive for *Salmonella* (61%), 11 presumptive positives for *E. coli* O157:H7 (14%), and 7 lots were presumptively positive for both (9%). The frequency of lots presumptive positive for *Salmonella* was statistically different ($p<0.05$) than the frequency of presumptive positive for *E. coli*. On the other hand, lot size and gender were not significant. During the month, 9 providers were sampled at least twice. From those 9 providers, 3 were found presumptive positive in all lots sent either for *Salmonella* or *E.coli* O157:H7

Significance: By the quantification of pathogenic bacteria on hides, the packing plants may detect cattle ranchers with a high or low incidence of pathogens. With this information, ranchers' practices can be improved and packing process protocols can be adjusted to process those animals.

P1-86 Effectiveness of Surface Sanitizers Against *Salmonella* Typhimurium in Hydroponic Lettuce System

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❖ ***Developing Scientist Entrant***

Introduction: There has been an increase in hydroponic leafy green production but there are no validated sanitation protocols to ensure food safety of hydroponic crops.

Purpose: To determine the effectiveness of sanitizers to mitigate contamination of *Salmonella* Typhimurium on contaminated surfaces in commercial leafy green NFT production.

Poster

Methods: Commercial hydroponic surfaces (ABS plastic embedded with UV inhibitors, food grade UV stabilized PVC, and PVC) were inoculated with *Salmonella* Typhimurium (~1x10^4 CFU/mL) and treated with commonly used sanitizers. Pathogen enumeration and surface swabs were collected for 13 different treatments.

Results: Sanitizers: Zerotol (5%/10min), SaniDate12.0 (200ppm/5min), Virkon (1%/10min), KleenGrow (2%/10min), and GreenShield (5%/10min) eliminated *Salmonella* Typhimurium (100% reduction achieved) from all surfaces when applied for the label recommended contact time. SaniDate12.0 at 100ppm/5min eliminated the pathogen from ABS/w/UVinh (water reservoir) and food grade UVstPVC (channel top/bottom). *Salmonella* Typhimurium was recovered from PVC (2.91 ± 0.30 Log/cm^2). Sodium hypochlorite (100ppm/200ppm/10min) and aqueous chlorine dioxide (10ppm/50ppm/10min) achieved only limited reduction (range 72.07-97.55% reduction). Chlorine was the least effective on food grade UVstPVC of NFT channels that commonly contact edible portions of leafy green crops.

Significance: We have demonstrated that *Salmonella* Typhimurium can be eliminated from commercial hydroponic surfaces using Zerotol (5%), Virkon (1%), KleenGrow (2%), GreenShield (5%), and SaniDate 12.0 (200ppm) when applied at the labeled rate for the recommended time. However, Virkon, KleenGrow, and GreenShield are not approved for food use. Although all sanitizers achieved 3 log reduction, sodium hypochlorite, aqueous chlorine dioxide, SaniDate 12.0 (100ppm) were not effective in eliminating *Salmonella* Typhimurium from all materials. Ineffectiveness of chlorine-based sanitizers in ridding the hydroponic surfaces from the pathogen is concerning due to their widespread use in industry. These findings are critical in the development of food safety guidelines for hydroponic growers.

P1-87 Beneficial *Pseudomonas* spp. Protected Kale from Salt Stress and Influenced Association with *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: Seawater intrusion and residual chlorine in soils from water chlorination can impose salinity stress on plants, triggering plant responses to this abiotic stress. Beneficial plant growth-promoting rhizobacteria (PGPR) promote plant health and can protect plants from abiotic stress. The impact that PGPR have on *Salmonella*-plant associations under salinity stress merit investigation.

Purpose: To evaluate the effects of PGPR on salinity stress responses in plants and the influence on *Salmonella*-kale interactions.

Methods: Greenhouse-grown kale 'Improved Dwarf' (23°C, 16h L:8h D) were root-inoculated twice with *Pseudomonas* spp. (10^8 CFU/mL) (PGPR+) or 0.1% peptone water (PGPR-, negative control), 2-days and 9-days post-germination. All plants were then watered for 12 days before being subjected to 15 g/L (high salt), 5 g/L (medium salt) or regular water (control) for 5 days. About 10$^{6\ Salmonella}$ Newport (SeN) or Javiana (SeJ) were inoculated onto marked leaves used for bacterial enumerated 24 hours post-inoculation. The remaining plant tissue was flash-frozen, ground and extracted in methanol/formic acid for biochemical analyses. Plant biomass data were collected separately.

Results: PGPR- control plants had lower proline (p<0.001) and flavonoid (p<0.1) levels and antioxidant capacity (p<0.05) than medium and high salt plants (p<0.05). PGPR+ high salt plants had higher proline (p<0.001), phenolics (p<0.05) and glucosinolates (p<0.001) than control plants. PGPR colonization did not have an effect on SeJ, but SeN counts were lower on PGPR+ control plants (p<0.01). There were higher SeN counts in PGPR- control than medium salt plants (p0.05). In controls, SeN survival in PGPR- plants correlated (R=0.80, p<0.05) with antioxidant capacity in kale. SeJ survival in PGPR+ plants under high salt correlated with glucosinolates (R=0.65, p<0.05). PGPR+ medium salt plants had higher biomass than high salt plants (p<0.05) and were not different from controls.

Significance: PGPR did not promote growth but provided protection against salt stress and altered phytochemical profiles, impacting kale-*Salmonella* associations.

P1-88 *In Vitro* Antagonistic Activity of Indigenous Microbiota of Spinach Grown in Soil with Different Nitrogen Content Against Common Outbreak Foodborne Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: Several spinach-related outbreaks of foodborne illness occur every year. Spinach leaves are inhabited by a variety of microorganisms, some of which may have antagonistic activity against foodborne pathogens if present.

Purpose: To quantify microbial groups native to spinach grown in soil with different nitrogen contents, and to determine antagonistic activity of isolates from these groups against foodborne pathogens.

Methods: Populations of mesophiles (MS), psychrotrophs (PY) coliforms (CL) and lactic acid bacteria (LAB) were quantified in 68 spinach samples grown in soil with various levels of nitrogen content by plate counts using adequate culture media. Individual colonies were randomly selected for each sample and media type to determine the inhibitory effect of the selected isolates towards 3 strains of Shiga toxin-producing *Escherichia coli* (*E. coli* O157:H7 K3999, *E. coli* O104:H4 TY2482 and *E. coli* O104 ATCC 2192), 2 strains of *Salmonella enterica* (*Salmonella* Poona and *Salmonella* Saintpaul) and 3 strains of *Listeria monocytogenes* (*L. monocytogenes* LIS 10072, LIS 0089 and LIS 0104) using the spot agar test.

Results: Counts of MS, PY, LAB and CL on spinach grown in soil with low nitrogen level were 6.5, 5.9, 4.5 and 6.0 log CFU/g, respectively, while counts for spinach grown in high-nitrogen level soil were 6.5, 6.1, 4.7 and 6.0 log CFU/g, respectively. These counts did not differ between nitrogen levels in soil used for growing spinach (P > 0.05). A total of 1,788 isolates were randomly selected from the microbial groups tested. From these, 122 isolates presented antagonism against at least one of the 3 pathogens tested. Most antagonistic isolates were members of the LAB group.

Significance: The results from this study suggest that some spinach epiphytic bacteria can be used as potential biocontrol agents, able to reduce the proliferation of common outbreak food-borne pathogens.

P1-89 Assessing *Salmonella enterica* Dynamics on Lettuce over Time in Relation to Contamination Levels

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella* in irrigation water can transfer to leafy greens pre-harvest. The relationship between persistence and growth of *Salmonella* on Romaine lettuce in relation to initial bacterial population density is not well understood.

Purpose: Assess *Salmonella* establishment on Romaine lettuce over time when applied to plants in varying concentrations, to better understand food safety risks in relation to *Salmonella* levels in water.

Methods: Romaine lettuce 'Parris Island Cos' were greenhouse-grown for 4 weeks (23°C, 16 h light: 18°C, 8 h dark photoperiod) and transferred to a BSL-2 growth chamber to acclimate for 5 days at 21°C, RH ~85%. A cocktail of *Salmonella* Newport, Typhimurium and Enteritidis was prepared and inocula were made to four concentrations (10^1, 10^2, 10^3 and 10^4) to apply 0, ~36, 360, 3600 and 36000 CFU to plants (n=4/treatment). Plants were sampled destructively 1, 24, 72 and 192 h post-inoculation and bacteria enumerated using an MPN method. Microbial data were analyzed with JMP Pro 15.2.0.

Results: Both timepoint and concentration were factors in *Salmonella* counts retrieved from Romaine lettuce (p≤0.01), with no interaction between time and concentration. For all concentrations tested, *Salmonella* grew on lettuce by at least ~2 log/plant between 1 and 72 h, then declined by 192 h, but never below counts obtained at 1 h. Shifts were not significant for the 10^1 concentration. The highest count was obtained at the 10^4 concentration at 72 h,

which was different from all other counts ($p \leq 0.05$). Increases were detected between the 1 h and 72 h timepoints for 10^2, 10^3 and 10^4 concentrations ($p < 0.05$). Significant difference between 1 and 192 h was only detected for the highest concentration (10^4) with a 1.3 logCFU/plant increase.

Significance: Data suggest that even low-level contamination in irrigation water can be a risk for food safety in lettuce grown under high relative humidity.

P1-90 Sugar Levels in Tomato Fruit Do Not Explain Differential Ability of Modern Cultivar and Heirloom Tomato Fruit to Support *Salmonella* Newport

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Introduction: Fruit of various tomato varieties differ in their favorability to *Salmonella*. Varying sugar levels among tomato cultivars (cvs.) could explain these differences but no data exist on sugar accumulations in fruit and how they relate to *Salmonella* interaction.

Purpose: Measure sugar levels in mature tomato fruit of modern and heirloom varieties and relate to *Salmonella*-fruit association data.

Methods: Fourteen tomato varieties (8 modern cultivars, 6 heirloom) were grown in high tunnels or greenhouse (26°C, 16 h L:18°C, 8 h D). At peak ripeness, fruit surface washes were collected by placing fruit in 5% methanol/water solution for 3 h. Around 10^3 *Salmonella* cocktail of Newport, Javiana and Braenderup cells were inoculated into 900 µL of fruit wash and incubated at 35°C with shaking at 200 rpm for 20 h, then enumerated by serial dilution and plate counting. A modified sulfuric acid-phenol spectrophotometric method was used to measure total sugars in fruit washes. Data were adjusted for fruit surface area. Microbial data was analyzed with JMP Pro 14.1.0.

Results: *Salmonella* counts in the modern cultivar fruit group were higher than the heirloom group ($p < 0.05$) but total sugars were lower ($p < 0.05$). Differences in *Salmonella* counts were detected among the heirloom $p < 0.05$) but not the modern cultivars. The modern cvs. 'Dixie Red' and 'Red Pride' had the highest, and 'Charger' the lowest *Salmonella* counts and sugar levels, but results were not statistically supported. However, a positive correlation was detected between bacterial counts and sugars (R=0.26, $p < 0.01$). Heirloom varieties 'Purple Bumble Bee', 'Green Zebra', 'White Tomesol' and 'Black Icicle' supported lower *Salmonella* populations than 'Emerald Evergreen' ($p < 0.05$), but these differences could not be explained by sugar levels.

Significance: Sugar amounts, including metabolizable and unusable sugars, do not explain variety-dependent differences in heirloom tomato fruit-*Salmonella* associations. Other phytocompounds could be limiting or favoring *Salmonella* on fruit and should be investigated.

P1-91 Effect of UV-C Light Treatment Against *Listeria monocytogenes* on Hydroponically Grown Lettuce and Its Effect on Quality

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❖ ***Developing Scientist Entrant***

Introduction: Controlled environment agriculture like hydroponic system is a growing industry and an alternative agriculture production system. However, studies have shown potential of microbial growth and internalization onto roots, stems and leaves of hydroponically grown produce from contaminated seeds or fertilizer solutions.

Purpose: This study investigated the impact of UV-C light on inactivation of *Listeria monocytogenes* on hydroponically grown lettuce and its impact on quality. The effect of treatment on germination of seeds for hydroponics was also evaluated.

Methods: Hydroponically grown lettuce leaves was spot inoculated (100 µL) at 5 log CFU/g with *Listeria monocytogenes* (101M, V7, LCDC and Scott A) and were exposed to UV-C light for 10, 20, 30, 40, 50, 60, 120, 180, 240, and 300s at 0.28 mW/cm^2 to avoid burns found higher doses. Samples were diluted in 1X PBS solution and enumerated on oxford agar after incubation at 37°C (24h). Effect of the treatment on quality (color, weight, and visual observation) of lettuce was evaluated. Lettuce seeds (n=20) were treated with UV-C light (0.28 mW/cm^2) prior germination for 0-60min and the germination rate (%) was measured on Day 8. The experiments were done in duplicate.

Results: UV-C light significantly reduced *Listeria monocytogenes* on lettuce heads when treated for 20s (1.33±0.13 Log CFU/g) and onwards. The reduction increased up to 3.15±0.12 log CFU/g when treatment time was increased up to 50s after which reduction was below the detectable limit (1.7 log CFU/g). The treatment showed no significant ($P > 0.05$) effect on the color of lettuce, and the weight decreased ($P > 0.05$) over time, irrespective of the treatment when exposed up to 60s. However, when treated above 60s, UV-light significantly impacted the visual quality of lettuce. UV-treated lettuce seeds had higher ($P < 0.05$) germination rate 77.5% (60 mins) than control (52.5%).

Significance: UV-C light could be a potential intervention strategy for maintaining safety of produce in hydroponic setting.

P1-92 *Listeria monocytogenes* Biofilm Formation on Coated and Non-Coated Stainless-Steel Coupons in Lettuce Juice as Affected by Environmental Microbes

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Introduction: Biofilm formation on food contact surfaces is one of the main concerns about *Listeria monocytogenes* (*Lm*) contamination in fresh produce processing.

Purpose: To investigate if *Lm* biofilm formation on stainless-steel coupons in lettuce juice is affected by Dursan coating and environmental microbes.

Methods: A cocktail of three *Lm* strains, two environmental bacteria (*RB*), including *Ralstonia insidiosa* and *Brevundimonas naejangsanensis*, and undetermined microflora (*UM*) collected from romaine lettuce and produce processing pilot plant surfaces were prepared for inoculation. An initial attachment was performed by incubating the coated and non-coated stainless-steel coupons in 10% sterile lettuce juice (SLJ, pH ~6.5) with 7 log CFU/mL Lm at 25°C for 5 h. After incubation, each coupon was washed in sterile water, and transferred to SLJ, SLJ with 5 log CFU/mL *RB* or *UM* solution at 25°C for three days. Bacterial biofilm populations on coupons and planktonic populations in SLJ were enumerated by plate count. *Lm* reduction after sanitation using 200 mg/L quaternary-ammonium-compounds, or 10 mg/L free-chlorine was evaluated.

Results: *Lm* grew in SLJ to over 8 log CFU/mL at 25°C, but its population was suppressed in the condition with UM. The average biofilm populations of *Lm* on stainless-steel coupons after incubation with *RB* increased from ~ 5.6 log CFU/cm^2 to 7.1 log CFU/cm^2, while *Lm* biofilm level was not significantly changed after incubation in SLJ. Incubation in SLJ with *UM* resulted in *Lm* reduction of 1.28 log. *Lm* biofilm population on Dursan-coated coupons with bare surfaces was significantly lower (> 0.6 log) than that on uncoated coupons. Biofilm population of *Lm* on stainless-steel coupons reduced 1-2.2 log after sanitation. The efficacy of sanitation was significantly affected by the formation of Lm biofilms with *RB* (decreased reduction) and *UM* (increased reduction).

Significance: Results of this study bring clues to improve the prevention and management of *Lm* biofilm formation in produce processing environments.

P1-93 Shift in *Listeria monocytogenes* and Microbiome on Whole Avocado, Fresh-Cut Cantaloupe and Romaine Lettuce during Storage at Refrigerated and Abused Temperatures

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Introduction: In recent years *Listeria monocytogenes* (*Lm*) outbreaks and recalls associated with fresh produce have heightened concerns and demands from industry. However, little is known about the interactions between *Lm* and the indigenous microbiota on fresh produce during storage.

Purpose: To examine the dynamics of *Lm* inoculum and indigenous bacteria on fresh produce during storage at refrigerated and abusive temperatures.

Methods: A cocktail of three *Lm* strains (4-7 log CFU/mL) was inoculated on whole avocado, fresh-cut cantaloupe and romaine lettuce. The shift in *Lm* and indigenous bacteria on inoculated and non-inoculated samples held at refrigerated (4 °C) and abusive (10-24 °C) temperatures during storage (0-17 days) was determined by both culture-dependent and independent methods. Composition and dynamics of bacterial communities on *Lm* inoculated and non-inoculated samples were analyzed by 16S rRNA high-throughput sequencing.

Results: The initial indigenous microbial populations on whole avocado, fresh-cut cantaloupe and romaine lettuce were about 5 log CFU/fruit, 2 log CFU/g, and 5 log CFU/g, respectively. Inoculated *Lm* population decreased on the surface of avocado but increased on fresh-cut cantaloupe and romaine lettuce after storage. Fresh-cut cantaloupe provided favorable growth conditions for *Lm* proliferation (1.7 and > 6 log increase at refrigerated and abusive temperatures, respectively) to overtake indigenous bacteria. The growth rate of *Lm* on fresh-cut lettuce was lower than that of the total mesophilic bacteria, resulting in 0.4 and > 2 log increase at refrigerated and abusive temperatures. Microbial diversity analyses indicated *Lm* growth might be associated with fresh produce microbiota. The highest microbial diversity was found on whole avocado, followed by fresh-cut romaine lettuce, and fresh-cut cantaloupe displayed the lowest microbial diversity.

Significance: Data derived from this study could contribute to better understanding of the relationship between *Lm* and indigenous microbiota on fresh produce during storage.

P1-94 Evaluation of a Filtration System for the Detection of *Cyclospora cayetanensis* in Water during Cabbage Processing

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❖ ***Developing Scientist Entrant***

Introduction: Routine water examination used in facilities does not include testing for *Cyclospora cayetanensis* which is an ongoing problem within the produce industry.

Purpose: In this study, we determined the potential of a proposed protocol that uses an elution buffer and hollowfiber filters to detect the presence of *C. cayetanensis* in a produce processing system.

Methods: The first method consisted of spiking chlorinated tap water (96L) of different turbidity levels with 10^5 *C. cayetanensis* oocysts and then filtering with hollowfiber filters for eight hours. The second method consisted of mixing clean shredded green cabbage with shredded red cabbage spiked with 10^5 *C. cayetanensis* oocysts and washing in tap chlorinated water. Oocysts were recovered from the cabbage mixture in two sample sizes: 100g of the cabbage mix using elution buffer and 25g using 0.1% Alconox (n=244, each) after 1 hour and 8 hours of wash. Nested-PCR and real-time PCR assays targeting the *Cyclospora* 18SRNA gene were used and compared in all experiments to determine which PCR method detected more positive samples.

Results: Hollowfiber filters captured the oocysts during the first 4 hours at different water turbidity levels. Cross-contamination was observed between the red cabbages, the green ones and the wash water. Detection rates for the Alconox and elution treated samples were not statistically different ($p>0.05$): 6.15% (15/244) and 9.43% (23/244), respectively.

Detection rates for the real-time and the nested PCR assays were significantly different ($p<0.05$): 1.64% (4/244) and 13.93% (34/244) positive cabbage samples, respectively.

Significance: This project presents a suitable protocol to test for *Cyclospora* in environmental settings for routine testing at a fresh produce facility. The presence of a robust and well-suited method for *C. cayetanensis* surveillance and regulatory testing in processing settings will be the first step in preventing the introduction of the parasite into fresh cut-produce sold at the market.

P1-95 Impact of Sanitizers on Nutrient Film Technique (NFT) Grown Lettuce and Basil

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❖ ***Developing Scientist Entrant***

Introduction: Contamination of nutrient solution in hydroponic production can pose serious risks to food safety hydroponic crops and lead to severe losses in the hydroponic fresh produce industry. The produce industry commonly uses chemical sanitizers for sanitation of pre- and post-harvest production water (nutrient solution). Because of this, any chemical added to the nutrient solution can profoundly affect plant health and reduce yield. The industry widely uses sanitizers, but their effect on plant health is unknown.

Purpose: The objective of this study was to evaluate the impact of sanitizers, previously shown to eliminate pathogens on the health of lettuce and basil grown in hydroponic NFT systems.

Methods: SaniDate and sodium hypochlorite (100 ppm and 200 ppm) effects were determined by the fresh weight of edible leaves and roots of the crops. A split-plot within a randomized complete block study design, where each greenhouse room was considered a block, was used to compare the treatments (SaniDate and 100ppm, 200ppm-sodium hypochlorite) and controls. We will measure total Carotenoid and Chlorophyll as variable for plant health(still working on it) and variables analyzed using SPSS.

Results: Average yield at the end of the plant cycle was reduced from 136.77 ± 2 8.41g/basil plant to 21.30 ± 19.74 g/ basil plant (200ppm-hypochlorite on basil), 65.32 ± 11.41 g/basil plant (100ppm-sodium hypochlorite) and 101.82 ± 22.73 g/basil plants (SaniDate treatment). Also, lettuce yield was reduced from 181.65 ± 10.89 g/lettuce plant to 33.04 ± 3.11 g/lettuce plant,14.37 ± 2.85 g/ lettuce plant, and 140.84 ± 14.6 g/lettuce plant for 200ppm-hypochlorite, 100ppm-hypochlorite, and SaniDate treatments, respectively. Crops treated with SaniDate recovered significantly with a p-value of 0.05 (lettuce treatment) and 0.03 (basil treatment).

Significance: SaniDate had little effect on crop health and yield and should be further validated against human pathogens in NFT systems during the growth cycle of crops.

P1-96 Effect of Ultraviolet Light Treatment on Microbial Reduction and Quality of Lettuce Varieties

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Introduction: Despite their nutritive and health benefits, leafy green vegetables (LGVs) such as lettuce have been identified as the commodity group of highest concern from a microbiological safety perspective. Hence, LGVs have been linked to serious outbreaks of infections which are caused by foodborne pathogens and their toxic compounds. Although interventions which include washing fresh produce with chemical sanitizers aim to remove pathogens, scope for improved methods for effective sanitisation are still needed especially at postharvest.

Purpose: This study evaluated the potential of UV irradiation (254 nm) on microbial decontamination and quality of lettuce varieties during storage.

Methods: Sixty (60) samples including iceberg (IL), little gem (LG), romaine hearts (RH), round (RL) and sweet gem (SG) lettuce leaves were randomly spot inoculated (1 mL) with a cocktail of model foodborne pathogens (*Escherichia coli* ATCC 25922 and *Listeria monocytogenes* NCTC 11994); a population of ~8 log CFU/g. Treated samples were subjected to UV irradiation (254 nm) for 5 min, 10 min and 20 min) while untreated samples served as control. All samples were stored at 4 °C for 5 days while colour readings and pathogen levels were determined at 2-day intervals using a colorimeter and by counting bacterial growth on tryptic soy agar (TSA) plates respectively.

Results: At day 0, mean microbial reduction ranged from 0.3 to 1.8 $\log_{10}$ CFU/g; 0.53 to 2.1 $\log_{10}$ CFU/g; and 0.9 to 3.0 $\log_{10}$ CFU/g after UV treatment of 5 min, 10 min and 20 min respectively. Also, the order of mean microbial reduction among the lettuce varieties indicated IL<RL<SG<LG<RH. However, there was regrowth of pathogens during storage. Results (in triplicates) further revealed a slight but not significant ($p > 0.05$) mean reduction in the colour quality of lettuce varieties.

Significance: Results of this study suggest that UV irradiation (254 nm) is an effective tool for improving fresh produce safety. However, irregularities of surface structures can limit the exposure to UV and efficacy of decontamination.

P1-97 Effectiveness of UVC Light Treatment in Controlling *Listeria monocytogenes* in Hydroponic Fertilizer Solutions

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❖ ***Developing Scientist Entrant***

Introduction: Hydroponic systems use and recirculate liquid fertilizer solutions containing all the necessary nutrients to grow plants. One of the major food safety concerns in recirculated nutrient solutions is the growth of *Listeria monocytogenes* and the spreading throughout the cultivation system. UVC light has been shown to be an effective chemical free treatment to reduce microbial load from water.

Purpose: This study examined the effect of UVC light treatment on the survival of *Listeria monocytogenes* in Hydroponic fertilizer solutions.

Methods: Three hydroponic fertilizer solutions (strawberry, tomato, and lettuce) were adjusted to the electrical conductivity of 1600 µs/cm. and pH 6 were inoculated with a cocktail of *Listeria monocytogenes* strains (101M, Scott A, LCDC (81-861, 4b), and V7 ½a) to get an initial level of 5.7 Log CFU/ml. Contaminated fertilizers solutions were treated with UVC light with an intensity of 1.32 mW/cm^2 for 5, 10, and 15 seconds. *Listeria monocytogenes* counts on the fertilizer solutions were enumerated using oxford agar plates and incubated for 24h at 37°C.

Results: *Listeria monocytogenes* levels on Hydroponic fertilizer solutions were significantly reduced. UVC light treatment for 5, 10, and 15 seconds on lettuce fertilizer solution significantly reduced *Listeria* levels ($p<0.05$) from 5.7 log CFU/ml to 4.61, 2.26, and 1.38 log CFU/ml, respectively. Similarly, on tomato and strawberry hydroponic fertilizer solution to 4.49, 2.14, and 1.04 log CFU/ml, and to 4.50, 2.19, and 1.75 log CFU/ml, respectively. Treatment time was a significant factor ($p<0.05$) in reducing *Listeria monocytogenes* on the fertilizer solutions. Therefore, there was a significant difference in *Listeria* reduction for the time in each fertilizer solution.

Significance: UVC light treatment of fertilizer solution is effective to minimize food safety risk associated with *Listeria monocytogenes* in a Hydroponic systems.

P1-98 Efficacy of Ozonated Water Delivered via Nanobubble Technology to Inactivate *E. coli* O157:H7 on Fresh-Cut Lettuce during Centrifugal Drying

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❖ ***Developing Scientist Entrant***

Introduction: The need for a sanitizer to inactivate pathogens on fresh produce is apparent given recent foodborne outbreaks linked to fresh-cut leafy greens. Current commercial practices primarily rely on flume washing with chlorine-based sanitizers. One promising alternative sanitizer to chlorine is ozone: ozone is a potent, non-toxic, residual-free, FDA designated GRAS antimicrobial.

Purpose: This study investigated the ability of ozone delivered via nanobubble technology to inactivate *E. coli* O157:H7 on shredded Romaine lettuce during pilot-scale centrifugal drying.

Methods: Three 22.7 kg batches of Romaine lettuce heads were immersed in a 4-strain avirulent cocktail of ampicillin resistant GFP-tagged *E. coli* O157:H7 (6980-2, 6982-2, CV2b7, and 43888) to contain 5.6±0.4 log CFU/g, drained, and stored for 24 h at 4°C before processing. On the day of the experiment, each batch was shredded, then dried in a 22.7-kg capacity centrifugal Spin Dryer while being sprayed or sprayed then dried with tap water containing 0 or 6ppm ozonated nanobubbles generated using En Solución's Ozone Nanobubble Generator. Lettuce samples (25g) were collected in triplicate after shredding and from three levels of the dryer bin after drying. Samples were diluted and homogenized in D/E buffer and plated onto TSA containing 0.6% yeast extract and 100 ppm ampicillin. Fluorescent colonies were counted after overnight-incubation at 37°C. *E. coli* O157:H7 counts on shredded lettuce before and after each treatment were used to determine the decrease in population

Results: *E. coli* O157:H7 populations on lettuce treated with water while spinning decreased by 0.18±0.28 log CFU/g, while the reduction of *E. coli* O157:H7 on lettuce treated with 6ppm ozone nanobubbles before and during drying led to log reductions ranging from 0.7 to 0.2, with lettuce in the top third of the dryer basket with the greatest reduction.

Significance: Based on these results, ozone nanobubbles should be explored at additional stages of leafy green processing.

P1-99 The Effect of Natural Plant-Based Antimicrobials on Inactivating *Salmonella* and MS2 Bacteriophage in Cucumbers

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Introduction: Foodborne illness outbreaks of *Salmonella* and norovirus associated with fresh produce are a significant human health concern. Natural water antimicrobials, which are more acceptable to consumers and more environmentally friendly than chemical antimicrobials, have become a new trend in controlling pathogens in foods.

Purpose: The objective of this research is to evaluate the efficacy of natural plant-based antimicrobials (NPA) on inactivating *Salmonella* and MS2 bacteriophage (surrogate for norovirus) on Kirby cucumbers.

Methods: MS2 and a cocktail of two *Salmonella* strains (produce outbreak strains) were spot inoculated onto the surface of cucumbers to obtain 5.62 PFU/g and 4.88 CFU/g, respectively. The inoculated cucumbers (n=2 for each treatment) were washed with tap water, 3% (V: V) NPA, and an antimicrobial Fruits & Vegetable Treatment (AFVT) for 90 seconds. Washing by Zemea® was set as a positive control. MS2 population after each treatment were evaluated by plaque assay while *Salmonella* was detected by XLT-4 media plate.

Results: Washing of Kirby cucumbers with 3% NPA significantly reduced the *Salmonella* population, achieving a 3.33 log CFU/g reduction ($P<0.05$) compared with tap water treatment. Efficacy of the 3% NPA was comparable to the commercial chemical antimicrobial (AFVT) for inactivating *Salmonella*, resulting in 1.55 and 1.66 log CFU/g reduction, respectively. No significant difference in reduction of *Salmonella* was observed between 3% NPA and Zemea® treated cucumbers. A 1.64 log PFU/g reduction of MS2 bacteriophage occurred on cucumbers treated with 3% NPA, which was not significantly different ($P>0.05$) from water and AFVT treatments.

Significance: Results of the study suggest that NPA may be a suitable alternative to the use of chemical antimicrobials during washing of fresh fruits and vegetables.

P1-100 Microbubbles Remove *Listeria monocytogenes* from the Surface of Stainless Steel, Cucumber, and Avocado

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce may be contaminated by bacterial pathogens including *Listeria monocytogenes* during harvesting, packaging, or transporting. Consumers may be at risk of foodborne illness if produce becomes contaminated.
Purpose: In this project, a cavitation process (formation of bubbles in water) was studied to determine the efficacy of microbubbles at inactivating the pathogen *L. monocytogenes* on stainless steel and the surface of fresh cucumber and avocado.
Methods: Stainless steel coupons (1"2"), cucumber, and avocado surfaces were inoculated with *L. monocytogenes* (LCDC strain). After 1, 24 or 48 h, loosely attached cells were washed off, and inoculated areas were targeted by microbubbles (~0.5 mm dia.) through an air stone (1.0 L air/min) for 1, 2, 5, or 10 min. After treatment, samples were transferred to sterile containers and serial diluted in peptone water, and plated on Oxford agar. Plates were incubated for 48 h at 35.
Results: For stainless steel, the mean log reduction of *L. monocytogenes* (48 h drying) peaked at 2.95 after 10 min of microbubbles when compared to a no bubble treatment. After 48 h pathogen drying, cucumbers treated for 10 min resulted in a 1.78 mean log reduction of *L. monocytogenes*. For avocados, the mean log reduction of *L. monocytogenes* (24 hr drying) peaked at 1.65 after 10 min of microbubbles. This cavitation treatment (10 mins) reduced over 95% of *L. monocytogenes* on the surface of stainless steel, cucumber, and avocado.
Significance: Microbubble applications may be an effective, economical, and environmental-friendly way to remove *L. monocytogenes* and possibly other bacterial pathogens from food impact surfaces and the surface of whole, intact fresh produce.

P1-101 Survival of *Listeria monocytogenes* on Avocado Skin and Potential for Transfer and Growth in the Pulp after Cutting

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❖ ***Developing Scientist Entrant***

Introduction: The potential of avocados as vehicles for transmission of *Listeria monocytogenes* (*Lm*) was recently recognized by CDC and FDA after a survey detected *Lm* in 17% of skin samples. However, avocado has never been directly linked to listeriosis outbreaks. The potential for transference of *Lm* from skin to pulp had not been investigated.
Purpose: This study was conducted to determine the *Lm* survival on avocado skin during storage, its transference to pulp by cutting, and post-cutting growth on pulp during refrigerated storage.
Methods: Avocado skins were spot-inoculated with five-strain *Lm* mixtures grown in non-selective media. Cells were dried overnight and avocados were stored at room temperature (RT) and at 4 ºC, and sampled over time to determine counts by plating on complex media, and incubation at 37°C for 24 h. To determine the transfer from skin to pulp, avocados were cut with smooth or serrated knives after skin inoculation and drying. Avocados were stored at 4 ºC for 21 days, and viable counts were determined from skin and pulp portions exposed to cutting. Statistical analysis was conducted by Tukey's test.
Results: *Lm* viability was gradually reduced on avocado skins during storage. Reductions of more than 2 Log CFU/spot after 8 days at RT and 5 Log CFU/spot after 49 days at 4 ºC were observed. From 0.1 to 10% of initial skin counts (8 Log CFU/spot) were detected immediately after cutting, in pulp samples. After 21 days storage at 4 ºC, the pulp counts at cutting sites were almost 8 Log CFU and 6 Log CFU with high and low inoculum levels, respectively ($P<0.05$). There was no significant difference between two types of knives ($P>0.05$).
Significance: These findings suggest that *Lm* can survive on avocado skins and transferred to pulp during cutting with subsequent growth during refrigerated storage.

P1-102 Comparison of Two Triple-Wash Processes with a Combination of Peroxyacetic Acid and H2O2 to Reduce Populations and Mitigate Cross-Contamination of *Salmonella* Typhimurium and *Enterococcus faecium* on Tomatoes

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Introduction: The West-Virginia-Small-Farm-Center suggests the use of a PAA-H_2O_2 mixer (SD) with triple-wash method to mitigate microbial growth and reduce cross-contamination of pathogens to produce.
Purpose: To compare the efficacy of triple-wash methods with SD to reduce populations and prevent cross-contamination of *Salmonella typhimurium* and *Enterococcus faecium* on tomatoes.
Methods: Nalidixic-acid (NaL-200 ppm) resistant *S*. typhimurium or *E*. *faecium* was dip-inoculated on 4-tomatoes (2-untreated control) followed by triple-washing with 4-uninoculated-tomatoes using water+water+antimicrobial (WWA) or water+antimicrobial+water (WAW) with 0, 0.0064, 0.1, 0.25, 0.50, 0.70, and 1.0% of SD for 45-s. *S. typhimurium* or *E. faecium* contaminated tomatoes were added to 150 ml of TSB and vigorously shaken for 30s-followed by MPN-method analyses. The turbidity of each well after incubation (35ºC, 24-h) was confirmed on NaL-200 ppm TSA (*Salmonella*) and BEA (*Enterococcus*). MPN values of each treatment were determined by an MPN-calculator followed by mixed model statistical analyses by SAS (n=16, 4 repeats, P=0.05).
Results: Initial population of *S. t*yphimurium and *E*. *faecium* on inoculated tomatoes were 5.11 and 5.39-$\log_{10}$MPN/g, respectively. WAW process indicated similar ($P > 0.05$) reductions of *S. typhimurium* (2.15-3.51 vs 1.53-3.54 $\log_{10}$MPN/g) and *E. faecium* (2.61-3.47 vs 2.36-3.65 $\log_{10}$MPN/g) with 0.0064-0.70% of SD. Reductions of *S*. typhimurium on tomatoes were 2.47-3.71 (WAW) and 1.53-3.54 (WWA) $\log_{10}$MPN/g for 0.0064 to 0.70% of SD, which were less than ($P < 0.05$) or similar to the reductions of *E. faecium* with 2.61-3.38 (WAW) and 2.36-3.56 (WWA) $\log_{10}$MPN/g. Although cross-contamination of *E. faecium* was prevented at 0.5% of SD in WAW and WWA, cross-contamination of *S. t*yphimurium was not prevented until 0.7% and 1.0% of SD concentrations in WWA and WAW, respectively.
Significance: *Salmonella* appears to be more susceptible to SD treatments than *Enterococcus* and may warrant further research to verify if *Enterococcus* is an acceptable surrogate for *Salmonella* when validating antimicrobial washing systems on produce.

P1-103 Efficiency of Power Ultrasound-Based Hurdle Technology to Reduce *Listeria monocytogenes* on Grape Tomatoes

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Introduction: In recent decades, *Listeria monocytogenes* has been repeatedly implicated in foodborne outbreaks associated with fresh produce. Commonly used washing practices may not be sufficient to reduce pathogens. Combined use of power ultrasound with chemical sanitizers may further reduce pathogen load on fresh produce surface; however, there is a knowledge gap in the literature on how *L. monocytogenes* responds to such hurdle treatments.

Purpose: To evaluate the efficacy of hurdle treatment and examine global gene expression profiles of *L. monocytogenes* on fresh produce using combined sanitizer and ultrasound treatment.

Methods: Tomatoes (100 g) were spot inoculated with rifampicin-resistant *L. monocytogenes* LS810 at 8 log CFU/g, air-dried for 1 h, followed by ultrasound treatment at 25 kHz with either water, 50 ppm chlorine solution, or 40 ppm peracetic acid (PAA) solution for 1, 2 or 5 min. After treatment, *L. monocytogenes* was enumerated on BHIA^{rif}. Two technical and four biological replicates were included. Data were analyzed by Student's t-test, $p \leq 0.05$. Treated tomato samples were prepared for RNA-sequencing using the TruSeq Stranded mRNA kit (Illumina) and run on a MiSeq. Differentially regulated genes were identified when |log2 fold change| >1, FDR ≤0.1.

Results: The inoculation level on tomato was 7.71±0.75 log CFU/g. A 1 min 25 kHz treatment with water, chlorine, and PAA resulted in 1.81±0.51, 2.45±0.93, and 3.16±0.43 log CFU/g reductions, respectively. No significant difference was observed among the treatment times. Four genes in LS810 were upregulated and 16 downregulated in response to ultrasound treatment in water. Twelve genes, encoding proteins of metabolic functions (*ptxT*) and enzymes responsible for protein breakdown (*addB*) were upregulated in response to ultrasound/chlorine treatment.

Significance: The results of this study demonstrated the efficacy of power ultrasound treatment combined with chemical sanitizers and revealed the stress response mechanisms of *L. monocytogenes* to this hurdle technology.

P1-104 Modeling Effects of Hydrogen Peroxide Concentration, Treatment Time and Dwell Time on the Efficacy of Cold Plasma-Activated Hydrogen Peroxide Aerosol Against *Salmonella* Typhimurium and *Listeria innocua* on Tomatoes

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Introduction: Better and more effective intervention technologies are needed to reduce populations of pathogens during postharvest handling of fresh produce.

Purpose: The objective of the present study was to evaluate the efficacy of cold-plasma-activated hydrogen peroxide aerosols in inactivating Salmonella and Listeria on the smooth surface and stem scar area of tomatoes.

Methods: Cherry tomatoes inoculated with cocktails of *Salmonella* Typhimurium and *Listeria innocua* on the smooth surface and stem scar area were treated for 10, 20 and 30 s followed by 15 and 30 min dwell times in a treatment chamber with cold plasma-activated aerosols generated from 0, 1, 2, 3, 4 and 6% and 7.8% H_2O_2. The survival populations of inoculated bacteria were enumerated on tryptic soy agar with 200 mg/mL pyruvate acid and 100 mg/mL nalidixic acid and PALCAM agars for the enumeration of *S*. Typhimurium and *L. innocua*, respectively.

Results: Exponential or sigmoidal regression analysis indicated that at H_2O_2 concentrations ≥ 4.2 %, the treatments reduced populations of L. innocua to non-detectable levels (detection level: 0.70 log CFU/piece) achieving 5 log reductions on the smooth surface of tomatoes for all tested treatment times and dwell times. To achieve an average 5-log reduction of *Salmonella* populations on the smooth surface, H_2O_2 at concentrations ≥ 5.7% H_2O_2 was required. On the stem scar area of tomatoes, populations of *Listeria* and *Salmonella* were reduced by less than 2 logs.

Significance: The efficacy of cold plasma-activated hydrogen peroxide aerosol mainly depended on the concentration of H_2O_2, and *L. innocua* was more sensitive to the treatment than *S*. Typhimurium. This information would help the produce industry to employ the novel technology to minimize the risk of human pathogens associated with tomatoes.

P1-105 Gas Phase-Hydroxyl Radical Treatments to Decontaminate and Extend the Shelf Life of Fruit and Vegetables

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Introduction: Fresh produce causes the highest proportion of foodborne illness outbreaks amongst the commodity groups and is responsible for 30% of food waste caused by premature spoilage. To date, the post-harvest wash applied to remove field acquired contamination has been shown to have limited efficacy and can result in cross-contamination between batches if not controlled. Washing of produce can introduce disinfection by-products along with decreasing shelf-life compared to intact products. Consequently, there is a need for technologies that can reduce human pathogens on fresh produce with a positive impact on quality/shelf-life. To this end, we have developed a produce decontamination method based on the generation of hydroxyl-radicals through the UV-C mediated breakdown of hydrogen peroxide and ozone.

Purpose: To assess and optimize a vapor phase hydroxyl-radical treatment to inactivate toxigenic *E. coli*, *Salmonella* and *Listeria monocytogenes* on model fresh produce (leafy greens, soft fruit and stone fruit) and assess the impact on shelf-life.

Methods: The hydroxyl-radical reactor consisted of a conveyor with hydrogen peroxide mist and ozone gas being introduced into the chamber with overhanging UV-C lamps. The treatments were optimized with respect to hydrogen peroxide concentration, UV-C dose and ozone concentration. The dependent factors were log count reduction of pathogens and quality metrics (color, texture) of the test fresh produce.

Results: The hydroxyl-radical decontamination efficacy was dependent on the hydrogen peroxide concentration and UV-C dose. A 1.5% hydrogen peroxide, 11 mg ozone and 113 mJ/cm2 UV-C typically supported a 3-5 log count reduction of pathogens on fresh produce. In addition, there was a positive effect on the shelf-life of fresh produce, especially strawberries and avocado which achieved a 75% increase compared to controls.

Significance: The gas-phase hydroxyl radical process provides a risk management approach to enhance the food safety and shelf-life of fresh produce.

P1-106 Microbial Load on Fresh Blueberries Harvested by Different Methods

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Introduction: Currently, more and more growers are transitioning to over-the-row (OTR) machine harvesters for harvesting fresh market blueberries.

Purpose: This study assessed the microbial load on fresh blueberries harvested by different methods.

Methods: Samples (n=336) of 'Draper' and 'Liberty' blueberries, harvested with a conventional OTR harvester, a modified OTR harvester, ungloved but sanitized hands, and hands wearing sterile gloves, were collected from a blueberry farm in the Pacific Northwest at 9 am, 12 noon, and 3 pm on four different harvest days separated by two months in the harvest season of 2019. Eight replicates of each sample were collected at each sampling point and transported to our laboratory under refrigerated conditions. Samples were evaluated for the populations of total aerobes (TA), total yeasts and molds (YM), and total coliforms (TC), as well as the incidence of fecal coliforms and enterococci. Data were fit into a GLIMIX arrangement, and Tukey's HSD test was used to separate the means.

Results: Mean microbial load was statistically similar ($P>0.05$) between the two blueberry cultivars. Mean TA counts on berries harvested at different time points were also similar, however, samples collected at 12 noon and 3 pm had significantly lower ($P<0.05$) mean YM counts than the 9 AM samples. The 3 pm samples also had significantly lower mean TC counts. Blueberries harvested by the conventional and modified OTR harvesters had significantly higher ($P<0.05$) TA counts, but not YM counts than those harvested by the other methods. Berries harvested by the modified OTR harvester also had significantly higher TC counts. Seven fecal coliform isolates (2.08%) were recovered from samples harvested by the modified OTR harvester, but no enterococci were detected.

Significance: Results show that the populations of TA and TC on fresh blueberries are affected by harvest methods. The research highlights the importance of routine cleaning and sanitation of OTR harvesters.

P1-107 Efficacy of Dry Heat Treatment in Reducing *Salmonella* Population on Artificially Inoculated Mung Beans

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Introduction: Decontamination of seeds by dry heat has been examined, however, with inconsistent results among published studies. Research is needed to understand factors that may affect treatment efficacy.

Purpose: Evaluate the efficacy of dry heat in reducing *Salmonella* on mung beans, as affected by treatment time (6, 16, 24 h), temperature (60, 70, 80°C), relative humidity (20-80%), and treatment scale (10 g, 1 kg). Determine the impact on germination, sprout yield and the extent of *Salmonella* re-growth during sprouting.

Methods: Ten g of mung beans inoculated with *Salmonella* or 1 kg of beans spiked with 10% of inoculated beans were subjected to dry heat treatment in a humidity-controlled chamber. Treated beans were analyzed for *Salmonella* by plate count and culture enrichment. Fifty beans were germinated in a petri dish and percentages of germinated beans were recorded for 5 days. Sprout yields were determined after 7 days of germination. Two hundred g of treated beans were subjected to sprouting in glass jars for 4 days and levels of *Salmonella* and background microflora were analyzed daily.

Results: A greater log kill was observed when treatment was conducted at higher temperatures, under higher relative humidities (RH), or for a longer time. Treatment at 60°C/80%RH or 70°C/60%RH for 16 h reduced *Salmonella* by > 3 logs to below detection (detection limit 0 log CFU/g) while maintaining germination and sprout yield at > 90% of that of untreated controls. A similar log kill was achieved whether 10 g or 1 kg of beans were treated under these treatment conditions. *Salmonella* re-growth was observed during sprouting of treated beans, although could be delayed. In one trial where beans were treated at 60°C/80%RH for 16 h, *Salmonella* was undetectable after 2 days of sprouting but increased to 5.0 logs after 4 days.

Significance: Dry heat treatment could reduce *Salmonella* on mung beans to below detection, but pathogen re-growth could occur during sprouting and may not be detected.

P1-108 Application of Cinnamon Oil Nanoemulsion to the Control the *Salmonella* spp. in Mungbean Seeds and Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks due to *Salmonella* in mungbean sprouts have been increased recently. Essential oils extracted from plants have been recognized for their effectiveness in food preservations. It is due to their strong antifungal, antiviral, and antibacterial properties. Utilization of cinnamon oil nanoemulsion may help to develop novel technique to prevent food borne outbreaks.

Purpose: The purpose of this study was to find the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cinnamon oil nanoemulsions against *Salmonella* species. Furthermore, to check the efficacy of antimicrobial nanoemulsions as a decontaminate for reducing *Salmonella* on mung bean seeds with sprouting and post-harvest storage.

Methods: Cinnamon oil nanoemulsion was prepared with ultrasonication using Tween 80 as emulsifier. Particle size of oil droplets were characterized using dynamic light scattering. Minimum inhibitory Concentration assay was performed on six strains of *Salmonella* strains to find out the antimicrobial efficacy of cinnamon oil nanoemulsion. Cell-membrane integrity has been performed to conform the results of MIC and MBC.

Results: Minimum inhibitory concentration (MIC) of cinnamon oil nanoemulsion for *salmonella* was 0.31%. The minimum bactericidal concentration was similar to MIC i.e., 0.31%. Scanning electron microscope images showed distortion of bacterial cell membrane after treatment with cinnamon oil nanoemulsion.

Significance: The data suggest that cinnamon oil nanoemulsion may be used as an effective natural antimicrobial agent to decontaminate the mungbean seeds and sprouts against *salmonella spp*.

P1-109 Efficacy of Hydrogen Peroxide for Disinfection of Sprout Seeds Inoculated with *Salmonella,* as Affected by Sanitizer Concentration, Treatment Time and Seed Type

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Introduction: Treatment with 6-10% hydrogen peroxide (H_2O_2) for 10 min has been recommended for disinfection of seeds for sprouting. Published studies focused primarily on alfalfa seeds. Research is needed to determine the efficacy of H_2O_2 in reducing pathogens on other types of seeds.

Purpose: Evaluate the efficacy of H_2O_2 on *Salmonella* reduction and impact on germination, as affected by H_2O_2 concentration (6, 8, 10%), treatment time (10 min, 1 h) and seed type (alfalfa, clover, broccoli, radish, onion, mung beans).

Methods: Ten grams of seeds inoculated with a cocktail of five *Salmonella* serovars were pre-rinsed twice with sterile tap water and treated with 40 ml of sterile tap water or H_2O_2. After treatment, seeds were rinsed with water twice and analyzed for *Salmonella* by plate count. Germination percentages of treated seeds were recorded daily up to 7 days (or 14 days for onion seeds). Sprout yields were determined by measuring the weight of sprouts on Day 7 (or Day 14 for onion).

Results: Treatment with 6% H_2O_2 for 10 min reduced *Salmonella* on the six types of seeds by 0.6-1.9 logs. Increasing H_2O_2 concentration from 6% to 10% resulted in a similar log kill (0.6-2.3 logs). Extending treatment time to 1 h (at 10% H_2O_2) led to a slight increase in log kill (1.0-3.3 logs). H_2O_2 was the most effective on alfalfa seeds, reducing *Salmonella* by 2.5 or 3.3 logs when seeds were treated with 6% or 10% H_2O_2, respectively, for 1 h. Seed germination or sprout yield was not affected under most conditions except treatment with 10% H_2O_2 for 1 h, where germination was reduced for all but mung beans and the yield was lowered by > 30% for clover seeds.

Significance: Except for alfalfa, treatment with H_2O_2 was not able to achieve a > 3-log reduction in *Salmonella* population on seeds (the minimum level EPA will consider registering an antimicrobial).

P1-110 Probing the Bacteriophage-*Salmonella enterica* Interaction on Alfalfa Sprouts

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Introduction: *Salmonella enterica* (*S. enterica*) is a causative agent of multiple outbreaks associated with alfalfa sprouts. Current industrial antimicrobial interventions have shown microbial reductions by <90%, allowing the survival of the remaining population and subsequent outbreaks. Bacteriophages (phages) have been suggested as an alternative to chemical sanitizers to control *S. enterica* on sprouts.

Purpose: (1) Determine the optimal phage multiplicity of infection (MOI) against *S. enterica* and (2) assess the efficacy of repeated phage applications against *S. enterica* on sprout seeds.

Methods: MOIs of 1, 10, 100, 1,000, 10,000 and 100,000 plaque-forming units (PFU) of phages per colony-forming units (CFU) of four *S. enterica* strains were tested in 96-well plates. *S. enterica* populations were estimated on Xylose Lysine Deoxycholate (XLD) agar from day 0 – 3. Sample size was n = 6. For

(2), the same four *S. enterica* strains were inoculated onto alfalfa sprouts and treated with two phage cocktails or individual phages on day 0. Half of the sprouts received water washes and the other received phage washes daily from day 1 – 7. Populations were estimated on XLD on day 0, 1, 3 and 7. Sample size was n = 4.

Results: For (1), MOI 1000 and 10000 were shown to have the highest efficacies at reducing *S. enterica* populations ($P < 0.05$) with cocktail SE14, SE20 and SF6. MOI 1,000 and 10,000 with cocktail SE14, SE20 and SF6 was most effective for 2 of 4 strains ($P < 0.05$). For (2), daily phage washes further reduced *S.* Enteritidis populations by 1 – 2.5 log CFU/ml compared to a single treatment and further reduced *S.* Muenchen, *S.* Newport and *S.* Typhimurium populations by <1 log CFU/ml ($P < 0.05$) for both cocktails and individual phages.

Significance: Optimal MOI should be tested when utilizing phages. Multiple phage treatments can further reduce populations on sprout seeds, but the magnitude of the effects was both strain- and phage-dependent.

P1-111 Efficacies of Chlorine and Peroxyacetic Acid Against *Listeria monocytogenes* in Simulated Apple Dump Tank Water

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❖ ***Developing Scientist Entrant***

Introduction: Chemical sanitizers are commonly added in recirculated apple dump tanks and flumes to mitigate microbial risks; however, their efficacies *against Listeria monocytogenes* in dump tank water systems in commercial apple packing lines remain unclear.

Purpose: To evaluate anti-*Listeria* efficacies of chlorine and peroxyacetic acid (PAA) in simulated apple dump tank water (SDTW).

Methods: *Listeria* spiked SDTW was treated with sanitizer solutions for up to 5 min. To evaluate the effectiveness of sanitizers in controlling water to apple cross-contamination, uninoculated apples were introduced to *Listeria* spiked SDTW supplied with chlorine or PAA solution sanitizers for 2 min. Survivors in the spent wash solution and on apples were enumerated. Each study was repeated three times.

Results: Efficacies of chlorine against *L. monocytogenes* in SDTW were concentration and organic load-dependent: its efficacies diminished more rapidly and significantly at lower initial free available chlorine (FAC) and a higher level of chemical oxygen demand (COD) ($P < 0.05$). At 1000 and 4000 ppm COD, chlorine at 100 ppm FAC led to 6.9 and 5.7 log CFU/ml reductions after 5 min, respectively. PAA of 40, 60, and 80 ppm were not influenced by organic matter in SDTW and led to over 8 log CFU/ml reductions after 5 min at all COD levels. PAA of 20 ppm only led to 3.2 log CFU/ml reduction after 5 min. The chlorine and PAA in SDTW with 1000 ppm COD lowered cross-contamination risk but failed to prevent cross-contamination from spiked SDTW to apples during the 2-min wash. The most effective treatment was 80 ppm PAA, which still resulted in 2.63 log CFU/apple cross-contamination to apples and 1.39 log CFU/ml in the spent wash solution.

Significance: Data provides the industry with practical information about the effectiveness of sanitizer intervention in controlling *L. monocytogenes* in apple dump tank water.

P1-112 Isothermal Inactivation of *Salmonella, Listeria monocytogenes,* and *Enterococcus faecium* NRRL B-2354 in Plant-Based Butters

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Introduction: We determined and compared bacterial thermal inactivation rates of two pathogens and a surrogate across three different plant-based butters under isothermal conditions.

Purpose: The characterization of differences in thermotolerance of key microorganisms in low water activity foods supports thermal process validation studies to achieve global food safety objectives.

Methods: In this work, peanut oil, almond oil, or sunflower oil-based bacterial inoculums were implemented to determine decimal reduction times (*D*-values) for *Listeria monocytogenes*, *Salmonella*, and *Enterococcus faecium* NRRL B-2354 (a *Salmonella* surrogate), in corresponding peanut, almond, and sunflower butters under isothermal conditions. Uniform plant-based butter samples inoculated using six-strain cocktails of *Salmonella* spp. or *Listeria monocytogenes*, or *E. faecium* alone were sandwiched in bags between immobilized copper plates. Uniform rapid heat-treatments were performed via water immersion at 70°C, 75°C, 80°C or 85°C. Thermal Death Time (TDT) studies were conducted in triplicate using corresponding oil-based inoculums in the three plant-based butters. Bacterial survival in peanut butter (46% fat, 0.15 a_w @ 25°C), almond (50% fat, 0.28 a_w @ 25°C), or sunflower (56% fat, 0.09 a_w @ 25°C) was determined by direct plating on tryptic soy agar with 0.6% yeast extract.

Results: *Salmonella* spp. had higher *D*-values than *L. monocytogenes* in all but one of the treatments. However, pair-wise comparisons found no statistical difference when assessing the thermotolerance of the two pathogens in the individual plant-based butters tested ($p > 0.005$). *E. faecium* in peanut butter was more heat resistant than what was observed characterizing the surrogate in almond butter or sunflower butter.

Significance: These data support *Salmonella* as the primary pathogen of concern in low water activity foods and add to a growing body of work that indicates the heat resistance of *L. monocytogenes* can approximate destruction kinetics observed for *Salmonella* spp. in matrices containing > 45% fat (wt./wt.). *E. faecium* proved to be a conservative and appropriate validation surrogate for the plant-based butters tested.

P1-113 Relative Humidity Influences Survival of *Salmonella enterica* in Minimally Processed Broccoli Stored at Different Temperatures

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Introduction: Broccoli is widely marketed after minimal processing. Recalls and salmonellosis outbreaks linked to minimally processed broccoli have increased worldwide. Relative humidity (RH) is known to affect *S. enterica* survival, but little is known about RH influences *S. enterica* survival in broccoli stored at different temperatures.

Purpose: To assess the survival of *Salmonella enterica* strains in minimally processed broccoli (MPB) stored at different temperatures and RH conditions.

Methods: Broccoli (*Brassica oleracea* var. italica) pieces (3 cm^3) were spot inoculated with a *S. enterica* cocktail (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Agona and *S.* Anatum; ~3.5 log CFU/g of broccoli). Samples were stored in desiccators with saturated salt solutions of lithium chloride, magnesium chloride, calcium nitrate and potassium sulfate (15, 35, 65 and 95% RH, respectively) at 7, 14, and 21 °C. *S. enterica* was enumerated on *Xylose-Lysine Deoxycholate* agar from 0 to 144 h (1.5 log CFU/g detection limit).

Results: *S. enterica* counts increased after 144 h in broccoli stored at 21°C at 95% RH (~1 log CFU/g), did not change in broccoli stored at 21 ºC at 65 or 35% RH and decreased at 21 ºC at 15% RH (~1.5 log CFU/g). Counts did not change in broccoli stored at 14 ºC at 95% RH, and decreased at 14 ºC at 65% and 35% RH (~1.2 log CFU/g). *S. enterica* counts decreased ~1.8 log CFU/g in MPB stored at 7 °C at 95% RH after 144 h, at 7 °C at 65% RH after 96 h, and at 7 °C at 35% RH after 72 h. No viable cells were detected in MPB stored at 14 and 7°C at 15% RH after 96 h and 48 h, respectively.

Significance: RH influences *S. enterica* survival in minimally processed broccoli. These results may contribute to the development of risk models for *S. enterica* in broccoli.

P1-114 Microbial Characteristics and Chemical Composition of Fermented Olives Performed at Home

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Introduction: With increasing popularity of home fermentations, studies of the differences in microbial populations, salinity, pH, and sensory profiles introduced by individual or environmental variability are needed to provide consumers with reliable and safe resources for fermentation.

Purpose: To assess the variation of olive fermentation characteristics across different home production environments and develop Extension guidance based on data and practical experience.

Methods: Nine individual batches of olives from the same origin were fermented in six different households in Davis, CA. Olives were fermented following a standard procedure in 1-gallon plastic containers for 182 days in brine containing 6.7% salt and 10.7% vinegar. At 24 timepoints, brine and olive samples were collected. Brine pH, salinity, and temperature were measured in real-time and additional brine and olive samples were stored for microbial community analysis. At the fermentation end point olive color (Hunter L,a,b) was measured.

Results: Initial (day 0) average pH of all nine brines was 2.6±0.1 and the pH of brine increased by an average value of 1.57±0.2 by day 63 at which point brine was supplemented with additional fresh brine. At day 0, the brine salinity measurements varied between all individual ferments, ranging from 55ppm to 75ppm and brine salinity decreased over time. Visual differences in the appearance of yeast pellicle and olive color were observed between all nine batches of olives. Two batches had observable mold growth and resulting a brine pH above 4.6.

Significance: This study highlights variation in pH, salinity, yeast pellicle, mold growth, and final color between nine batches of in-home fermented olives. The pH of olive brine increased significantly during the first two months of the fermentation, therefore it is advisable for home fermenters to add additional acid during olive fermentation. Two of nine batches (22%) had mold growth, indicating inconsistencies in quality and potentially safety of in-home olive fermentation.

P1-115 Transcriptome Profiling of *Listeria monocytogenes* Growth in Cantaloupe Juice Compared to Laboratory Growth Medium (TSB)

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Introduction: A multistate outbreak of listeriosis occurred in the USA in 2011 linked to contaminated whole cantaloupe. Cantaloupe juice not only supports *Listeria monocytogenes* (*Lm*) growth but also causes a different phenotype of Lm compared to lab media. RNA-Seq technology can be used to understand the bacterial transcriptomic response to environmental changes.

Purpose: This study is to understand the impact of cantaloupe juice on Lm gene expression and physiology by comparing the transcriptome of Lm 2025 after growth in cantaloupe juice or widely used laboratory medium Tryptic Soy Broth (TSB).

Methods: The cantaloupe outbreak strain *Lm* 2025 (serotype 1/2b) was grown in filter-sterilized cantaloupe juice and TSB for 48h at 24 °C statically. The cell pellet was resuspended in *RNALater* solution after centrifugation and stored at -80 °C after snap freezing. The experiment was repeated three times with two technical replicates per trial. Total RNA of those 12 samples was extracted using the Invitrogen RiboPure™ Bacteria Kit. The library was prepared with Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero™ Plus kit and sequenced using Illumina platform NextSeq 500. RNA-Seq analysis was applied to compare the RNA expression profiles of Lm after growth in cantaloupe juice and TSB.

Results: *Lm* 2025 showed a strong autoaggregation phenotype after static growth in cantaloupe juice. Transcriptomics revealed a total of 1247 differentially expressed genes (DEGs) were significantly up-/downregulated with at least 2-fold change (568 and 679 genes, respectively, P<0.05). The results of functional annotation analysis of DEGs indicated that these genes mainly participate in metabolism, two component system, stress response, and virulence.

Significance: These data highlight the profound differences in the global transcriptome after growth in cantaloupe juice and provide the basis for future functional characterization of genes with potential roles in autoaggregation, and virulence or stress regulation of *Lm* in cantaloupe juice.

P1-116 Let's Cut the Rug: Investigating Food Safe Alternative Materials for Watermelon Harvest Activities

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Introduction: Carpet used in watermelon harvesting activities functions to cushion the product during transport from field to packing, however, this absorbent material provides a niche for unwanted microbial growth and is difficult to effectively clean, sanitize, and maintain in a sanitary manner. Further, competing priorities and limited resources on farms render it difficult to perform in-house evaluation of suitable alternatives for watermelon harvesting buses.

Purpose: To find an alternative food contact surface for watermelon harvesting buses that protects melon quality, is durable, is sanitizable, and is economically feasible.

Methods: Partnering with Grow USA, two prototypes were piloted on mid-Atlantic watermelon farms; 1) 610.30 GSM (g/m^2) non-porous liner over existing wall and 2) pre-formed foam encased in 610.30 GSM liner, both with 1.27 cm thick rubber floor mats (semi-nonporous). These materials were installed on buses, then followed throughout harvest (n=18 visits). To evaluate sanitary quality of the surface, walls and floors were sampled with Hygiena EnSURE Touch and assayed for ATP, Total coliforms, and generic *E. coli*. To evaluate melon quality, individual hauls were counted to gauge the number of cracked or broken melons, with a correction added for melons discarded for other quality reasons (n=33 hauls). Swabs and harvest counts were performed on buses with no intervention to serve as a negative control.

Results: Buses with intervention returned significantly lower ATP, coliform, and *E. coli* levels compared to control (p<0.05), driven by presence of the non-porous liner walls. Both prototypes returned significantly fewer busted and cracked melons compared to negative controls (p<0.05). While prototype two had significantly fewer melons damaged compared to prototype one (p<0.05), the latter is more economically feasible for small to mid-size farmers.

Significance: Investigating suitable alternative materials and developing effective cleaning and sanitizing approaches for equipment can reduce food safety hazards during watermelon and other specialty crop harvesting activities.

P1-117 Attachment Strength of Foodborne Pathogens on Melon Hybrids from Various Regions in the United States

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Introduction: Foodborne pathogens may contaminate melons at any production stage prior to consumption; it is therefore, important to understand which melon hybrids/varieties are more susceptible to bacterial attachment.

Purpose: The objective was to measure the attachment strength of foodborne pathogens on various melon hybrids.

Methods: Melon hybrids from six locations (Arizona, Indiana, North Carolina, Georgia, Texas, and California) were evaluated for attachment abilities of *Salmonella* Newport and *Listeria monocytogenes* to their rinds. To determine the attachment strength, discs were cut from the melon rinds, inoculated with overnight cultures of either *S.* Newport or *L. monocytogenes* (7 log CFU/ml) and allowed to attach for 30 minutes under a bio-hood. The discs were then

vortexed for 15 seconds to remove loosely attached bacteria and sonicated for 2 minutes to remove strongly attached bacteria. Bacteria in the suspension were enumerated by serially diluting the samples and plating on xylose lysine deoxycholate agar, for *S.* Newport, and modified oxford formulation agar, for *L. monocytogenes*.

Results: The attachment strength was calculated as the CFU of the strongly attached bacteria, over the total CFU of attached bacteria. Hybrid TH9 had the lowest attachment strength for *S.* Newport (0.05±0.01) and the highest attachment strength for *L. monocytogenes* (0.32±0.06). Hybrid TH5 showed the highest attachment strength for *S.* Newport (0.23±0.095) while Hybrid TH3 the lowest attachment strength for *L. monocytogenes* (0.17±0.02). Overall, the average attachment strength of *S.* Newport (0.17) on all hybrids tested was lower than that of *L. monocytogenes* (0.24). For comparison, the average attachment strength on commercial varieties (Infinite Gold and Davinci Sakata) for *S.* Newport and *L. monocytogenes* were higher (0.29, 0.23).

Significance: Results show that melon hybrids may be less susceptible to contamination than commercial varieties. Additional studies can aid growers in selecting melon hybrids that are more resistant to the attachment of *S.* Newport and *L. monocytogenes*.

P1-118 Relative Growth of *Listeria monocytogenes* in Unpasteurized and Pasteurized Low Acid Produce Juices

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (*Lm*) is known for its survival in produce environments and in cold storage. Less is known about the impact of natural microbiota of different produce on growth of *Lm* within cold-holding temperature abuse.

Purpose: Quantify growth of *Lm* in pasteurized and unpasteurized cantaloupe, watermelon, cabbage, and romaine lettuce juice under cold-holding temperature abuse.

Methods: Cabbage, romaine lettuce, watermelon, and cantaloupe were juiced using a handheld juicer. Pasteurized (63°C, 30 min; pH<4.6) and unpasteurized juices (pH<4.6) were inoculated with a cocktail of eight *Lm* strains (2 log CFU/mL), in triplicate and incubated at 7°C, 15 days. Total plate counts (TPC) and *Lm* were enumerated on tryptic soy agar + 0.6% yeast extract and Harlequin *Listeria* chromogenic agar, respectively, with incubation at 37°C, 24-48h.

Results: *Lm* and TPC reached high cell density (7-8 log CFU/mL) in all four pasteurized juices. Growth was comparable between pasteurized and unpasteurized juices for watermelon (*Lm*: 8.25±0.66 vs. 7.48±1.11 log CFU/mL; TPC: 8.51±0.33 vs. 9.29±0.34 log CFU/mL) and cantaloupe (*Lm*: 8.50±0.31 vs. 8.14±0.18 log CFU/ml; 8.56±0.34 vs. 8.32±0.21 log CFU/mL). In unpasteurized romaine lettuce juice, highest counts were seen at day 6 (*Lm*: 4.78±0.17 log CFU/ml; TPC: 6.41±0.23 log CFU/mL), followed by a decline, with no *Lm* recovered at day 15 (<-1 log CFU/mL) and low TPC (2.20±1.90 log CFU/mL). In unpasteurized cabbage juice the population decreased from day 1 to 6 (*Lm*: 2.31±0.05 to 1.96±0.54 log CFU/ml; TPC: 6.58±0.63 log CFU/mL), with no *Lm* recovered at day 15 (<-1 log CFU/mL) and low TPC (1.46±0.15 log CFU/mL).

Significance: Our data demonstrated that pasteurized produce juices provide a favorable growth environment for *Lm* at colder temperatures. Contrarily, decline in *Lm* population in unpasteurized romaine lettuce and cabbage juices suggest presence of inhibitory microorganisms or substances, warranting further research into their antilisterial properties.

P1-119 Efficacy of Free Chlorine and Peracetic Acid Against *Listeria monocytogenes* in Spent Citrus Wash Water

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❖ ***Developing Scientist Entrant***

Introduction: Citrus packhouses commonly use chlorine and peracetic acid (PAA) to minimize the risk of cross-contamination in recirculated wash steps. Research on the efficacy of these sanitizers in citrus-specific applications is limited and additional data is needed to better understand the concentrations required to adequately reduce cross-contamination risk.

Purpose: The purpose of this research was to investigate the efficacy of free chlorine and PAA in reducing *L. monocytogenes* (LM) in spent citrus wash waters.

Methods: Spent high-pressure (HP) and fungicide flooder (FF) water from three citrus packhouses were tested. HP treatments were done at ambient temperature with 15, 30 ppm free chlorine for 15, 30 s. FF treatments were done at 16, 32, 45°C and 18, 30, 60 ppm PAA for 0.5, 1, 2 min. After reaching the target temperature, samples were inoculated with LM (~6-log CFU/mL). Samples were neutralized with Dey-Engley broth and enumerated for LM on Tryptic Soy Agar with a Modified Oxford Agar overlay. As appropriate, turbidity, pH, ORP and/or Imazalil were measured. Seven HP and six FF water samples were tested in duplicate. LM populations before and after treatment were log transformed and used to determine the log reduction.

Results: After a 15 s exposure to 15 ppm free chlorine, 7/7 HP samples achieved a >5-log reduction of LM. PAA at 18 ppm and 45°C, resulted in a 2.2 to >6.4 LM log reduction at 1 min. At 45°C both 30 and 60 ppm of PAA at 1 and 0.5 min, respectively resulted in 6/6 FF samples achieving a >5-log reduction.

Significance: These data suggest that in HP washers a free chlorine concentration of 15 ppm is sufficient to eliminate LM. For PAA only the highest temperature tested (45°C) consistently yielded a 5-log reduction of LM at 30 and 60 ppm.

P1-120 Effect of Fine Bubbles and Electrochemical Disinfection on Efficacy of Chlorine Against Bacterial Pathogens on Bell Peppers

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Introduction: The use of fine bubbles during washing could facilitate the removal of bacteria from the surface of the fresh produce. Additionally, the application of electrical current in wash water has been proven to increase the efficacy of chlorine solution.

Purpose: In this study, we evaluated the effect of fine bubbles and electrochemical disinfection alone and in combination on the efficacy of chlorine against three bacterial pathogens on bell peppers.

Methods: Bell pepper surfaces were spot inoculated with 200 µL of *Listeria monocytogenes, Salmonella enterica, and E. coli* O157:H7 (~6 log) and washed with water or chlorine solution (10 or 100 ppm) for 10 minutes. Washing was performed using fine bubbles (micro and nanobubbles) or an electrochemical system (direct current of 500 mA and potential differences of 0V, 5V, or 20 V) or a combination of both. The tested pathogens were enumerated using the plating technique on selective agars. All the experiments were performed in duplicate.

Results: The results indicated that 10 ppm of chlorine was able to reduce the *S. enterica* count by 2 log CFU/g and *L. monocytogenes* and *E. coli* O157:H7 count by 1 log CFU/g. The combination of fine bubbles and the electrochemical system was found to be more effective (P<0.05) in reducing all three pathogens than using chlorine alone. Fine bubbles alone removed 1.1 log CFU/g of *L. monocytogenes*, 1.27 log CFU/g of *E. coli* O157:H7, and 2.7 log CFU/g of *S. enterica*. The potential difference of 5 Volt and electric current of 500 MA applied to 10 ppm of chlorine solution in combination with fine bubbles resulted in the reduction of 4 log of *S. enterica* count and 2 log of *L. monocytogenes* and *E. coli* O157:H7 count.

Significance: The combination of fine bubbles and electrochemical disinfection could be used as an alternative method to conventional chlorine washing.

Poster

P1-121 Characterization of *Listeria monocytogenes* Isolated from Frozen Vegetables Processing Plant: Subtyping, Biofilm Formation Capacity and Quaternary Ammonium Compounds Resistance

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* has been implicated in some outbreaks associated with consumption of frozen vegetables. The persistence of the pathogen in process food environments has been associated to their ability to form biofilms and to the resistance to chemical disinfectants.

Purpose: To characterized *L. monocytogenes* strains isolated from frozen vegetables processing plant environment based on their PFGE pulsotype, biofilms production capacity and presence/absence of quaternary ammonium compounds (QACS) resistance genes.

Methods: *L. monocytogenes* strains (25) isolated from a frozen vegetables processing environment were subtyped using Pulsed-Field Gel Electrophoresis (PFGE) method. Biofilm production (BP) was quantified using crystal violet assay in microtiter plate incubated at 35°C during 72 h; optical density (OD) was measured at 595 nm. The BP was classified in four categories: none, weak, moderate, and strong. Genes qacH and *bcrABC* associated to QACS resistance were investigated by PCR.

Results: Among the strains 6 pulsotypes were found: A (64%), C (12%), I (8%), Q (8%), V (4%) and Y (4%). Optical density values showed high variability at 24 h (0.1129-0.9418), 48 h (0.1904-1.6365), and 72 h (0.1688-4.88261). According to BP production strains were classified in none (16%), weak (24%), moderate (25%), and strong (35%). The bcrABC gen was detected in 13 (52%) strains belonging to A pulsotype, while qacH gen was not present. The pulsotype and the strain influenced the BP ($p<0.05$), meanwhile the time and the presence of QACS resistance genes were not statistical significative.

Significance: The presence of QACS resistance genes in *L. monocytogenes* strains in combination with a moderate/strong biofilm formation capacity can be a critical risk for persistence of the pathogen in food plant environments.

P1-122 *Salmonella enterica* and *Listeria monocytogenes* Growth Kinetics during Rehydration of Dehydrated Corn and Subsequent Storage

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❖ ***Developing Scientist Entrant***

Introduction: Dehydrated vegetables, including corn, are often used in restaurants and at retail grocers. Once rehydrated, these foods have neutral pHs and high water activities which may allow the survival and proliferation of foodborne pathogens, including *Salmonella enterica* and *Listeria monocytogenes*.

Purpose: To examine the extent to which dehydrated corn supports the growth of *S. enterica* and *L. monocytogenes* during rehydration and subsequent storage.

Methods: Fresh corn was dehydrated at 60°C for 24 h. Dehydrated corn was inoculated with a 4-strain cocktail of *S. enterica* or rifampicin-resistant *L. monocytogenes*, resulting in 4 log CFU/g, and dried at ambient for 24 h. Corn was rehydrated using 5 or 25°C water for 24 h. Throughout rehydration, samples were removed and drained for 10 min. To enumerate *S. enterica*/*L. monocytogenes*, samples were homogenized with BPB/BLEB and cultivated on TSAYE with overlaid XLD/BHIA^{rif}. Rehydrated corn was stored at 5, 10 or 25°C and enumerated at intervals up to 7 d. Three independent experiments with triplicate samples for each timepoint were conducted. Data were analyzed by Student's t-test, $p \leq 0.05$.

Results: After 24 h rehydration at 5 and 25°C, the population of *S. enterica* on corn was <2.4 and 2.66±0.05 log CFU/g, respectively. The population of *L. monocytogenes* was <2.4 log CFU/g for both temperatures. During storage, *S. enterica* proliferated on corn only at 25°C when rehydrated at 5°C: 4.58±0.16 log CFU/g at 7 d, lag phase 3.45 d, and growth rate 0.61±0.09 log CFU/g per d. *L. monocytogenes* proliferated at all conditions except 5 and 10°C when rehydrated at 5°C. The populations after 7 d at 25°C were not significantly different between rehydration temperatures: average population 7.51±0.80 log CFU/g and growth rate 0.64±0.007 log CFU/g per d.

Significance: The results of this study will help inform discussions on the safety of dehydrated corn once rehydrated and stored.

P1-123 Prevalence of Foodborne Pathogens in Food Products Acquired from Farmers' Markets in Central Virginia during COVID-19 Pandemic

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Introduction: Recognizing the importance of food safety education toward stakeholders, continued research is necessary regarding the actual food safety and microbiological implications associated with the unique retail environments in which farmers' market products are sold.

Purpose: The aim of this study was to assess the prevalence of foodborne pathogens in potential at-risk products identified from VSU preliminary study and procured from farmers' markets in Central Virginia.

Methods: A total of 490 food products produced by 60 farms and sold at 11 registered farmers' markets were obtained between March and November 2021. Bacterial isolation and identification have been performed following AOAC-approved or performance-tested methods.

Results: Aerobic mesophilic bacterial counts ranged from < 1.4 to 8.2 log CFU/g, with the lowest and the highest counts recorded for egg and kale, respectively. The level of coliforms > 5.0 log MPN/g was detected in arugula, green onion, green pepper, lettuce, kale, tomato and chicken. Detection of 18.1% *E. coli*, 8.0% *Listeria,* 10.1% *Campylobacter*, and 1.1% *Salmonella* were discovered in tested samples.

Significance: This study demonstrated a potential health hazard arising from farmers' market-acquired local food products and emphasizes the importance of food safety training for food producers, farmers' market vendors, and consumers as a whole to prevent foodborne illness. Findings in this study will contribute to developing and disseminating future food safety training and educational programs for stakeholders, including farmers, farmers' market vendors, and consumers, supporting the healthy development of farmers' markets. The research is regional in execution but has national relevance because it portrays a scalable and integrated food safety research model to understand microbial ecology in a retail farmers' market setting.

P1-124 Characterization of Foodborne Pathogens Isolated from Select Fresh Produce Marketed in Food Desert Areas of Central Virginia

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Introduction: Lack of large chain supermarkets (LCM) close to food desert areas may result in overreliance of low-income residents on small neighborhood convenience store retailers to acquire fresh foods. Studies demonstrate that small, independently owned markets (SIM) incur more critical and non-critical code violations in food safety than LCM.

Purpose: The purpose of this study was to assess microbiological quality differences of select fresh produce sold at SIM and LCM within identified food desert areas of Petersburg and Colonial Heights in Virginia.

Methods: A total of 122 fresh produce samples procured from registered 10 SIM, and 9 LCM between September 2018 and April 2019 were evaluated.
Results: Higher aerobic mesophile counts were found in all samples acquired from SIM compared to those procured from LCM. Regardless of food outlet source, *Campylobacter*, *E. coli*, and *Listeria* were detected in 10.7%, 4.9%, and 3.3% of the samples. Most of the isolated *Campylobacter* (76.9%) were from SIM. Twenty-eight bacterial isolates consisting of *Campylobacter*, *E. coli*, and *Listeria* were tested for their susceptibility to 12 antimicrobials. Ampicillin resistance showed the highest frequency among *Campylobacter* (84.6%), while nalidixic acid resistance was the highest in *Listeria* isolates (100%). Approximately 85% of *Campylobacter* and 27% of *E. coli* isolates exhibited multidrug resistance (MDR).
Significance: Findings in this study document the safety of fresh produce procured from food outlets in the food desert environment in Virginia and critical food safety associated with economic viability in food desert areas. Continued research efforts on a larger-scale sample size with wider varieties are needed to validate the observed presence and MDR of foodborne pathogens associated with food products marketed at food outlets in food desert areas and to determine and intervene their cause(s) to support the healthy development of food products sold in the area.

P1-125 Molecular Characterization of Pathogenic *Escherichia coli* Associated with Street Vended Ready-to-Eat Fresh Produce in Lagos and Ogun States, Southwest Nigeria

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Introduction: Ready-to-eat fresh (RTE) produce can serve as an important vehicle of enteric pathogens transmission to humans and public health threat. Pathogenic *Escherichia coli* (PEC) are an important cause of gastrointestinal diseases in Sub-Saharan Africa with concomitant mortality mainly in children. Due to the lack of microbiological safety supervision in the fresh produce sector in Nigeria, the quality of RTE produce is of concern.
Purpose: The focus of this study is to investigate the prevalence of PEC strains in street vended RTE fresh produce Lagos and Ogun States by determining the existence of virulence genes in the associated *E. coli* isolates.
Methods: A total of 163 RTE fresh produce samples (carrot, cabbage, lettuce, cucumber, pineapple, watermelon, and pawpaw) were purchased from various street vendors and farmers' markets in Lagos and Ogun state. The *E. coli* were isolated on sorbitol-MacConkey (SMAC) agar while PCR was used to determine the presence of virulence factors.
Results: The characterization of the *E. coli* isolates (*n*=199) confirmed the presence of four *E. coli* pathotypes, with at least one pathotype recognized in 87 fresh produce samples. About 50 fresh produce samples (62%) were positive from Ogun state while 37 (38%) were positive from Lagos state. ETEC77%), followed by EIEC (9.19%), EPEC (6.89%) and STEC (6.89%). The most prominent *E. coli* pathotype per fresh produces were ETEC for cabbage, EPEC and STEC for cucumber; and EIEC for carrots.
Significance: The presence of PEC in street vended RTE fresh produce poses a public health risk to consumers in Ogun and Lagos state. The awareness about the safety level of these fresh produce and the need for proper food safety management system in the fresh produce chain of Lagos and Ogun states should be emphasized.

P1-126 Characterization and Antimicrobial Resistant Profiles of *Salmonella* Species Associated with Ready-to-Eat Fresh Produce Sold in Open Markets of Lagos and Ogun States, Nigeria

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Introduction: Fresh cut ready-to-eat (RTE) fruits and vegetables sold in open markets are readily available in Nigeria. Outbreaks of *Salmonella spp.* linked to fresh produce have been reported globally. However, in Nigeria consumers are unaware of the possible *Salmonella* spp infections from fresh produce.
Purpose: This study endeavours to examine the prevalence, molecular and antibiotic resistance profiles of *Salmonella spp.* isolated from RTE fresh cut fruit and vegetable samples obtained from open markets in Lagos and Ogun states, Nigeria.
Methods: A total of 204 fresh produce samples (Cabbage, Carrot, Lettuce, Cucumber, Watermelon, Pineapple, and Pawpaw) were tested for the presence of *Salmonella spp.* using the media based method and molecular characterization. Presumptive *Salmonella spp*. were characterized using multiplex PCR while Kirk-Bauer disc diffusion method was performed for antibiotic susceptibility.
Results: A total of 89 presumptive *Salmonella spp.* were isolated from the RTE fresh cut produce from Lagos and Ogun State; of which 69 isolates were confirmed belonging to *Salmonella enterica* subsp. I group using PCR. Overall *Salmonella* spp. detection was most prevalent in the carrot (32%) samples. *Salmonella enterica* subsp. Typhimurium was detected only in pineapples from Sagamu market. Antibiotic susceptibility performed on 16 isolates of the 69 PCR confirmed *Salmonella spp.* revealed that 7 isolates have resistant to at least two or more of the twelve antibiotics tested. Resistance to ceftriaxone and gentamicin was most prominent in 87% and 57% of the tested isolates respectively.
Significance: Fruits and vegetables sold in open markets in Lagos and Ogun states, Nigeria are not safe for consumption and may contain potentially pathogenic multidrug resistant *Salmonella spp*., which may pose a public health risk. To avoid food poisoning from this pathogen, appropriate government agencies should ensure food safety and hygiene measures is enforced.

P1-127 Impact of Temperature, Concentration, and Contact Time on Bacterial Reduction in Surface Waters by Chlorine

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Introduction: Agricultural water may carry human pathogens, and when it directly contacts the harvestable portion of the crop during irrigation or protective sprays it could pose a human health risk.
Purpose: Our objective was to assess the effectiveness of Calcium Hypochlorite against *Salmonella* (SAL), Shiga-toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes* (LM), and naturally occurring *E. coli* and coliform populations in agricultural water.
Methods: Water (ground and two ponds) was inoculated at 6 log CFU/ml with a rifampicin resistant 5 strain cocktail of either SAL, STEC, or LM and held at 4, 10, or 15°C (n=6). Free Chlorine concentrations (0, 2, 4, 10, 20, and 50 ppm) were tested at six contact times (0, 30, 60, 120, and 300 s). Sodium thiosulfate was added to quench the reaction. Water samples were diluted and plated onto non-selective (TSA-SAL, STEC; or BHI-LM), and selective (XLT4, SAL; SMAC, STEC; MOX, LM) media supplemented with rifampicin (80 µg/ml). Uninoculated water sources were treated in the same manner to enumerate generic *E. coli* and coliform populations (log MPN/100ml) using Colilert Quantitrays.
Results: For all water sources, temperatures, and organisms, the longer the contact time and the higher the concentration the greater the microbial reduction. SAL, STEC and LM populations declined to below the limit of detection (0 log CFU/ml) when treated at 50 ppm chlorine for 300s. Population declines ranged from 0.4 log CFU/ml (SAL 4, 10, and 15°C, 2ppm chlorine, 10 s) to > 6.3 log CFU/ml (LM 4°C, 50 ppm chlorine, 5 min). Indigenous *E.coli* and coliform populations declined to below the limit of detection (<0 log MPN/100ml), when treated at 10 ppm chlorine, 300s. Increasing water temperatures did not significantly increase ($p \geq 0.05$) reductions in bacterial populations.
Significance: Chlorine used to treat agricultural water is impacted by concentration, contact time, but not by temperature.

P1-128 Antimicrobial Resistant *Escherichia coli and Enterococcus* spp. in Irrigation Water: Ponds, Creeks, and Streams in Small-Scale Produce Farms

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Introduction: Fresh produce growers in small-scale farms often use surface water for irrigation. Often these farms are adjacent to animal farms; hence surface irrigation water nearby might be contaminated with antimicrobial resistant bacteria, posing risks to public health.

Purpose: The purpose of this study was to determine antimicrobial resistance of *Escherichia coli* and *Enterococcus* spp. in irrigation water from ponds, creeks, and streams adjacent to small-scale produce farms in Tennessee.

Methods: Pond, creek, and stream irrigation water (n=111) was collected over 3 months and screened for *Escherichia coli* and *Enterococcus.* Eosin-Methylene Blue and Enterococcosel agar plates were used for recovery of *Escherichia coli* and *Enterococcus*, respectively. Biochemical and PCR methods were also applied for bacterial confirmation. The antimicrobial susceptibility test for bacteria (*Enterococcus* =163, *Escherichia coli* =79) was conducted according to Kirby-Bauer disk diffusion method.

Results: Overall, the presence of *Enterococcus* (54.05%,) was significantly (P<0.05) higher than *Escherichia coli* (26.12%) in all water samples. Inclusively of all water from ponds, creaks, streams and farm, *Enterococcus* significantly (p<0.05) showed higher resistance to nalidixic acid (80.37%) and cefixime (76.07%) as compared to erythromycin (28.22%), ampicillin (9.82%), chloramphenicol (6.13%), and gentamicin (1.84%). *Escherichia coli* resistance was significantly (p<0.05) higher to vancomycin (100.00%), bacitracin (100.00%), and erythromycin (88.61%) as compared to azithromycin (17.72%), cefixime (8.86%), and ampicillin (3.65%). *Enterococcus* in ponds, creeks, stream, and farm water showed high resistance to nalidixic acid (100%; 80.0%; 78.8%; 74.2%) and cefixime resistance (66.7%; 73.3%;77.3%; 77.3%), respectively. No Enterococcus was resistant to imipenem. *Escherichia coli* in all surface water was highly resistant to vancomycin (100%), and bacitracin (100%).

Significance: This study indicates that pond, creek, stream and, farm irrigation water is a reservoir of antimicrobial resistant *Enterococcus* and *Escherichia coli*. Information on antibiotic resistant bacteria in surface irrigation water in small-scale farms provides insights needed to educate growers on mitigation of antimicrobial resistance.

P1-129 Influence of Seepage Irrigation Systems on Microbial Water Quality

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Introduction: Seepage irrigation is an irrigation method where a network of canals and v-ditches surround fields and field blocks. The water seeps into the field; the lateral movement of water elevates the water table uniformly to irrigate crops directly to the roots. There is a lack of research regarding the impact seepage irrigation systems on microbial water quality, and subsequently where growers should collect samples that are representative of use, as required by the current Produce Safety Rule.

Purpose: The purpose of this study is to compare the microbiological quality of surface waters to that of subsurface water used for seepage irrigation on Florida farms.

Methods: Water samples (500ml) were collected from 6 seepage irrigated produce fields over 2 months. In each field, surface water samples are collected from the main canal (n=1) and the v-ditch (n=1); subsurface seepage samples are collected from seepage wells (ca. 50 cm deep into planting beds, 9 wells/field) located at different distances from the canal and v-ditch. Generic *E. coli* and coliforms populations (MPN/100 ml) were enumerated by Colilert and Quanti-trays.

Results: Average coliform populations in seepage (n=95), canal (n=11) and v-ditch (n=11) surface water samples are: 2.17±1.17, 3.73±0.3, and 3.98±0.36 log MPN/100 ml, respectively. High variability was seen amongst seepage samples with coliform levels ranging between <1 MPN/100 ml to >3419.6 MPN/100 ml. Average *E. coli* populations in canal and v-ditch surface waters was 0.62 log MPN/100, with populations ranging between <1 to 161.55 MPN/100 ml. *E. coli* was detected 3 times in seepage well water samples (3/95, 3.2%). When detected in seepage well waters, *E. coli* population levels were 2, 19.85, and 62.9 MPN/100 ml.

Significance: Microbial water quality of water samples from canals and v-ditches is not representative of the microbial water quality that contacts the crop via seepage irrigation.

P1-130 Agricultural Water Microbial Baseline of Indicator Organisms and Produce Safety Assessments of Honduras Farm Exporting Produce to the U.S. Market

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Introduction: Agricultural water (AW) is one of the primary sources of bacterial contamination of produce and annually costs $1.4 billion in the USA.

Purpose: The objective of the study was to develop indicator microorganisms' baseline of AW sources to assess the microbial quality of water used in Honduras for produce production and packing farms exporting to the USA.

Methods: Main AW sources for 21 produce farms and packing houses were evaluated. Total sampling sites per facility varied from 1-15 according to size; a total of 3-5 sampling events were conducted to account for seasonal variability. Water samples were taken with 24oz sterile Whirlpack™ bags, collecting a minimum of 100mL of water. The outlet where the sample was collected was cleaned, disinfected, and AW flowed for 1-3 minutes prior to sample taking. Four indicator microorganisms were evaluated, aerobic counts (AC), enterobacteria (EB), coliforms (CC), and *E. coli* (EC) using Hygiena's MicroSnaps® following manufacturer's recommendations.

Results: Farms J, K, and H were observed to have significantly highest AC with 4.0±1.3, 3.5±1.2, and 3.3±0.8 LogCFU/ml, respectively. Farms R and T had significantly highest EC counts with 1.4±0.4 and 1.0±0.7 LogCFU/ml, respectively. Comparatively, Farms K and F had the significantly highest CC observed with 1.8±1.0 and 1.7±1.1 LogCFU/ml, respectively. Finally Farms G, T, and A had the significantly highest counts on EB with 3.0±0.4, 2.4±0.9, and 2.3±0.8 LogCFU/ml.

Significance: According to the Produce Safety Rule published in 2015 and proposed for modifications in 2020, 19/21 farms would have AW with *E. coli* counts higher than the geometric mean and statistical threshold value proposed. This study serves as an effort to develop a preliminary national baseline to assess the microbial risk of AW sources and to establish recommendations and action plans that will lead to overall improvement of AW safety used for US-bound produce produced and packed in Honduras.

P1-131 Investigating the Prevalence of *Salmonella* and *E. coli* in Florida's Soil and Identifying Key Environmental Factors

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Introduction: Multiple produce outbreaks have been linked to soil contamination in specific growing regions indicate a need to further investigate regional, environmental factors that impact pathogen prevalence in the soil.

Purpose: The purpose of this study is to determine the prevalence of generic *E. coli* and *Salmonella* in Florida's agricultural soils to help understand the microbial contamination of produce at the pre-harvest level.

Methods: A longitudinal field study was performed on produce production in three geographically distributed agricultural areas across Florida. At each location, 20 unique 5 m by 5 m field blocks were selected and soil was collected for *Salmonella* detection and isolation (25g) and *E. coli* and coliform enu-

meration (5g). Composite soil samples (n=60) were collected with complementary data on the weather, adjacent land-use, soil micro-nutrients, and field management practices in the fall of 2021.

Results: The overall prevalence of *Salmonella* in the soil is 3.3% (2/60) in 25 g. Farm C has the highest prevalence at 10% with 2 presumptive positives from the 20 samples obtained while both Farm A and Farm B had no presumptive positives. The average *E. coli* count is 0.18±0.60 CFU/g and the average coliform count is 2.02±1.05 CFU/g. Counts ranged between the farms, with an average *E. coli* and coliform counts of 0 and 3.29±0.22 CFU/g for Farm A, 0.124±0.45 and 1.53±0.87 CFU/g for Farm B, and 0.08±0.35 and 1.88±0.66 CFU/g for Farm C, respectively.

Significance: A low prevalence of *Salmonella* and generic *E. coli* in Florida's soil was found in the fall when many planting activities are taking place. This suggests that the risk of pre-harvest produce contamination via soil in these Florida growing regions is low and major changes in production management practices are not necessary to further mitigate the risk of pre-harvest contamination from soil.

P1-132 Biological Soil Amendments of Animal Origin Extend the Survival of *Escherichia coli* in Soils in the Southeastern U.S.

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❖ ***Developing Scientist Entrant***

Introduction: Extended survival of foodborne pathogens in soils can affect their transfer to produce. The use of Biological Soil Amendments of Animal Origin (BSAAO), including heat-treated or composted products, can affect survival of pathogens in soils.

Purpose: To evaluate the effect of different BSAAOs (composted poultry litter [CPL] and heat-treated poultry pellets [HTPP]) on the survival of *Escherichia coli* in soils.

Methods: Nonpathogenic, rifampicin-resistant *E. coli* TVS 353 (9 logCFU/ml) was applied to 1m × 3m plots of sandy loam soil at a rate of 1 L/plot. Replicate plots (n=3) containing either CPL, HTPP or which were unamended (UN) were evaluated for *E. coli* survival over 56 days. To quantify *E. coli*, 30g soil samples were collected, homogenized and direct-plated onto tryptic soy agar with rifampicin on each sampling day. A most probable number (MPN) enumeration method was employed when plate counts fell below 0.7 logCFU/g. Moisture content of soils were recorded on each sampling day. One-way ANOVA ($p \leq 0.05$) was used to compare *E. coli* survival in BSAAO plots.

Results: There were no significant differences ($p > 0.05$) observed in the reduction of *E. coli* in CPL- (2.36±0.17 log CFU/g), HTPP- (2.87±0.21 log CFU/g), and UN- (2.59±0.1 log CFU/g) plots over 56 days. Across all BSAAO treatments, *E. coli* levels decreased significantly from day 0 (4.61±0.68 log CFU/g) to day 56 (2.21±0.65 log CFU/g). Greater survival of *E. coli* was observed in CPL-plots (2.71±0.05 log CFU/g) and HTPP-plots (2.73±0.43 log CFU/g) compared to UN-plots (1.77±0.65 log CFU/g). Soil moisture content affected *E. coli* survival significantly ($p < 0.05$) with an average moisture content of 3.2% during sampling period.

Significance: Survival of *E. coli* TVS 353 in soils was extended by the addition of HTPP or CPL. These results will be useful for mapping pathogen transfer from soil to crops and pre-harvest risk assessment studies of fresh produce.

P1-133 On-Farm Food Safety Practices Assessment in Texas

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Introduction: The Food Safety Modernization Act (FSMA) is a federal mandate that shifted focus from responding to foodborne illness to preventing it and established seven final rules which include the produce safety rule (PSR). PSR establishes science-based minimum standards for safe growing, harvesting, packing, and holding of vegetables for human consumption.

Purpose: The purpose was to evaluate the results of initial on-farm assessments of food safety practices to identify patterns and challenges that resulted in non-compliance to the PSR.

Methods: Large farms (n=69) in Texas were assessed by field specialists from the Texas Department of Agriculture Office of Produce Safety (TOPS) over two growing seasons. Large farms are defined by FDA as covered farms for which, on a rolling basis, the average annual monetary value of produce the farm sold during the previous 3-year period is more than $500,000. Covered commodities produced by the farms included leafy greens, citrus, summer squash, watermelons, cantaloupe, mushrooms, and tomatoes. On-farm assessments were conducted using PSR to determine non-compliance issues and data was reported by subpart of PSR. The data and results were tabulated using Microsoft Excel anonymously.

Results: The results of the initial assessments showed a total of 110 non-compliance issues related to Subpart O record keeping (46/110), followed by Subpart L equipment and buildings (27/110) and Subpart C personnel qualifications and training (20/110). More specifically, the assessment demonstrated that for Subpart O, growers may perform the practices but not include or log records of the completed work. Examples for subpart L were that few growers were not cleaning and sanitizing equipment used to transport produce and some farms did not dispose of handwashing waste water effectively.

Significance: Study results can determine the opportunity or need for training, education, or development of additional resources to specifically address patterns and/or underlying challenges related to non-compliance.

P1-134 Formation of *Listeria monocytogenes* Persister Cells in the Produce Processing Environment

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Introduction: *Listeria monocytogenes* (*Lm*) is a foodborne pathogen that has been involved in several produce-related outbreaks. Persisters are a subpopulation of phenotypic variants that exhibit high tolerance to stresses such as antibiotic treatments.

Purpose: The objective of this study was to investigate if *Lm* could form persister cells in produce processing environment.

Methods: Three *Lm* strains (483, 480 and 485) isolated from produce processing plants were used in this study. Washed overnight *Lm* culture was centrifuged and resuspended in 5 ml of sterile water, brain heart infusion broth (BHI) or artificial lettuce washing water with high (HCOD) or low organic loads (LCOD) (1,332 vs. 652 mg/L) and inoculated onto stainless-steel coupons. The formation of persister cells were determined after 0, 3, 5 and 7 days of storage at 4 ℃ by exposing *Lm* recovered from the coupons to 100 ppm of gentamicin for 4 h and plating onto tryptic soy agar.

Results: *Lm*483 generated a significantly ($p < 0.05$) higher percentages of persisters in lower nutrient conditions (0.006% in sterile water; 0.003% in LCOD) than in nutrient-rich environments (0.001% in BHI; 0.001% in HCOD) after 7 days of storage at 4 °C. In contrast, *Lm*485 and *Lm*480 formed a significantly ($p < 0.05$) higher amount of persisters in BHI and HCOD compared to those in LCOD and sterile water. Persister cells fell below the limit of enumeration (1 Log) after 3 days of incubation in water for *Lm*485 and *Lm*480, and 5 days in LCOD for *Lm*480. Storage duration did not significantly impact the percentages of persister cells formed during storage ($p > 0.05$).

Significance: Information about the formation of persistent pathogenic cells in various dormant status helps us better develop control strategies to prevent and control the formation of those cells.

P1-135 The Effect of Physico-Chemical Treatment in Reducing *Listeria monocytogenes* Biofilms on Lettuce Leaf

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Introduction: *Listeria monocytogenes* is an anaerobic, rod-shaped, gram-positive pathogen. The water, soil, silage, animal feces and so on are the good sources of *L. monocytogenes*. It causes food contamination due to its growth ability at low temperatures (>1–10 °C).

Purpose: In this study, 22 strains of *Listeria monocytogenes* collected from different clinical (10 strains) and environmental sources (12 strains) were analyzed by PCR analysis conducted on one Listeria-specific gene and five virulence genes.

Methods: The biofilm formation on lettuce exposed to physicochemical treatments under various conditions was investigated. Bacterial growth conditions, PCR analysis, Preparation of lettuce samples and biofilm formation, Chemical treatment (NaOCl and ClO_2), Physical treatment (ultrasound), Simultaneous physicochemical treatment, Detachment of the biofilm from the lettuce samples, Field emission scanning electron microscopy (FESEM), TEM analysis, Confocal laser scanning microscopy (CLSM) were performed in this study.

Results: The results indicated that the individual effects of 5-min ultrasound treatment and 300 ppm sodium hypochlorite (NaOCl) were similar, with a reduction of 0.82 log CFU/cm^2. For chlorine dioxide (ClO_2) at 60 ppm, the biofilm reduction rate was approximately 5.45 log CFU/cm^2. Combined treatments of 5 min of ultrasound plus 300 ppm NaOCl or 40 ppm ClO_2, which proved even more effective, eradicated *L. monocytogenes* biofilm on the lettuce surface in non-detected level.

Significance: ClO_2 has the potential to replace NaOCl in food industries for the disinfection of food products, which has been reported to affect human health, during the sanitization process of fresh food products.

P1-136 Isolation and Characterization of Two Specific Phages to Control *Pectobacterium carotovorum* subsp. *carotovorum*

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❖ ***Developing Scientist Entrant***

Introduction: *Pectobacterium carotovorum* subsp. *carotovorum* (PCC) is a main pathogen causing soft rots in various vegetables. Since the use of agricultural antibiotics to control PCC during harvest has increased the emergence of antibiotic-resistant PCC, the necessity of the lytic phage has increased as a novel alternative substance for preventing the occurrence of antibiotic-resistant PCC.

Purpose: The purpose of this study was to isolate and investigate the characteristics of novel PCC-specific phages for using as an alternative.

Methods: Two lytic phages infecting PCC were isolated and purified from soils of agricultural fields in Korea. Each phage morphology was observed using TEM and the specificity of each phage was investigated using thirteen PCC strains, nine non-PCC strains, and twelve foodborne pathogens by dot assay. Bactericidal effects of two phages were compared by incubating each phage with its host at MOIs of 0.01 to 10 and measuring viable cells during 30 h. Each phage stability at the exposure of UV-A and six major agricultural antibiotics was compared using plaque assay.

Results: Two PCC-specific phages, named as PC1 and PC2, were isolated and purified from soil for tomatoes and napa cabbages, respectively, with the high final concentration. In morphological observation, PC1 was classified into a Myophage whereas PC2 into a Siphophage. The specificity of both phages demonstrated the narrow host range with a slightly different host range. The bactericidal effect of PC2 was superior to maintain the duration time than that of PC1. The stability of PC2 to against UV-A was greater than that of PC1 due to the smaller reduction of phage (0.18 log PFU/mL), however, the stability of PC1 against six agricultural antibiotics was greater than that of PC2, significantly stable in its titer against all antibiotics.

Significance: This study demonstrated the characteristics of two isolated phages from fresh produce soil for developing novel PCC control substances.

P1-137 Efficacy of Silica Powders on Mortality and Progeny Production of the Lesser Grain Borer, *Rhyzopertha dominica* (F.) (*Coleoptera: bostrichidae*)

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❖ ***Developing Scientist Entrant***

Introduction: The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera:Bostrichidae), is a serious insect pest of stored grains and is managed using synthetic insecticides. Such use has resulted in development of insect resistance. Inert dusts are chemically unreactive, are safer alternatives to synthetic insecticides, and resistance in insects is not an issue.

Purpose: This study evaluated efficacy of two silica powders for managing *R. dominica* on concrete surfaces.

Methods: The particle size and shape of the two silica powders, obtained from Imerys Chemicals, Lompac, California, were analyzed using a Malvern Morphologi G3 SE instrument. Bioassays were performed by exposing adults to silica powders-treated concrete arenas in 9 cm Petri dishes at 13 concentrations ranging from 0-5 g/m^2 with exposure times of 4, 8, 12, 24, 36 and 48 h. After exposure adults were transferred to 30 ml round plastic containers with 30 g of wheat to determine mortality at 7 d and adult progeny production at 42 d. Mortality and progeny production data were analyzed using three and one-way ANOVA at $\alpha = 0.05$ and mean separations were done using REGWQ procedure.

Results: The particle size diameter of silica powder 2 was significantly greater compared to silica powder 1 at D_{10}, D_{50} and D_{90} µm diameters. Between the silica powders, adults were extremely susceptible to silica powder 2 leading to complete mortality of adults and inhibition of adult progeny production at a concentration of 0.5 g/m^2 after the 4 h exposure, but with silica powder 1, complete mortality and inhibition of progeny production occurred at the same concentration after a 24 h exposure.

Significance: The implications of the present study indicated that the application of silica powders to concrete surfaces such as empty bins can be very effective in managing *R. dominica* population prior to storage of new grains.

P1-138 Survival of Generic *Escherichia coli* on Different Material Types of Tree Fruit Picking Bags

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❖ ***Developing Scientist Entrant***

Introduction: Data on the survival of microbial indictors on picking bags is lacking. Microbial indicators are often used in food safety to assess cleanliness.

Purpose: The objective was to evaluate survival of generic *Escherichia coli* (TVS353) on four different material types of tree fruit picking bags (100% Canvas, 100% Nylon, 100% Cordura, and a Cordura-nylon blend).

Methods: Coupons of each material type were sterilized by UV light for 10 min and inoculated with a single strain of RIF resistant (80 ppm) generic *E. coli* (TVS353) by spot application of a wet inoculum (100 µL) at 6 to 7 log CFU per coupon. Coupons were dried for 1 h, and held at ca. 22ºC and 30% RH for 7 d. *E. coli* was enumerated at seven different time-points including 0, 4, and 8 h, and 1, 2, 3, and 7 d. Coupons were subjected to rub-shake-rub for 60 s

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with 0.1% peptone (20 mL) and plated in duplicate on selective (MAC-R) and non-selective (TSA-R) media in triplicate experiments with triplicate replicates (n=9). Significant differences (P<0.05) were evaluated by Tukey's HSD test in R studio (version 4.1.1).

Results: Reduction in generic *E. coli* population was dependent on material-type and storage duration. Within 24 h, populations on all material-types declined. By 7 d, survival of *E. coli* was greatest on Canvas (5.7±0.2 log CFU/coupon; P<0.0001), compared to all other material-types. Nylon, Cordura, and Cordura-nylon, each had significant reductions in *E. coli* populations of 1.9, 1.8, and 2.0 log CFU/coupon, respectively by 7 d (P <0.0001).

Significance: Preliminary findings suggest picking bag material-type and storage duration (which aimed to mimic time between cleaning/sanitizing) impacted recovery of *E. coli*. Canvas picking bags may pose a greater risk for produce contamination, compared to other material-types; thus, growers may wish to use bags with minimal canvas.

P1-139 Efficacy of Sanitizer Treatments in Simulated Dump Tank Water Against *Listeria monocytogenes* on Apples

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❖ ***Developing Scientist Entrant***

Introduction: Chlorine and peroxyacetic acid (PAA) are commonly applied sanitizers in recirculated dump tank systems during commercial apple packing; however, little is known about their efficacies against *Listeria monocytogenes* in this application.

Purpose: To evaluate the efficacies of chlorine and PAA in inactivating *L. monocytogenes* on fresh apples and preventing cross-contamination in simulated dump tank water (SDTW).

Methods: Granny Smith apples were inoculated with *L. monocytogenes* at 6.0 $\log_{10}$ CFU/apple, then subjected, alone or along with uninoculated apples, to different solution treatments in SDTW with 1000 ppm chemical oxygen demand. Survival of *L. monocytogenes* on inoculated apples, uninoculated apples, and spent washing solution were enumerated.

Results: Efficacies of chlorinated water with an initial free available chlorine (FAC) of 25-100 ppm against *L. monocytogenes* on apples were significantly impacted by the presence of organic matter, especially for chlorine with 25 ppm FAC. Chlorine wash at 50-100 ppm FAC for 2-min in SDTW reduced *L. monocytogenes* on apples by 0.9 $\log_{10}$ CFU/apple. However, 25 ppm FAC in SDTW for 2-5-min wash only led to 0.3 $\log_{10}$ CFU/apple reduction of *L. monocytogenes* on apples. Efficacies of PAA at 20-80 ppm against *L. monocytogenes* were less influenced by organic matter; PAA at 80 ppm and 2-5-min contact caused 1.7-1.8 $\log_{10}$ CFU/apple reduction. Sanitizers at tested concentrations reduced the counts of *L. monocytogenes* transferred to uninoculated apples in SDTW but could not prevent it even for 100 ppm chlorine or 80 ppm PAA, regardless of contact time.

Significance: Data highlight the need for alternative intervention strategies for dump tank operation to ensure the microbial safety of apples.

P1-140 Impact of Gas Ultrafine Bubbles on the Efficacy of Commonly Used Antimicrobials for Apple Washing

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❖ ***Developing Scientist Entrant***

Introduction: Ultrafine bubble (UFB) technology is a novel tool in food safety with the potential to improve the potency of antimicrobials during produce washing.

Purpose: This research was conducted to investigate the impact of incorporating gas [air and carbon dioxide (CO_2)] UFBs on the potency of chlorine (Cl_2; 100 and 200 ppm) and peracetic acid (PAA; 40 and 80 ppm) antimicrobial solutions against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on inoculated Gala apples.

Methods: Apples were dip inoculated with either *E. coli* or *L. monocytogenes*, and then allowed to dry at room temperature (~22°C) for 1 h. The apples were treated by dipping into Cl_2 or PAA solutions with or without UFBs for 1 or 2 min. Apples were then transferred directly into stomacher bags containing Dey-Engley neutralizing broth, in which they were hand messaged for 90 s. Microbial enumerations were performed using injury recovery media (brain heart infusion agar overlaid with respective selective media).

Results: The incorporation of CO_2 UFBs in antimicrobial solutions resulted in significantly greater *E. coli* and *L. monocytogenes* reductions (2.1 and 2.4 Log CFU/apple, respectively) on apples compared to solutions without UFBs (1.4 and 1.9 Log CFU/apple, respectively). However, the incorporation of air UFBs resulted in similar log reductions of *E. coli* and *L. monocytogenes* (1.9 and 2.2 Log CFU/apple, respectively) on apples compared to antimicrobials with CO_2 UFBs and without UFBs. The 2 min treatment time for various antimicrobials resulted in significantly greater *L. monocytogenes* reductions (2.4 Log CFU/apple) compared to 1 min treatment time (2.0 Log CFU/apple), but no differences in log reductions based on treatment time were observed for *E. coli*.

Significance: This study demonstrated that using CO_2 UFBs in Cl_2 and PAA solutions significantly increases their potency against *E. coli* O157:H7 and *L. monocytogenes* on Gala apples.

P1-141 Fates of *Listeria monocytogenes* on Waxed Apples and Brushes Contaminated during Wax Coating

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Introduction: *L. monocytogenes* has been reported to be isolated from waxing coating lines in apple packing facilities, highlighting the potential risk of microbial cross-contamination to fresh apples. However, little is known about the fate of *L. monocytogenes* on waxed apples and waxing brushes during storage.

Purpose: To assess the persistence of *L. monocytogenes* on waxed apples contaminated before wax coating or cross-contaminated during wax coating application, as well as on waxing brushes.

Methods: Fresh Fuji apples were dip-inoculated with *L. monocytogenes* followed by wax coating application or introduced by waxing brushes. Waxed apples were then subjected to 12-week cold storage mimicking commercial storage conditions. The fate of *L. monocytogenes* on waxed apples and wax brushes was further evaluated.

Results: After 12 weeks of cold storage, there were about 1.8-2.0 $\log_{10}$ CFU/apple reductions of *L. monocytogenes* on apples pre-contaminated with *L. monocytogenes* before waxing coating regardless of coating types. A similar die-off of *L. monocytogenes* was observed on unwaxed Fuji apples. There was a 1.8-1.9 $\log_{10}$ CFU/apple reduction of *L. monocytogenes* on waxed apples contaminated during wax coating after 12-weeks of cold storage, which was comparable to that of *L. monocytogenes* on waxed apples contaminated before wax coating. *L. monocytogenes* transferred to waxing brushes were gradually die-off during the first four weeks of ambient storage, then remained relatively stable level at ~3 $\log_{10}$ CFU/brush during the subsequent eight weeks of storage.

Significance: This study provides the apple industry with information about the persistence of *L. monocytogenes* on waxed apples and waxing brushing, highlighting the need for potential intervention strategies to ensure microbial safety.

P1-142 Fates of *Listeria innocua* on Fuji Apples with Commercial Wax Coating

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❖ ***Developing Scientist Entrant***

Introduction: Wax coating is widely used on apples during commercial packing; however, little is known about the fate of *Listeria* spp. on waxed apples during commercial cold storage.
Purpose: To assess the fate of *Listeria innocua*, a well-known surrogate of *Listeria monocytogenes* on wax-coated Fuji apples during commercial cold storage.
Methods: Fuji apples were inoculated with *L. innocua* at 5-6 $\log_{10}$ CFU/apple, dried at ambient temperature (~22°C) unless specified, then coated with three commercial apple fruit waxes, PrimaFresh 360 HS, PrimaFresh 606 EU, or Shield-Brite AP-450. The waxed apples were subjected to commercial cold storage at ~ 1°C for up to 18 weeks and sampled every 2-3 weeks for *L. innocua* survival*,* and yeasts and molds enumeration with four independent replicates (8-10 apples/replicate).
Results: The drying temperature at 22 - 60 °C had no impact on the survival of *L. innocua* on wax- coated apples. During 18 weeks of commercial cold storage, *L. innocua* was reduced by 1.9 and 2.3-2.5 $\log_{10}$ CFU/apple on unwaxed apples and wax-coated apples in year 1. In year 2, the reduction of *L. innocua* on waxed apples, regardless of wax type, was reduced by 1.9-2.0 $\log_{10}$ CFU/apple, which was not different from that on unwaxed apples after 18 weeks of commercial cold storage. Furthermore, the wax coating had no impact on the survival of yeasts and molds on apples; there was a 0.4-0.5 $\log_{10}$ CFU/apple increase after 18 weeks of cold storage regardless of wax treatment.
Significance: Data provide the apple industry with information about the fate of *Listeria* on waxed fruits during storage and holding.

P1-143 Effect of Conditioning and Storage Practices on 'Honeycrisp' Apple Interactions with *Salmonella enterica* and STEC Implications for Food Safety

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Introduction: The 'Honeycrisp' apple is a chilling sensitive cultivar that can develop chilling injury when cooled immediately after commercial harvest. Apples are typically subjected to temperature conditioning to prevent physiological disorders from chilling during extended storage. The impact of these practices on food safety is not known.
Purpose: To investigate the effect of conditioning and storage practices on 'Honeycrisp' apple association with enteric pathogens.
Methods: 'Honeycrisp' fruit was harvested at commercial harvest and randomly split into groups for immediate processing or subjected to a conditioning step (1 week storage at 10°C before transferring to 3°C) or not (immediately stored at 3°C). Stored fruit were evaluated after 2 and 4 months of storage (n=18 per treatment). 'Honeycrisp' apples were inoculated with cocktail strains of *Salmonella enterica* (Se) *s*erotypes Newport, Enteritidis and Javiana, and Shiga toxin-producing *Escherichia coli* (STEC) O124:H19, O103:H11. Sections of peel or flesh were inoculated with ~4.5 LogCFU/sample at harvest, 2 and 4 months of post-storage. Samples were incubated in bags for 24 h at room temperature and bacteria were enumerated by serial dilution and plate counting.
Results: For peel and slices, Se counts were lower ($p \leq 0.05$) from apples stored for 2 and 4 months compared to harvest, regardless of conditioning. Se levels on peel were 0.5 and 3.4 logCFU/peel lower on conditioned and 1.1 and 3.1 logCFU/peel lower on non-conditioned apples at 2 and 4 months, respectively. In addition, Se levels of the flesh among conditioned apples were 2.2 and 4.8 logCFU/slice lower, and among non-conditioned apples 1.2 and 3.4 logCFU/slice lower at 2 and 4 months, respectively, compared to apples processed at harvest. For STEC, no effect was detected for peel, but counts on non-conditioned apple slices were 1.5 logCFU/flesh higher ($p \leq 0.05$) than conditioned apples at 4 months of storage.
Significance: Data suggest that storage length and possibly conditioning affect enteric pathogen associations in apples.

P1-144 Evaluating the Effect of the Plant Growth Regulator Retain® on 'Honeycrisp' Apple Association with *Listeria monocytogenes* and *Salmonella enterica*

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Introduction: Fruit of the 'Honeycrisp' apple grown in Maryland tend to drop from the tree before maturation. The plant growth regulator ReTain® is often applied to extend harvest time and reduce abscission that results in fruit loss before picking. The influence of delaying ripening on food safety is not known.
Purpose: To investigate whether ReTain® application to 'Honeycrisp' apple fruit effects enteric pathogen association with fruit peel and flesh.
Methods: 'Honeycrisp' fruit were harvested from trees treated with the plant growth regulator ReTain® (n=18) or without (control, n=18) at commercial harvest and 2-weeks post-commercial harvest. Fruit was sliced and inoculation experiments of 'Honeycrisp' flesh and peel were performed using strain cocktails of two enteric pathogen species: *Listeria monocytogenes* (Lm) ATCC 19115, Lm F2025, and Lm F2030 and *Salmonella enterica* (Se) serotypes Newport, Enteritidis and Javiana*.* Sections of peel or flesh samples were inoculated with ~4 LogCFU Lm or Se. Samples were incubated in bags for 24 h at 35°C (Se) and 48 h at 30°C (Lm). After incubation, bacteria were enumerated by serial dilution and plate counting.
Results: There were statistically significant differences ($p<0.05$) in the recovery of bacteria from the peel and flesh of apples treated with ReTain® versus no ReTain® at the time of commercial harvest. Lm levels were 2.2 logCFU/peel higher but 0.8 logCFU/flesh lower on ReTain®-treated fruit than control, respectively ($p<0.05$). At commercial harvest, both ReTain®-treated fruit peel ($p<0.05$) and flesh ($p<0.05$) were less favorable for *Salmonella* than control, with counts being 1.9 log CFU/peel and 0.3 logCFU/flesh lower. These differences between ReTain®-treated and untreated fruit were not detected in fruit collected 2-weeks post-commercial harvest.
Significance: Data suggest that the ability for apples to support enteric pathogens may be impacted by fruit ripening inhibitors if collected at commercial harvest, but not if harvested past this date.

P1-145 Application of Atmospheric Cold Plasma (ACP) for *E. coli* O157:H7 Inactivation on Gala Apples

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Introduction: Fresh produce consumption in the United States has increased in recent years as consumers search for a healthier lifestyle. However, microbial contamination of produce has become a major food safety challenge. Atmospheric cold plasma (ACP) is on the rise as an emerging non-thermal technology for microbial decontamination.
Purpose: The purpose of this study was to evaluate the efficacy of a dielectric barrier discharge ACP system as a non-thermal decontamination technique to inactivate *Escherichia coli* O157:H7 on Gala apples.
Methods: Approximately 100 µl aliquots of *Escherichia coli* O157:H7 were spotted on the denoted areas on the apples. About 9 $\log_{10}$ CFU/ml of *E. coli* O157:H7 was inoculated on the surface of apples and air dried for 1 hour. Subsequently, contaminated apples were subjected to ACP treatment for 10, 20, 50, 100, 150, 200 seconds at a fixed input power of 200 W and at 50 mm from the electrodes. Treated apples were then analyzed to determine *E. coli* O157:H7 inactivation, each treatment was performed in triplicates.
Results: Results obtained showed a significant ($p < 0.05$) positive correlation between exposure time and log reduction. A significant ($p < 0.05$) log reduction of 0.4, 1.0, 1.7, 2.1, 2.7 and 3.7 CFU/ml was observed when exposure time increased from 0 to 10, 10 to 20, 20 to 50, 50 to 100, 100 to– 150 and

Poster

150 to 200 seconds, respectively. Microbial inactivation data was modeled by using Weibull distribution. According to the model, predicted time to achieve a 5-log reduction was estimated to be 335 seconds. Our results also demonstrated that no physical changes in color and texture occurred on the surface of apples after ACP treatment.

Significance: This study demonstrated that ACP could be a promising technology for inactivation of foodborne pathogens on the surface of fresh produce without altering the physical characteristics.

P1-146 Evaluation of Nontoxigenic *Clostridium* spp. as Proteolytic *Clostridium botulinum* Surrogates for Growth Inhibition Challenge Studies

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Introduction: Identifying a surrogate for *Clostridium botulinum* is imperative since its use in growth inhibition challenge studies is restricted to authorized laboratories. Pass/fail criteria for *C. botulinum* growth inhibition studies is determined by toxin (BoNT) production, which complicates the implementation of nontoxigenic surrogates.

Purpose: To correlate growth of nontoxigenic *Clostridium* spp. as potential surrogates with growth and neurotoxin production by *C. botulinum* in a model food system.

Methods: Ground turkey (75±1.0% moisture, 1.5±0.2% NaCl, pH 6.4±0.1) was supplemented with sodium lactate (0, 2, 2.5, 3 and 3.5%) and inoculated with 2-log CFU/g of a 10-strain *C. botulinum* (serotypes A and B) or 4-strain nontoxigenic *Clostridium* spp. (*C. tyrobutyricum*; *C. sporogenes*) spore cocktail. Samples were vacuum packaged, cooked to 75˚C for 10 minutes, chilled in ice-water, and incubated at 27˚C for 7 days. Triplicate samples of each treatment were enumerated daily for *Clostridium* using Differential Reinforced Clostridial Agar. BoNT was detected in *C. botulinum*-inoculated samples using the mouse bioassay. Each experiment was conducted twice.

Results: BoNT production was delayed with increasing concentrations of sodium lactate; BoNT was detected at days 3, 3, 4, 5 and 5, for Control, 2, 2.5, 3, and 3.5% lactate, respectively. Population increases of *Clostridium* surrogates was comparable to that of *C. botulinum* for all treatments ($p<0.05$) except for the 3 and 3.5% lactate treatments on day 4 and 5 respectively, in which *C. botulinum* exhibited better growth (~1 log greater increase). The lowest average growth of *C. botulinum* associated with toxin production was ~3.1 log (~6 log CFU/g) observed in the 3.5% treatment and corresponded to ~3.8 log (~5 log CFU/g) for *Clostridium* surrogates.

Significance: These data suggest the nontoxigenic *Clostridium* spp. analyzed in this study may serve as suitable screening surrogates for proteolytic *C. botulinum* growth inhibition studies with pass/fail criteria of no more than a 2-log increase of growth.

P1-147 The Efficacy of Conventional Garden Spray, Electrostatic Spray, and Dip with a Peroxyacetic Acid and Hydrogen Peroxide Mixer to Inactivate *Listeria monocytogenes* on Apples

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❖ ***Developing Scientist Entrant***

Introduction: Due to multi-state outbreaks of listeriosis on apples from 2015-2019, *Listeria monocytogenes* has been deemed an emerging, under researched pathogen by the United States Department of Agriculture -National Institute of Food and Agriculture.

Purpose: This study evaluated the efficacy of a PAA+H_2O_2 mixer's (SD) ability to inactivate *L. monocytogenes* on apples when delivered by electrostatic sprayer (ES), garden sprayer (GS), and dipping.

Methods: Organic *Honey Crisp* (HC), *Fuji* (FJ), and *Pink Lady* (PL) apples were dip-inoculated with *Listeria* (2-strain) for 5 min and then dried for 10 min. The inoculated apples were then untreated (control), sprayed with water only, or treated with SD-0.0064, 0.1, 0.25 and 0.50% for 20 s via GS, ES, or dip. Then the apples were drained (2 min) and placed in a sterile sample bag with 100 ml tryptic-soy-broth and shaken for 30 sec. Samples were then 10 or 100-fold serially diluted in 0.1% buffered- peptone-water and spread plated on MOX agar plates, followed by incubation for 48 h at 37°C and enumeration. The Mixed Model Procedure of SAS (p=0.05) was used to analyze the reduction of *L. monocytogenes* on cultivars (2 replicates/6 samples/replicate) under different delivery methods, concentrations, and their interactions.

Results: Results indicated dip was the most effective ($P < 0.05$) antimicrobial delivery method compared to GS and ES, as shown by the reductions, which decreased from 2.41 (dip) to 1.54 (GS) and to 1.06 (ES), from 2.31 (dip) to 1.79 (GS) and to 1.20 (ES), and from 2.41(dip) to 1.44 (GS) and to 0.84 $\log_{10}$CFU/apple (ES) on HC, FJ, and PL apples, respectively. Reductions of *L. monocytogenes* were greatest ($P < 0.05$) when apples were treated with 0.50% SD.

Significance: Results suggested that applying SD through dipping in a 0.5% concentration level is effective at inactivating pathogens on apples, thus reducing the risk for future listeriosis outbreaks.

P1-148 Aggregative Bootie Cover Soil Sampling Shows Similar Indicator Bacteria Recovery Ability Compared to Grab Soil Sampling from Commercial Romaine Fields

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Introduction: Soil is a potential contamination source of produce. However, typical grab soil sampling may be underpowered due to sampling location and size limitations. Soil sampling by testing bootie covers might be more representative as it aggregates across a field.

Purpose: The purpose of this study was to develop a bootie cover soil sampling method and to compare it with soil grab sampling.

Methods: Sampling was performed on two days, in eight beds per day, in a commercial Romaine lettuce farm in Salinas, California. Three sampling methods were used to take soil samples: high resolution grabs (HR), composite grabs (CS), and bootie cover sampling (BC, wearing disposable bootie covers while taking grab samples). The number of HR, CS, and BC samples was 72, 28, and 28, respectively. Twenty-five grams of well-mixed soil sample, or the whole bootie, was processed to enumerate generic *Escherichia coli*, coliforms, and aerobic plate counts (APC). ANOVA and *F*-tests were used to compare means and variances, respectively.

Results: There was no generic *E. coli* detected in all samples. For coliforms, recovery on BC, CS, and HR were 3.51 ± 0.57, 2.63 ± 0.61, and 2.56 ± 0.92 log CFU/g, respectively. Bootie cover showed a higher ($P < 0.05$) mean than CS and HR, whereas HR showed a higher ($P < 0.05$) variance than CS and BC. For APC, recovery on BC, CS, and HR were 7.03 ± 0.26, 7.12 ± 0.18, 7.32 ± 0.15 log CFU/g, respectively. The means of CS and BC were lower ($P < 0.05$) than HR, whereas the variances of CS and HR were lower ($P < 0.05$) than BC.

Significance: These data suggest that aggregative bootie cover testing performs similar to, at least not worse, than grab sampling for indicator organism testing. Future work is planned to determine if this is a more powerful method for pathogen detection.

P1-149 Use of a Novel Ozonated Water Generation System to Reduce Surrogate *E. coli* on Leafy Greens

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Introduction: The use of ozone as a produce disinfectant has been affected by application shortcomings, , such as low or unstable concentration generation, poor oxidation reduction potential (ORP), poor stability among other parameters; however, its use for RTE produce such as high-risk leafy greens and its benefits when compared to other options require a stable generation system that consistently lowers bacterial contamination.
Purpose: The aim of this study was to evaluate the use of novel ozonated water generator, Biosafe™ system as an anti-microbial for leafy greens by the use of pathogen surrogates.
Methods: Leafy green samples were purchased from local retail stores. A total of 330 sample units of 25g each were analyzed for the study (120 lettuce, 120 spinach and 90 Kale). Samples were divided into 3 groups, n= 110 was cultured for natural flora (total aerobic counts), n= 220 were submerged in an 8-log cocktail of surrogate *E. coli* -ATCC® MP-26™. This group was further divided into 2 (n=110 were cultured as controls (attachment) and n=110 were treated then cultured (treatment)). Highly reactive (>810 mV ORP ozonized water at 3ppm was sprayed on to each side of the leaves for five (5) seconds. Samples were analyzed for microbial reduction by plating dilutions on to 3M petrifilms.
Results: Overall, data shows that the ozonated water generating system reduces microbial levels ranging from 6 logs to between 5 and 4.5 logs on each leafy green respectively when using a surrogate cocktail to simulate natural contamination.
Significance: The data suggests that Biosafe™ ozonated water generating system is effective in deactivating surrogate *E. coli* (ATCC® MP-26™) on leafy green surfaces.

P1-150 Biomapping of Indicator Organisms in Controlled Environment Agriculture Vertical Hydroponic Leafy Greens Production Facility to Support Food Safety Management Systems

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Introduction: Controlled Environment Agriculture is becoming a viable option for the production of leafy greens to improve food safety and sustainability; however, the microbial levels of indicator organisms and potential for generating contamination sources in these environments is largely unknown.
Purpose: The study aims to establish statistical threshold values, that will support food safety management decisions and the identification of antimicrobial intervention locations for controlled environment agriculture/greenhouses that grows leafy greens vertically in hydroponics.
Methods: A vertical indoor agriculture facility for the production of leafy greens (lettuce varieties) was sampled during full production for 6 months on a bi-weekly basis. Environmental (vines, floors, walls, door handles, equipment, tools, wares, fertigation, etc.) and product samples were collected. A total of 939 environmental and 203 product samples were evaluated for this study. Samples were analyzed for Aerobic Counts (AC), *Enterobacteriaceae* (EB) and *E. coli* counts (EC) using the 3M Petrifilm™ system, the TEMPO™ system and the MicroSnap™ system according to manufacturer's recommendations. Microbial levels were converted to Log10 CFU/surface-area/swab/cm^2/100mL for environmental samples and Log10 CFU/g for product and analyzed using the R® Statistical Software.
Results: Contamination levels varied significantly during the production cycle. The environmental surfaces with high contamination levels were: drains, nursery floor, cradles, and vines in decreasing levels from AC to EB and below the detection limit for EC. The product contamination levels with AC increased from 2logcfu/g at planting to 3logcfu/g at harvesting.
Significance: Despite the high control levels of greenhouse operations, there is evidence of foodborne outbreaks attributed to leafy greens grown in controlled environments. Developing microbial baselines of indicator organisms can serve the alternative agriculture industry by identifying potential sources of contamination and will also assist these operations in developing baselines to determine statistical process controls.

P1-151 Endophytic Bacterial Communities Associated with Berries and Leafy Greens May Contribute to Enteric Virus Persistence

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❖ ***Developing Scientist Entrant***

Introduction: Human norovirus (hNoV) contaminations of berries and leafy greens are a major cause of epidemic gastroenteritis worldwide. Several studies have already identified interactions between enteric viruses and commensal bacteria in the human gut, but limited work has been done with bacterial communities associated with plant surfaces and their role in hNoV persistence.
Purpose: The purpose of this study was to evaluate the adhesion of hNoV surrogates, murine norovirus (MNV-1) and Tulane virus (TV), to biofilm-forming bacteria associated with the surfaces of berries and leafy vegetables.
Methods: Bacteria associated with berries and leafy greens which also have the ability to form biofilms (*Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Pseudomonas fluorescens* ATCC 13525, *Pantoea agglomerans* ATCC 27155 and *Bacillus cereus* ATCC 14579) were grown *in vitro* in a 96-well plate with pegs built into the lid and used for the MNV-1- and TV-biofilm adhesion assay (10^5 PFU/mL, 25 °C for 30 min). RNA from viruses attached to biofilms was extracted (NucliSens magnetic extraction protocol) and then quantified by RT-qPCR. Recovery means (RNA genome copies) were then assessed.
Results: No significant differences (ANOVA 2-way, Tukey) in MNV-1 (5.8 ± 0.1) and TV (6.44 ± 0.05) genome copies/sample were detected in the presence of bacterial biofilms compared to their respective control (5.6 ± 0.2 and 6.23 ± 0.08). However, the unchanged recovery rate could indicate a positive interaction between the two viruses and bacteria under these conditions.
Significance: The data suggest that direct virus-biofilm interactions may protect MNV-1 and TV from viral inactivation treatments, such as pulsed light (PL) and thus, allow a greater persistence of the virus on food surfaces. PL treatments are being analysed. Eventually, this project could lead to the development of new biofilm control strategies and more efficient viral inactivation methods in the food industry.

P1-152 Prevalence of Hepatitis E Virus in Blueberries and Pork Liver Pâtés in Canada

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Introduction: The Hepatitis E Virus (HEV) causes sporadic hepatic cases in industrialized countries through a zoonotic transmission. Pigs and wild animals have been characterized as HEV-hosts. The main source of transmission of HEV to humans is the consumption of raw or undercooked pork. Another possible route of transmission could be the presence of wild animals carrying the virus in berry fields, that would contaminate the fruits by direct contact with infected feces.
Purpose: The aim of this study is to assess the potential level of HEV contamination in blueberries and pork liver pâtés sold in Canada.
Methods: One hundred and fifty samples of 25 g of blueberries were harvested in the provinces of Québec and New Brunswick (Canada) and processed according to the standard method ISO 15216-1:2017(E) (limit of detection : 100 genome copies/sample). Fifty samples of raw pork liver pâté and fifty samples of cooked pork liver pâté were collected in the province of Québec (Canada). All pork liver pâté samples were processed by crushing and successive washings with centrifugation, to recover a clarified suspension which was then filtered. For both food matrices, viral RNA extraction was achieved using the Nuclisens eGene-UP method. Molecular detection of HEV was then performed by RT-qPCR. Appropriate positive and negative controls were performed for each sample types.
Results: Out of the 150 tested blueberry samples, none showed HEV detection (95% CI 0.000 - 0.0243). Raw and cooked pork liver pâté samples are currently being analyzed.

Poster

Significance: According to our experimental plan, these results suggest that the risk of HEV contamination of Canadian blueberries is low or non-existent and thus, the risk of HEV transmission to humans through ingestion of zoonotic-contaminated blueberries is minimal. However, sampling plan to cover larger blueberry production fields could bring more precision on the risk assessment of HEV.

P1-153 Significance of Human Norovirus and Hepatitis A Virus in Cranberries Harvested in Quebec

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Introduction: Millions of foodborne illnesses are recorded each year worldwide and are mainly caused by enteric viruses, such as human norovirus (huNoV) and hepatitis A virus (HAV). Contamination routes can occur unintentionally by a discharge of contaminated water, like wastewater, close to the production environment or by irrigating food with this contaminated water. Berries are known to be involved in several outbreaks around the world due to their contamination with foodborne viruses. Cranberry production involves close contact with water throughout his harvesting and could potentially be contaminated by such viruses.

Purpose: The aim of this study was to evaluate the prevalence of huNoV and HAV in cranberries harvested in the province of Quebec (Canada), the second worldwide producer of cranberries.

Methods: Two hundred thirty-four samples of 2 x 25 g frozen cranberries were harvested all around Quebec, from 44 different cranberry producers and analyzed. With a statistical power of 80%, the expected results were a prevalence of less than 2% ($p<0.01$). The international standard ISO 15216-1:2019 method was performed to concentrate viruses using the mengovirus as the process control. Viral RNA was then extracted using Nuclisens buffers and eGene-UP apparatus and detected using RT-qPCR.

Results: To date, more than half of the samples (165/234) were analysed with the ISO 15216-1:2019 method. HuNoV genotype I RNA was detected in three samples by RT-qPCR. Further confirmation (Sanger sequencing) and infectious state (propidium monoazide (PMA) pretreatment) will be performed on these three samples with the second 25 g. No HAV genetic material has been detected so far.

Significance: Our results bring more information on foodborne viral risk assessment in cranberries, a Quebec local product in great demand. More investigation is needed to better understand the role of the harvesting method involving flooding fields in possible viral contamination of fruits.

P1-154 Risk of Norovirus Foodborne Illness by Raw Oyster Consumption in Korea

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Introduction: Oyster is occasionally consumed raw or undercooked, and many studies show the high prevalence of norovirus in oyster samples. Thus, the consumption of the oyster may cause norovirus foodborne illness.

Purpose: The objective of this study was to evaluate the probability of norovirus foodborne illness by the oyster consumption in Korea.

Methods: Oyster samples (N=156) were collected from markets, and the presence of norovirus was evaluated. The oyster samples inoculated with murine norovirus (norovirus surrogate), and the viral titers were enumerated during storage at 4°C−25°C. The norovirus titers were fitted to the Baranyi model to calculate shoulder period and death rate. To evaluate the temperature effect on these kinetic parameters, the parameters were fitted to a polynomial model as a function of temperature. Temperature and time for distribution of oysters were surveyed, and consumption amounts were also surveyed. A dose-response model for norovirus was also searched. With these data, the probability of norovirus foodborne illness was estimated by the Monte Carlo simulation in @Risk software.

Results: Of 156 samples, norovirus was detected 1 sample. Thus, the initial contamination level was estimated by the Beta distribution (2, 156), and the level was -5.3 Log PFU/g. The developed predictive models showed that norovirus titers decreased under the conditions of the Uniform distribution (0.325, 1.643) for time and the Pert distribution (10, 18, 25) for temperature. The Pert distribution [RiskPert (1.8200, 1.8200, 335.30), RiskTruncate (0, 236.8)] showed that the average consumption amount was 56.65 g at 0.98% of frequency. ${}_1F_1$ hypergeometric dose–response model [$1-(1+2.55\times10^{-3}\ \text{Dose})^{-0.086}$] was appropriate for the dose response of norovirus. The simulation showed that the probability of norovirus foodborne illness was 5.9×10^{-10} per person per day.

Significance: The results indicate that the raw oyster consumption may cause norovirus foodborne illness in Korea at 5.9×10^{-10} per person per day.

P1-155 Development of Small Interfering RNA to Knock-Down *irf7* Related to Anti-Viral Factor in FRhK-4 Cells

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Introduction: Hepatitis A is the inflammation of the liver caused by hepatitis A virus (HAV). It is one of the most frequent causes of foodborne infection. HAV should be cultured in fetal rhesus kidney-4 (FRhK-4) cells to increase the copy number of HAV prior to the application of molecular detection technique. However, the detection of HAV in samples is considered difficult because of the antiviral factor of FRhK-4 cells.

Purpose: The objective of this study was the development of small interfering RNA (siRNA) to knock-down interferon regulatory factor 7 (*irf7*) which plays a role in antiviral function in FRhK-4 cells.

Methods: We designed siRNA sequences to knock-down of *irf7* in FRhK-4 cells, using online program provided by Dharmacon Inc. The siRNA was transfected to FRhK-4 cells, and HAV was infected the cells at 10^5 PFU/mL. After the culture at 37°C and 5% CO_2 for 2 days, the expression levels of cytokines (*cxcl10* and *ccl4*) encoded by *irf7* in the siRNA-transfected FRhK-4 cells were measured through quantitative real-time PCR. *cxcl10* amplification with the primers and *ccl4* amplification with the primers were used in the qRT-PCR analysis. Cycle threshold (Ct) values were used to evaluate the relative expression of *irf7*. Relative gene expressions were calculated using $2^{-\Delta\Delta Ct}$ method.

Results: The appropriate siRNA sequences were 5'- GCA CCU GGA CGG ACA CUU A=UU- 3. Transfection of this siRNA into FRhK-4 cells were induced successfully. Subsequently, the cytokine levels of *cxcl10* and *ccl4* were significantly lowered ($p<0.05$) in siRNA-transfected FRhK-4 cells compared to the non-transfected FRhK-4 cells.

Significance: This finding suggests that siRNA to knock-down *irf7* in FRhK-4 cells was developed successfully, and thus, the cells can be used to culture more HAV by repressing *cxcl10* and *ccl4* expressions.

P1-156 Method Validation for the Recovery of Porcine Respiratory and Reproductive Virus, a Potential SARS-CoV-2 Surrogate, from Stainless Steel

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Introduction: Airborne transmission of SARS-CoV-2 within food manufacturing facilities is well documented. However, the potential for the virus to survive and persist on food contact surfaces has not been extensively evaluated. Therefore, identifying an appropriate surrogate is critical to studying viral transfer and decontamination.

Purpose: To compare virus recovery methods from soiled stainless-steel coupons at 4°C.

Methods: Porcine Respiratory and Reproductive Virus (PRRSV) (ca.7 logs tissue culture infective dose, TCID50) was inoculated onto stainless-steel coupons (1.5 cm x 1.5 cm) along with organic load (5% yeast extract, 5% bovine serum albumin, and 0.4% bovine mucin) and allowed to dry for 15 min at room temperature followed by incubation at 4°C and 35% relative humidity. At 1 h and 24 h post-inoculation, the coupons were processed for virus recovery by four methods: vortex, vortex with glass beads, elution buffer and vortex, elution buffer and shake. The rinsate was processed for virus 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: All four methods were equally effective to recover PRRSV from the soiled stainless-steel surfaces (> 79% recovery) and the amount of infectious virus recovered after 24 h was similar ($P \geq 0.05$) to that recovered at 1 h indicating that the virus survived well for up to 24 h without losing infectious potential.

Significance: The 'elution buffer and shake' method will be used in subsequent experiments with human coronavirus 229E (HCoV-229E) because it is the most economical and labor-saving method. Experiments are underway to determine the survival and persistence of HCoV-229E on food contact surfaces under simulated food industry conditions of temperature and relative humidity.

P1-157 Method Development to Determine Virus Contamination in Soil Types Collected from U.S. Farms

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Introduction: Soil has been considerably less studied than other vehicles of transfer of foodborne viral disease such as foods, surfaces or water. There is currently limited knowledge indicating contamination of produce through virus transfer from soil, application of soil amendments and limited data on the factors influencing this potential uptake.

Purpose: A study was undertaken to develop analytical tools to identify virus presence (focusing on norovirus and hepatitis A virus) in different soil types collected from several farms from two states (Ohio and Georgia) throughout two growing seasons.

Methods: Preliminary evaluation of the extraction method by seeding with a human norovirus GII.4 clinical isolate revealed high variability in recovery between different soil types. As a result, a direct nucleic acid extraction method was chosen to screen the close to two hundred farm samples collected during the 2019 growing season. Nucleic acids recovered were tested for individual virus RNA by real-time quantitative reverse transcription qPCR (RT-qPCR).

Results: Five-g samples were tested in duplicate from each batch of soil collected. Norovirus (genogroups GI and GII) prevalence was less than 2% and hepatitis A virus occurrence was around 7%. Positive samples had low virus load as measured in RNA copies, (corresponding to high RT-PCR Ct values of ≥ 35). Several of the samples exhibited significant PCR inhibition (36/140) highlighting the need to remove such inhibitory substances from the extracted RNA. Inhibition was most frequent in silt textured soil, however, since we have metadata for a limited number of the samples, we cannot draw further correlations regarding farming practices or other soil characteristics and PCR inhibition.

Significance: Having a readily available and versatile method that is compatible for virus recovery from diverse soil types coupled with molecular detection (by RT-PCR) is necessary to better understand presence, diversity, and stability of these viral pathogens in soil.

P1-158 Combination of Paper Membrane-Based Filtration and Ultrafiltration for the Enhanced Detection of Foodborne Virus from Post-Washing Water

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Introduction: Post-washing water (PWW) of root vegetables was considered a vehicle for foodborne virus transmission in the post-harvest stage.

Purpose: For supporting viral surveillance and control, this study aimed to optimize the methodology of concentrating viruses in high soil content PWW.

Methods: In the current study, PWW (soil content:0, 1, 2, 3, and 5%, w/v) seeded with approximate 10^5–10^2 PFU/ $TCID_{50}$ (10-fold serial diluted titer) of non-enveloped murine norovirus 1 (MNV-1, surrogate of human norovirus), hepatitis A virus (HAV) strain HM-175, and enveloped human coronavirus 229E (HCoV-229E, surrogate of SARS-COV-2), was conducted to estimate their recovery efficiency by utilizing the following three methods: (1) centrifugal concentrator device method, (2) negatively charged membrane adsorption-elution method, (3) paper membrane-based filtration-concentration method.

Results: Genome copies (GC) of each virus were quantitated by RT-qPCR to calculate their recovery efficiency [(recovered/ seeded) × 100%]. The third concentration method with a paper membrane filtration was the most efficient of the three methods and the total average recoveries efficiency (%) was determined to be 117.4 ± 58.5 (MNV-1), 118.2 ± 126.7 (HAV), and 14.3 ± 10.9 (HuCoV-229E), respectively. In contrast, unstable detection and lower recovery efficiency were verified in nonduplicate trials in methods 1 (< 6%) and 2 (< 16%). Moreover, in extreme conditions (5% soil content with viruses seeding at a concentration of 10^2 PFU/
$TCID_{50}$), method 3 exhibited exceeding stable and excellent recovery efficiency (%) which was 78.8 ± 13.3 (MNV-1), 44.5 ± 25.3 (HAV), and 4.01 ± 2.7 (HuCoV-229E), respectively. Overall, our results demonstrated that method 3 outperformed recovery efficiency compared to methods 1 and 2 in PWW.

Significance: This study provided an optimal protocol for virus recovery in post-washing water with high soil content.

P1-159 Residual Efficacy of Surface Sanitizing Wipes Against Two Pathogenic Bacteria, Human Norovirus, and Human Coronavirus

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Introduction: The anti-microbial effectiveness of surface sanitizing wipes (SSWs) is established, but they are presumed to provide immediate, not necessarily prolonged protection.

Purpose: To determine if there was residual efficacy of SSWs against representative bacteria and viruses on stainless steel (SS) coupons.

Methods: Five commercial SSWs were evaluated: Product A (benzyl ammonium chloride); B (dodecyl dimethyl ammonium chloride + ethanol + isopropyl alcohol); C (sodium hypochlorite); D (hydrogen peroxide); and E (ethanol). Viruses included human norovirus (hNoV) GII.4 Sydney (20% stool specimen) and human coronavirus (hCoV) 229E (ATCC-VR-740, cell culture lysate). Bacteria included *Staphylococcus aureus* ATCC6538 and *Klebsiella pneumoniae* ATCC4352. The study was done in accordance with EPA protocol #01-1A, with minor modifications. Briefly, sterile SS coupons were wiped with SSWs until saturated, then allowed to dry under ambient conditions. Coupons were inoculated with bacteria/virus, dried again, and left for contact times of 0min, 5min, 1h, 2h and 4h. Following exposure, viruses were recovered and enumerated using the sequential steps of elution, RNase pre-treatment, RNA extraction and RT-qPCR. Bacteria were eluted and quantified by cultural methods. Log_{10} reduction (LR) in genome equivalent copy (viruses) or bacterial count was calculated by comparison to the no-treatment control.

Results: Both bacteria were completely inactivated after exposure to SAW-treated SS coupons, corresponding to >4.0 LR. All SSWs demonstrated residual efficacy on SS against hCoV, irrespective of contact time. Products A and C (4.2±0.4 and 3.9±0.7 LR, respectively) outperformed B (3.5±0.1 LR), followed by D and E (2.9±0.2 and 2.9±0.1 LR, respectively). Only Products A and C had residual efficacy against hNoV which improved with contact time. For Product A, LR ranged from 0.5±0.7 to 1.9±1.0, and for C, ranged from 0.8±0.4 to 1.5±0.5.

Significance: SSWs can provide residual activity against viruses and bacteria. This has significance in choice of surface sanitizers for multiple applications.

P1-160 Synergistic Inactivation of Tulane Virus, a Human Norovirus Surrogate, Using UV-a Light Radiation and Curcuminoid(s) from Natural and Synthetic Sources

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Introduction: Human Norovirus (HuNoV) is the leading cause of foodborne illnesses in the United States and Tulane virus (TV) is a common surrogate for HuNoV. Curcuminoids could be excellent natural photosensitizers and curcuminoid(s) coupled with ultraviolet-A (UV-A) light radiation was effective in inactivating foodborne bacteria. However, studies on the curcuminoid coupled with UV-A light radiation in inactivation of HuNoV are lacking.
Purpose: The purpose of this study was to evaluate the effectiveness of the combination of UV-A light radiation and curcuminmoid(s) from natural and synthetic sources in inactivating TV.
Methods: Natural curcumin, synthetic curcumin, demethoxycurcumin, and bisdemethoxycurcumin were dissolved or oversaturated in DMSO, as well as ethanol to 2 mg/ml, respectively. In triplicate experiments for each treatment, TV in water including 5 ppm or 50 ppm curcuminoid(s) was treated under a UV-A light (365 nM) in the wells of a 48-well plate. Samples that did not contain any curcuminoid, as well as samples not exposed to UV-A light radiation were also included for comparison. Samples were treated for up to 3 hours at room temperature and the virus titers for the samples were quantified by plaque assay.
Results: After 3-hr treatments, 50 ppm of UV-A light-activated natural curcumin, synthetic curcumin, bisdemethoxycurcumin (over saturated), and demethoxycurcumin in 2.5% ethanol achieved 2.6 ($p<0.05$), 0.5, 3.4 ($p<0.05$), and 2.8-log ($p<0.05$) reductions of Tulane virus, respectively, and 1.0, 0.6, 2.0 ($p<0.05$), and 1.9-log ($p<0.05$) reductions of the virus were achieved, respectively, by the same treatments except that the compounds were in 2.5% DMSO; and some of those reductions are higher than the corresponding sums of the log reductions achieved by each tested curcuminoid alone and UV-A light alone, which were not higher than 0.6 log.
Significance: Combination of UV-A light radiation and some curcuminoid(s) respectively can effectively inactivated TV, and therefore can potentially be used to control HuNoV contamination.

P1-161 Efficacy of Ultraviolet-C Against Human Coronavirus 229E on Food Contact Surfaces and Foods

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Introduction: SARS-CoV-2, the cause of the COVID-19 outbreak, is transmitted by respiratory droplets and it became life-threatening viral pandemic in worldwide. The lack of research about survival and inactivation coronavirus on food contact surfaces and foods could raise concern about COVID-19. UV-C irradiation can be applied as a non-thermal sterilization method to food.
Purpose: This study investigated the effects of the physical (ultraviolet[UV]-C irradiation) inactivation method on various food contact surfaces (stainless steel[SS] and polypropylene[PP]) and foods (lettuce, chicken breast, and salmon) contaminated with human coronavirus 229E (HCoV-229E).
Methods: 50 µL of HCoV-229E were contaminated on food contact surfaces (1 cm diameter disk) and foods (1×1 cm for lettuce and 5×5×5 mm for chicken breast and salmon) . UV-C irradiation (0-60 mJ/cm^2 on food contact surfaces and 0-3600 mJ/cm^2 on foods) was performed on each sample. After recovery step, eluted virus was analyzed with $TCID_{50}$ assay.
Results: In the carrier tests, HCoV-229E titers on food contact surfaces were significantly decreased with increased doses of UV-C (0-60 mJ/cm^2). On food contact surfaces, virus was not detected by UV-C dose of 60 mJ/cm^2 (Detection limit: 1.0 $\log_{10}$ $TCID_{50}$/coupon). In food tests, UV dose at 3600 mJ/cm^2 achieved 1.71, 1.04, and 1.01 log10 reductions on lettuce, chicken breast and salmon, respectively.
Significance: This study suggests that UV-C irradiation was effective against coronavirus and it could be applied to food supply chain to inactivate coronavirus remain on the food contact surfaces and foods.

P1-162 Inactivation of Foodborne Virus by Novel Organic Peroxyacids-Based Disinfectants

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❖ ***Developing Scientist Entrant***

Introduction: The use of chemical disinfectants to inactivate foodborne microorganisms is common in the food industry. However, some disinfectants, such as peracetic acid (PAA), have a limited efficacy against a wide range of viruses and give off a strong odor. Therefore, the development of novel chemical disinfectants could improve these flaws.
Purpose: The objective of this study is to analyze the antiviral properties of perlevulinic acid (PLA) supplemented with or without 1% sodium dodecyl sulfate (SDS), PAA and a PAA-based disinfectant on hepatitis A virus (HAV), and murine norovirus 1 (MNV-1).
Methods: Disinfectants (50, 80, 250, 500 and 1000 mg L^{-1}) were evaluated in triplicate in aqueous solution of MNV-1 (10^7 PFU/mL) for contact times of 0.5, 1, 5, and 10 min. Optimal conditions were then tested in triplicate on MNV-1 and HAV on stainless steel surfaces. Tenfold dilutions of virus solutions were treated with the various disinfectants and then neutralized in Earle's Balanced Salt Solution containing L-cysteine hydrochloride. Virus infectivity was analysed with plaque assays.
Results: Contact time and concentration of all disinfectants significantly increased ($p<0.05$) the viral inactivation of MNV-1 in aqueous solutions (e.g., PLA 2.89 ± 0.21 log and PAA 4.04 ± 0.05 log at 50 mg L^{-1} for 5 min). However, results were different on stainless steel for MNV-1 ($p>0.05$) (e.g., PLA 0.42 ± 0.27 log and PAA 3.55 ± 0.55 log at 80 mg L^{-1} for 5 min) and HAV, respectively (e.g., PLA 0.11 ± 0.21 log and 0.14 ± 0.16 log at 250 mg L^{-1} for 5 min).
Significance: Overall, this project will allow the development of new chemical formulas that are more effective against major foodborne viruses and limit their impact on the consumers and the environment. Food matrices (berries) and impact on hepatitis E virus are currently being analysed.

P1-163 Survival, Transfer and Evolution of Bacteriophage Phi6 as a Lipid-Coated Virus Surrogate

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Introduction: Outbreaks caused by enveloped viruses have led to an interest in research on potential surrogates. These experiments expand on the use of Bacteriophage Phi6 (a double stranded RNA virus) as a surrogate.
Purpose: The purpose of this study was to evaluate how Phi6 survives on stainless steel, transfers to gloves, and to see if the virus would evolve in response to environmental stress.
Methods: Phi6 propagation and plaque-forming assays were performed using *Pseudomonas syringae* pathovar phaseolicola using the top agar overlay technique. Two different concentrations (100 ul or 10 x 1ul) at ~ 10^{10} PFU/ml were spotted onto stainless steel coupons (5x5cm) and held for up to one week at room temperature. Transfer efficiency testing used the same method, and coupons were evaluated by touching for 10 seconds and applying a pressure of ~250g/cm2 with nitrile gloves for more than 50 transfer events. Phi6 lysate was applied to coupons and held at 100% RH and 35°C for 15 minutes for stress testing. Coupons were sampled as described above. Surviving viruses were re-cultured and re-inoculated onto new coupons. Passage was repeated for 10 times on consecutive days using a fresh lysate derived from surviving viruses each day.
Results: Phi6 viability decreased linearly on stainless steel during the 7-day period by 0.54 $\log_{10}$/day (R2=0.92) for 100ul and 0.50 $\log_{10}$/day (R^2=0.95) for 10 1ul. The assay limit of detection was $\log_{10}$ 2.6. Transfers were detected for up to day 5 for both amounts, and $\log_{10}$ percent transfer was significantly different between the two inoculum sizes ($P<0.0002$). Phi6 showed increasing tolerance to temperature and RH stress after 5 passages compared to its initial population and were highly tolerant after 10 passages.

Poster

Significance: These data will be useful in characterizing the risks associated with enveloped virus survival, transfer as well as selection pressures for viral evolution.

P1-164 Transfer of Phi6 between Thumbpads and Surface Types Common to Food Service Environments

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Introduction: Fomite surfaces are an important viral pathogen transmission route; however, virus transfer between hands and surfaces is difficult to establish for BSL-2, or higher, pathogens. Phi6 (φ6) bacteriophage has emerged as a surrogate to study enveloped viral pathogens including SARS-CoV-2 due to structural similarities and BSL-1 status. Determining φ6 transfer rates on food service establishment related surfaces is needed.
Purpose: To determine the transfer rate of φ6 between thumbpads and surfaces relevant to food service settings based on surface type, contact time, and transfer direction.
Methods: Six 5x5cm surfaces (aluminum, plastic, stainless steel, touchscreen, vinyl, wood) were evaluated for surface-to-thumbpad and thumbpad-to-surface φ6 transfer. φ6 (2uL) was deposited on the donor surface then pressed on the recipient surface for 5 or 10s at 800±40 g pressure. Thereafter, φ6 was eluted with 2mL LC broth, diluted, and plated on LC agar via the double agar overlay assay. At least three experimental trials were performed, excluding artificial saliva surface-to-thumbpad transfer (n=1). A two- or three-way ANOVA with Tukey's test was performed to determine transfer rate differences based on inoculum matrix, surface type, and touch time (alpha=0.05).
Results: No significant differences were observed between transfer rates from thumbpad-to-surface (P>0.05). Among all surfaces and inoculum matrices, the transfer rate at 5 and 10s touch time from thumbpad-to-surface was 24.7±42.9% (median=6.70%) and 10.4±23.2% (median=0.84%), respectively. No significant differences in transfer rates were observed in tripartite from surface-to-thumbpad based on surface type or touch time (P>0.05). From surface-to-thumbpad in tripartite, the transfer rate at 5 and 10s contact time was 6.74±4.13 (median=6.58%) and 11.67±10.57 (median=7.19%), respectively.
Significance: To better characterize disease transmission, understanding how transfer direction, surface type, and contact time impacts virus transfer is needed. This data will help to determine effective disinfection protocols to control SARS-CoV-2 on fomite surfaces.

P1-165 Bacteriophage Phi6 as a Surrogate for SARS-CoV-2 Survival at Various Temperatures and Relative Humidities

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❖ ***Developing Scientist Entrant***

Introduction: A novel human Coronavirus (SARS-CoV-2) was identified in China in December 2019 after several atypical pneumonia cases emerged. This virus rapidly spread causing the WHO to declare a pandemic on March 11th, 2020.
Purpose: The purpose of this study was to determine the survival of bacteriophage Phi6 as a potential surrogate for SARS-CoV-2 on stainless steel in different environments.
Methods: Phi6 has been proposed as a non-pathogenic surrogate for SARS-CoV-2. Phi6 has several characteristics that resemble SARS-CoV-2 including a lipid envelope, membrane spike protein and RNA nucleic acid. The survival of Phi6 on stainless steel was measured under different conditions. Ten 1ul drops of lysogeny broth containing ~1E+10 plaque forming units of Phi6 were inoculated onto stainless steel surfaces, air dried and then stored at all combinations of three temperatures (7, 25 and 37˚C) and three relative humidity (RH) values (15, 50, 100%). Viable Phi6 was evaluated at days 0, 7, 21, 35, 49 and 63 (7˚C), days 0, 7, 14, 21, 35, 49 (25˚C) at days 0, 7, 14, 21, 28 and 35 (35˚C) and minutes 0, 15, 30, 45, 60, and 75 (35˚C, 100% RH) by recovering in 10ml lysogeny broth using one minute of the rub shake method. Each experiment was conducted in triplicate.
Results: Both temperature and humidity effect the survival of Phi6, with both lower temperature and humidity favoring survival. Regression analysis predicted a 1 log reduction in infectious virus after ~150 days at 7˚C and 15% RH and after ~5 days at 25˚C and 100% RH (R2=0.59 and 0.64 respectively). Inactivation at 35˚C and 100% RH was rapid (~6 log reduction within 30 min).
Significance: These data can inform estimation of the risk of fomite mediated transmission as a possible route for the spread of viral diseases.

P1-166 Persistence of Herpes Simplex Virus Type 1 on Non-Porous Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Herpes simplex virus type 1 (HSV-1) is an enveloped virus that causes recurrent and incurable diseases in 67% of the world population (WHO, 2022). Although HSV-1 is not considered a foodborne virus and is mainly transmitted through direct oral-to-oral contact, some studies have shown that it can persist on non-porous surfaces and ready-to-eat foods that can act as potential vectors.
Purpose: This study aims to investigate the persistence of HSV-1 on different surfaces under controlled conditions.
Methods: An HSV-1 viral suspension of 5 log (PFU/mL) diluted in PBS 1X was inoculated on stainless steel and aluminum coupons (1 cm^2). After the viral suspension has completely dried, the coupons were then incubated at various temperatures (-20 °C, 4 °C, 23 °C and 37 °C) and times (0 h, 0.5 h, 1 h, 4 h, 24 h and 7 days). After each treatment, the viral particles were recovered with Earle's Balanced Salt Solution (EBSS) 1X. The persistence of HSV-1 was then evaluated by plaque assay using Vero cells. Experiments were performed in triplicate for each condition.
Results: The persistence of HSV-1 on surfaces was significantly affected by the temperature and the incubation time ($P < 0.0001$). HSV-1 persisted for at least 4 h at - 20 °C, 4 °C and 23 °C but was not detected at 37 °C after 1 h. A significant difference ($P < 0.0001$) in the number of viruses recovered at cold temperatures after 7 days was also observed. At -20 °C and 4 °C, 2.5 and 2.4 log (PFU/mL) of HSV-1, respectively, were recovered from stainless steel compared to none on aluminum. However, prior to 7 days, no significant differences were observed between those surfaces.
Significance: Depending on the environmental conditions, indirect transmission of HSV-1 by surfaces is plausible. These results could support the food industry in its process of continuous improvement of food hygiene and safety.

P1-167 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; Bioneer, Daejeon, Korea) that is 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℃ and 5% CO_2 for 2days, 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. Relative gene expressions for *irf7*, *cxcl10* and *ccl4,* were calculated using the $2^{-\Delta\Delta Ct}$ method.

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: This finding suggests, 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

P1-168 Evaluation of a Laboratory Detection Method Using Sucrose Flotation to Concentrate *Cyclospora cayetanensis* Oocysts in Two Different Types of Soil

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Introduction: Contact of produce with soil contaminated with *Cyclospora cayetanensis* may represent an important vehicle in the transmission of this organism. There is a scarcity of detection methods for this parasite in soil. Our previous studies showed that a flotation concentration method followed by DNA extraction using commercial kits produced the best sensitivity for *C. cayetanensis* detection compared to using commercial DNA extraction kits for soil alone.

Purpose: To further evaluate the flotation concentration method for detection of *C. cayetanensis* in two different types of soil (rich in silt and rich in sand).

Methods: Ten grams of soil samples rich in sand (>50%) or rich in silt (>50%) were seeded with known numbers of *C. cayetanensis* oocysts [10 (n=10), 20 (n=10) and 100 oocysts (n=5)]. Unseeded soil samples were included as negative controls (n=5). Samples were processed by flotation concentration in a sucrose solution. The supernatant containing the top of the solution (20 ml) was then collected, diluted in water, and centrifuged. DNA was extracted from the pellet using a validated DNA extraction method with bead-beating and a qPCR for *C. cayetanensis* detection.

Results: All negative control samples were negative. No inhibition was observed in any processed samples. The flotation procedure showed a limit of detection of 10 oocysts in 10 g for both types of soil. The rates of positivity for samples seeded with 10, 20 and 100 oocysts were 30%, 70% and 100% for samples rich in silt, and 20%, 50% and 80% for samples rich in sand, respectively.

Significance: The flotation technique provides detection of as few as 10 oocysts in 10 g of soil samples rich in silt and soil samples rich in sand. The capability of detecting *C. cayetanensis* oocysts in soil will facilitate outbreak investigation and increase knowledge on the epidemiology of this important parasite.

P1-169 Examination of Gaseous Chlorine Dioxide as a Sanitizer to Reduce *Cryptosporidium parvum* on Produce

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❖ ***Developing Scientist Entrant***

Introduction: Protozoan oocyst contamination of fresh fruits and vegetables remains a concern after years of contamination resulting in outbreaks of gastroenteritis. *Cyclospora cayetanensis* oocysts are a major concern but are difficult to obtain, so surrogates, like those from the important pathogen *Cryptosporidium parvum*, can provide meaningful data, especially due to its increased resistance to disinfectants and normal mitigation methods.

Purpose: Investigate the effects of chlorine dioxide gas (ClO2) on *C. parvum* on fresh commodities under varying treatment times with respect to food quality.

Methods: In an enclosed 35L container, grape tomatoes (40g) and baby-cut carrots (35g) were inoculated with 100uL of a *C. Parvum* suspension (10^6 oocysts total), 50g of ClO2-precursors were then added. After 0, 1, and 3 hours of treatment each sample was rinsed with 3mL of 10% FBS-RPMI medium to collect oocysts. Oocyst infectivity was determined using a human cell culture assay (HCT-8 cells) coupled with qPCR. Product quality was assessed by pre- and post-treatment water activity and colorimetric measurements (n=3/product). Data are an average across three trials.

Results: After 1 hour of treatment, tomatoes showed a 4.8 log reduction in *C. Parvum* oocyst viability while carrots had a 3.6 log reduction, after 3 hours of treatment *C. Parvum* became undetectable on tomatoes and decreased by log of 5.2 on carrots. There was no significant difference in water activity pre- or post-treatment for either sample. The ΔE^*_{ab} (overall color change) for carrots is 14.8 after one hour and 43.5 after three, indicating a dramatic change in color. In contrast, tomatoes had a ΔE^*_{ab} of 4.0 and 1.9 after one and three hours respectively.

Significance: This study provides commercially useful information regarding effective concentrations and exposure periods for the reduction of *C. Parvum* on produce using ClO2. Effectiveness on other commodities continues to be evaluated, along with efficacy on other protozoa.

P1-170 Evaluation of Prevalence and Methods of Detection of *Cyclospora cayetanensis* in Irrigation Water in the United States

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Introduction: Recent outbreaks of cyclosporiasis associated with domestically grown produce have highlighted the need to determine the prevalence of the parasite, *Cyclospora cayetanensis*, in US produce growing environments, including agricultural water.

Purpose: We evaluated methods for *C. cayetanensis* detection in irrigation water samples to determine its prevalence and to aid in outbreak environmental investigations.

Methods: Large volume (50-L) irrigation water samples were collected by dead-end ultrafiltration. The resulting water concentrates were analyzed for the presence of *C. cayetanensis* 18S rDNA via the BAM 19B PCR protocol, modified with the use of master mix designed for environmental sample testing. Samples that tested positive in BAM 19B were further characterized using the recently published *C. cayetanensis* typing method for molecular-based epidemiological clustering of clinical cases.

Results: From September 2020 to May 2021, 132 irrigation water samples were collected and tested for the presence of *C. cayetanensis*. Of these, 17 yielded positive results with the modified BAM 19B protocol and were further characterized. Only one of the eight typing markers (part of the mitochondrial 16S rRNA gene) amplified successfully and was sequenced in 15 samples. BLASTN searches of the amplified sequences did not match any known organism. Phylogenetic analysis showed that the sequences detected in the environmental samples belonged to coccidia that were basal to the well-supported clades occupied by *C. cayetanensis* and *Eimeria* spp., indicating that the sequences were not from *C. cayetanensis*.

Significance: Results indicate that the current published assay used in FDA BAM 19B for molecular detection of *C. cayetanensis* may not have adequate specificity and needs further evaluation to assess its utility when applied to environmental samples. Typing methods used to link environmental and

Poster

clinical isolates have the potential to aid in confirming environmental detection of *C. cayetanensis* but require further evaluation to determine sensitivity in environmental samples.

P1-171 Comparative Evaluation of GENE-UP® *Campylobacter* Method for the Detection of *Campylobacter* Species in Ground Chicken and Chicken Carcass Rinse

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Introduction: *Campylobacter* is a leading cause of foodborne illness and hospitalization in the United States, and due to its high prevalence, an effective and rapid screening method is needed.

Purpose: The GENE-UP® *Campylobacter* method is a real-time PCR assay for the detection of *C. coli, jejuni* and *lari* in poultry products, and the effectiveness of this assay was compared to the USDA FSIS MLG 41.05 reference method for the detection of *Campylobacter* species in raw ground chicken and chicken carcass rinse.

Methods: 20 replicates of 25 gram raw ground chicken and 20 replicates of 30 mL of chicken carcass rinsate samples were analyzed using the candidate and reference methods. Each set of test portions was artificially inoculated with a fractional low level (0–2 CFU) of *Campylobacter* species, and each matrix included 5 high inoculated (2–10 CFU) and 5 uninoculated (0 CFU) samples. Raw ground chicken was combined with 225 of Bolton Broth and chicken carcass rinsate was combined with 30 mL of 2x Bolton Broth. Samples were incubated at 42 ± 1°C for 24 ± 2 hours under standard atmospheric conditions and all samples were confirmed culturally.

Results: For both the candidate and reference method, data from 30 replicates of raw ground chicken at 25 grams and 30 replicates of 30 mL chicken carcass rinsates were analyzed. Based on MPN values and no observed discrepant results between candidate and reference methods, there was no statistically significant difference observed between the candidate and reference method using a 95% confidence interval.

Significance: The candidate *Campylobacter* method is a rapid screening method for the presence of *Campylobacter* species in select poultry matrices and offers end users a next-day option to screen raw ground chicken and chicken carcass rinsates for target *Campylobacter* species.

P1-172 Presumptive *Cyclospora* Findings in Surface Waters

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Introduction: *Cyclospora* is a foodborne pathogen that causes gastrointestinal illness in humans and is associated with the consumption of contaminated fresh fruits and vegetables, often reported in tropical countries. In the recent years, outbreaks of cyclosporiasis were associated with fresh vegetables produced in the US.

Purpose: A total of 547 surface water samples were examined for the presence of *Cyclospora* during the months of October 2020 to August 2021 in the US.

Methods: Ten-liter water samples were collected from surface water from two locations (locations A and B) within the US. Water temperature, turbidity, and chemical composition were determined from each sample and water was filtered using hollowfiber filters and concentrated by centrifugation to a final volume of 0.5ml. Samples were examined using a nested PCR and qPCR to determine the presence of *Cyclospora*. Presumptive positive samples were further observed by microscopy and confirmed via gene sequencing.

Results: In location A, water turbidity was mostly below 20 NTUs and temperatures fluctuated from 18C to 34C. In location B, water turbidity varied from 0-45 NTUs, however during the months of May turbidity levels were as high as 226 NTU. Water temperatures ranged from 8C to 22C. Presumptive *Cyclospora* positive samples were observed more often in during the months of December and April with location A having 3.78% and 3.15% and in location B having 7.3% and 2.6%, respective to the months of December and April.

Significance: Identification of presumptive *Cyclospora* from surface water samples may be an indication that water is exposed to human fecal contamination.

P1-173 Persistence of *Cyclosporiasis* in Children from Regions of Morelia, Mexico

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Introduction: Cyclosporiasis is a foodborne often associated with acute and persistent diarrhea. In the U.S. most reported cases were associated with consumption of domestic and imported products that are consumed uncooked.

Purpose: In this study, samples from 8,646 children were examined to determine the persistence of cyclosporiasis in Michoacán, Mexico, during 2010-2019.

Methods: Coproparasitological examination was performed at the Diagnostic Parasitology Laboratory by direct observation, concentration by sedimentation (formol-ether), and flotation (Faust and Sheather's methods), followed with Gomori and Kinyoun staining. Presumptive positive samples were confirmed by observation of morphological characteristics, oocyst sporulation in 2.5% potassium dichromate, and PCR testing.

Results: During the period of 2010 - 2019, 8,646 patients with gastrointestinal illness provided a total of 26,364 stool samples for coproparasitological examination. Of those, 96 (1.1%) were positive for *C. cayetanensis* oocysts and confirmed by PCR assay. Children in pre-school (3 - 5 yr) had the highest infection rates (34.4%), followed by 6 to 11-year olds (31.3%), toddlers (22.9%), and teenagers (11.4%). Most of the cases with cyclosporiasis were observed during the months of June to August and more than one stool sample was needed to detect positive cases. Most of the cases came from the city of Morelia and live in 'colonias' where potable water, garbage collection services, or paved roads are not available, and habitants have poor sanitary conditions.

Significance: The prevalence of cyclosporiasis in the region has increased in the past 10 years and seasonality has persisted every year during the months of June to August.

P1-174 Inactivation of the Norovirus Surrogate Bacteriophage MS2 on Glass Surfaces by Ozonized Water

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Introduction: Noroviruses (NoV) are a common cause of foodborne disease outbreaks. Environmental surfaces contaminated with NoV can cross-contaminate foods. Little is known about the effects of ozonized water for inactivating viruses on glass surfaces, which have been increasingly used in domestic food processing.

Purpose: The purpose of this study was to evaluate the efficacy of ozonized water for inactivating the NoV surrogate bacteriophage MS2 on glass surfaces

Methods: MS2 was propagated using *Escherichia coli* C3000 as a host on tryptic soy agar plates using the double agar overlay method. The plates were incubated overnight at 37 °C for 4 h and viral titers were determined. Autoclaved glass surfaces (5×5 cm) were spot inoculated with MS2 (9.9 log PFU/cm^2) and dried for 5 h in a biosafety cabinet. Inoculated surfaces were immersed in ozonized water (6.25 ppm) produced by an ozone generator (AC 220V, Plug

Poster

UE 600 mg/Lh). Ozone concentration was confirmed using the iodometric method. MS2 titer was measured on surfaces before and after different contact times with ozonized water (0, 5 and 30 s, and 1, 5, 10, 15 and 20 min). Glass surfaces treated with un-ionized water were used as controls.

Results: MS2 inactivation increased with exposure time. Reductions of 1.7 and 2.7 log PFU/ cm^2 were observed after 30 s and 1 min of exposure, respectively. Reduction of MS2 on glass surfaces was ~3.5 and 4 log PFU/ cm^2 after 5 and 10 min, respectively. After 15 min of exposure, reduction of MS2 on glass surfaces was ~4.8 PFU/ cm^2 and reached >5 log reduction after 20 min of exposure.

Significance: The findings indicate that ozonized water is a possible strategy for controlling NoV on glass food contact surfaces.

P1-175 Emergence of Global Food Regulations to Combat Coronavirus Pandemic in Food Sector

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Introduction: Coronavirus pandemic (Covid-19) leaves wide-ranging and catastrophic effects at normal ways of living, has disturbed the global food supply chain, and have a damaging impact on food security as well. Food industry and the government come together and played vital role to ensure that the food sector should remain open for consistent and unhindered production of food, so consumers have continued access to safe, healthy, and nutritious food during this pandemic.

Purpose: The purpose of study was to focus on how immediately food regulatory authorities responded and took initiatives during pandemic, formulated regulations, developed precautionary measures, enforced specific standard operating procedures and best practices for the food sector to keep them safe and health.

Methods: In present study the data was collected from all prestigious food regulatory authorities by studying published articles, policies, instructions, guidelines, and available resources to search about emerging food regulations.

Results: Results of this study showed that food regulatory authorities' response on pandemic was so robust that were continuously updating the information's and guidelines for food sector staff trainings, cleaning and sanitizations, Covid-19 mitigation protocols, employee's health and control, personnel hygiene, managing operations in food sector and sources and transmission routes.

Significance: This study is quite helpful for food service, retail, food manufacturing and for catering and hospitality sector to get aware about current and updated food regulations which emerged during pandemic to limit the spread of coronavirus, and which are now mandatory to implement, and enforcement agencies have heavy penalty in case of violations.

P1-176 Zerovalent Iron Sand Filtration Markedly Reduces Water Contamination by Both Bacterial and Parasitic Contaminants

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Introduction: Bacterial and parasitic pathogens, such as shiga-toxigenic *E. coli* and *Cyclospora cayetanensis*, contaminate fresh produce, causing foodborne outbreaks. Commonly used chemical disinfectants are ineffective in reducing *Cyclospora* oocyst levels in irrigation water; zerovalent iron sand (ZVI) filtration may provide an alternative treatment to reduce pathogens in irrigation water.

Purpose: To determine if zerovalent iron sand (ZVI) filtration can simultaneously reduce contamination of water by *E. coli* and by a *C. cayetanensis* surrogate, *Eimeria tenella*.

Materials and Methods: Sterile, deionized water (4 L) inoculated with *E. tenella* (5 x 10^5 oocysts) and rifampicin-resistant *E. coli* TVS 353 (1 x 10^5 CFU/mL) was filtered at 0.5 L/min through a sand pre-filter (100% sand) and then a filter (50% zerovalent iron, 50% sand by volume). An additional 6 L of sterile deionized water transited the filters before backflushing with 4 L sterile water. *E. tenella* was quantified using microscopy after concentrating each sample using continuous flow centrifugation. *E. coli* were quantified using plate counts on MacConkey Agar supplemented with 80 ug/mL rifampicin. Abundance in influent, effluent, and backflush were compared using one-way ANOVA ($p < 0.05$) over four replicate experiments.

Results: ZVI filtration reduced *E. tenella* by at least 98% in effluents but only 17% in the backflush. *E. coli* levels were significantly ($p < 0.05$) reduced by over 97% in both the effluent and backflush.

Significance: ZVI filtration markedly reduced both bacterial and parasitic contamination from the effluent; physical obstruction may have prevented most *E. tenella* from passing through even the sand pre-filter, enabling their recovery in the backflush. Bacterial reductions, even in the backflush, suggest that *E. coli* were killed or bound by ZVI in filters because cells fully passed through the sand pre-filter and ZVI filter. Future studies should investigate whether parasites sustain injury when exposed to zero valent iron particles or treated by ZVI sand filtration.

P1-177 Prevalence and Genomic Analysis of *Listeria Monocytogenes* in Chilean Surface Water

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Introduction: *Listeria monocytogenes* (*Lm*) is the food-borne pathogen that causes listeriosis. This bacterium is widely distributed in the environment, and it can survive and proliferate under adverse conditions. *Lm* can contaminate irrigation water and go into the food production chain.

Purpose: To evaluate the presence and persistence of *Lm* in surface water across time and characterize the isolates genetically.

Methods: Thirty points were sampled during nine months in two rivers of the Metropolitan-Region and two from the Maule-Region, Chile. Water samples were obtained by filtrating water (10 L) through a Modified Moore's Swab. The presence of *Lm* was evaluated according to BAM/FDA. Isolates (n=142) were sequenced in the Illumina HiSeq instrument. The genomic analysis considered whole-genome annotation (Prokka V1.14.6), Core-Genome and Pan-Genome definition (Roary V3.11.2), phylogenetic relatedness (FastTree V2.1.3), and the detection of virulence and stress response genes (Proteinortho V5.16b).

Results: *L. monocytogenes* average prevalence 23% in waters from Metropolitan and 20% from Maule region. Rivers from the Metropolitan region showed more sites requently contaminated with the pathogen. Genomes contained 2,064 core genes and 4406 accessories genes. The most prevalent serogroups were IVb (48,5%), IIb (24,6%), and IIa (23,2%), and isolates were of lineage I (73,2%) and II (23,9%). Internalin genes (*intA* and *intB)* were detected in all genomes, and *Listeria* pathogenicity-island identified were LIPI-1 (100%), LIPI-3 (33%), and LIPI-4 (20%). The Stress Survival Islet (SSI) was detected in 21% of isolates. *Lm* isolates identified in the same river but on different dates were highly clonal, suggesting persistence of those strains in the rivers.

Significance: This study identifies the presence of *Lm* in surface waters and its presence persists over time. Results indicate the importance of controlling the presence of *Lm* in water and the need to establish environmental monitoring programs to prevent contamination of food with this pathogen.

P1-178 Dynamics, Serotypes, and Antimicrobial Resistance of *Salmonella* in a Karst Ground Water System

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Introduction: *Salmonella* is the leading cause of bacterial foodborne infections, hospitalizations, and deaths. While over two-thirds of human infections are foodborne, a quarter of cases are acquired from other sources, including water. Although most infections are self-limiting, severe infections in certain populations including children require antibiotic treatment which can be impacted by antibiotic resistance, posing serious public health threat.

Purpose: The purpose of this study was to monitor the dynamics of *Salmonella* karst ground water contamination, serotype distribution, and antimicrobial resistance.

Methods: Ten sites in the city of Bowling Green, Kentucky, were monitored by weekly water sampling over 46 weeks. Water samples were filtered and cultured for the isolation of *Salmonella* following standard methods. PCR confirmed isolates were whole genome sequenced for serotyping and genotypic resistance. Influence of sampling site, sampling resolution, and season on the prevalence and serotype distribution were analyzed using likelihood ratio test, exact chi-square test, and binary logistic regression.

Results: Overall *Salmonella* prevalence in the karst ground water samples (n=443) was 14.4%. The prevalence significantly varied by tier category (higher in the top tier sites), sampling sites (range: 3.5-26%), sampling weeks (range: 0-90%), month (range: 0-33%), and by season (range: 1.8-20%). Positive water samples (n=64) resulted in 176 isolates that belonged to 18 serotypes. Prevalence of genotypic resistance was 12.5% (n=176). Majority of the resistant isolates (82%; n=22) were multidrug resistant. Nine antimicrobial resistance genes belonging to aminoglycoside, phosphonic acid, sulfonamide, and tetracycline antimicrobial classes, as well as quaternary ammonium compounds were detected.

Significance: *Salmonella* was widespread across sites, weeks, months, and seasons. The highest prevalence was in the groundwater-fed surface water, the main source of drinking water for the city. Because karst groundwater is a complex system, weekly sampling is important for monitoring and understanding of environmental factors influencing *Salmonella* prevalence and its antimicrobial resistance to protect drinking water supply.

P1-179 Isolation of *Salmonella* spp. from Surface Water Potentially Used for Produce Irrigation in the Metropolitan Region, Chile, 2021

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Introduction: *Salmonella* is one of the most relevant agents causing foodborne diseases. This pathogen can survive for several months in the environment, and evidence has shown that irrigation water has been a vehicle for the contamination of produce.

Purpose: To identify and isolate *Salmonella* spp from surface water potentially used for irrigating produce in the Santiago Metropolitan Region, Chile, and identify factors linked to the presence of the pathogen.

Methods: We collected 480 samples from two river systems, Mapocho and Maipo, from April to July 2021. Monthly, we sampled 60 sites for each river system. Samples were obtained through a Modified Moore Swab by filtering 10 L of water. Water temperature and pH, total dissolved solids, ambient temperature, presence of animal feces, and presence of crops were recorded for association analyses. *Salmonella* isolation was performed following the FDA BAM method, and isolates were confirmed by a PCR detecting the *invA* gene. For data analysis, point biserial correlation coefficients were calculated in RStudio, and Chi-square analyses were analyzed in WinEpi.

Results: *Salmonella* was isolated every month from both rivers, with detection frequencies ranging from 25.0% (15/60) to 56.7% (34/60). The highest *Salmonella* prevalence was detected in April in both river systems: 56.7% (34/60) in Mapocho and 35.0% (25/60) in Maipo. *Salmonella* was persistently isolated in sites 15, 45, and 54 in Mapocho and site 37 in Maipo. Significant associations ($p<0.05$) were detected between *Salmonella* identification and the presence of crops in the Maipo river, and smaller but significant correlations between *Salmonella* presence and water temperature and pH in the Mapocho river.

Significance: This study provides information about the presence of *Salmonella* spp. in surface waters of the Metropolitan Region, Chile, which are used for the irrigation of produce and might represent a risk for human health.

P1-180 Efficacy of Ozone Against *Salmonella* Newport and *Escherichia coli* O157:H7 in Non-Traditional Sources of Water at Room Temperature and 4°C

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Introduction: The food and agricultural industries use large volumes of water. The upcoming drought conditions necessitate exploration of alternate irrigation water sources.

Purpose: Investigate the efficacy of ozone against *Salmonella* Newport and *Escherichia coli* O157:H7 in non-traditional water sources.

Methods: Five water samples were collected across Arizona: Tucson reclaimed (RW), Tucson reverse osmosis reject (ROR), Yuma return flow (Y-RF), Tucson rain catchment (T-RC) and Oracle rain catchment (O-RC). The water sources were inoculated with either *S.* Newport or *E. coli* O157:H7 and a pre-treatment sample was taken. The inoculated water at room and refrigeration (4°C) temperatures was treated with ozone at different times until target pathogens were below detectable levels (<1 log CFU/ml). Pre-treatment and post-treatment samples were plated on xylose lysine desoxycholate agar with antibiotics and cefixime tellurite sorbitol MacConkey agar. Post-treatment water was enriched in tetrathionate broth and R&F *E. coli* broth for enrichment of *Salmonella* and *E. coli* O157:H7, respectively.

Results: The minimum treatment times needed to inactivate *S.* Newport at both room and refrigeration temperatures in various water types ranged 2-4 min, while for *E. coli* O157:H7 the inactivation times ranged 30 sec-1 min. *Salmonella* consistently needed higher treatment times for inactivation below detection levels than *E. coli* O157:H7. The lowest treatment time for *Salmonella* at both temperatures was 2 min for ROR. Y-RF, T-RC and O-RC needed the lowest treatment times of 0.5 min for *E. coli* O157:H7 at both temperatures and 0.5 min for ROR at room temperature. The highest treatment time for *Salmonella* and *E. coli* O157:H7 was for RW at 4 and 1 min, respectively. *E. coli* also needed a 1 min treatment time in ROR at 4°C. Enrichments of post-treatment samples showed presence of *Salmonella* in ROR water at room temperature.

Significance: Ozone could potentially be used as an effective treatment for decontaminating non-traditional water sources.

P1-181 Isolation of Indicator and Foodborne Pathogenic Bacteria from Rainwater and Reverse Osmosis Reject Water Samples in Arizona

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Introduction: The agricultural industry is exploring alternate sources of irrigation water due to drought conditions being anticipated in the future. The quality of alternative non-traditional irrigation water sources could have a potential impact on the safety of agricultural production.

Purpose: The objective of this study was to evaluate the microbiological quality and safety of potential non-traditional irrigation water sources in Arizona by testing for the presence of indicator and pathogenic bacteria in these samples.

Methods: Rainwater samples were collected from two rainwater collection tanks and reverse osmosis (RO) reject water samples from two wastewater processing plants in Arizona. Standard membrane filtration methods were used for the detection of indicator bacteria- *Escherichia coli*, total coliforms and enterococci. Water samples (n=22; 10 rainwater and 12 RO reject water) were filtered through cellulose ester membrane filters and bacterial populations were enumerated by placing them on selective agar. For detection of pathogens (*Salmonella enterica, Listeria monocytogenes* and *E. coli* O157), water samples (100 ml, 1 liter, 10 liters) were filtered through Modified Moore swabs and enriched in Universal Pre-enrichment Broth, followed by selective enrichment broth for each pathogen. The enriched broth was streaked onto agar media selective for each pathogen. Presumptive colonies were confirmed by PCR/real-time PCR.

Results: Among the 10 rainwater samples, the ranges of *E. coli*, total coliforms, and enterococci were 0, 0.5-310, and 0.3-43 CFU/100 mL, respectively. In the 12 RO reject water samples, the ranges of *E. coli*, total coliforms and enterococci were 0, 0-23000, and 0 CFU/100 mL, respectively. *Salmonella*, *L. monocytogenes* and *E. coli* O157 levels were below the detection limit (0.03 MPN/L) in all the rainwater and RO reject water samples.

Significance: The non-detectable levels of pathogens and generic *E.coli* in rainwater and RO reject water samples indicated the potential of these water sources for the irrigation of food crops.

P1-182 The Application of Ultraviolet Light Technology to Enhance the Safety of Agricultural Water on Kansas Fresh Produce Farms

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❖ ***Developing Scientist Entrant***

Introduction: Ultraviolet (UV) light-based water treatment systems are an increasingly investigated alternative to chemical sanitizers for agricultural surface water disinfection as they are highly effective, user-friendly, and do not produce toxic by-products. However, the relatively high concentration of particulate matter in surface water which "shields" microbes from inactivation is a major challenge to expanding its application in agriculture.

Purpose: The objective of this project was to test the efficacy of two commercial UV reactors to reduce the microbial risk of agricultural water.

Materials and Methods: The microbial reduction efficacy of the Minipure MIN-9 system (1-9 gallons per minute (GPM), 1.34-gallon capacity) was investigated through in-lab validation trials using lab water inoculated with *Escherichia coli, Salmonella* Typhimurium, and *Listeria innocua*; superhume was used to adjust the UV-transmission (UVT) to 20%, 30%, and 40%. An on-farm study using three agricultural water sources was performed to determine the efficacy of the Minipure MIN-9 and SARIN (1-110 GPM, 4.75-gallon capacity) UV systems in natural agricultural water. Agar-based methodology (Limit of detection, LoD) 25 CFU/mL) and Colilert with Quanti-tray/2000 (LoD 1 MPN/100mL) was used to enumerate the surviving microbial population for the in-lab and on-farm trials, respectively. Flow rates of 6, 7, and 9 GPM were used for all trials.

Results: For in-lab validation, a log reduction of 0.43 to 2.96 was achieved using the Minipure MIN-9 system, and was dependent on flow rate (p<0.0001), UVT (p<0.0001), and target pathogen (p<0.0001). For on-farm trials, a log reduction of 0 to 3.38 was achieved, and was dependent on reactor type (p<0.0001), source (p<0.0001), and UVT (p<0.0001).

Significance: These results demonstrate the efficacy of UV light for reducing the microbial risk of agricultural water. Further studies are needed using different UV devices, flow rates and transmissions to develop guidance on the UV technology for conventional or hydroponic produce growers.

P1-183 Quantification of Survival and Persister Formation Among Shiga-Toxin Producing *E. coli* Strains in Water

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❖ ***Developing Scientist Entrant***

Introduction: Shiga toxin-producing *E. coli* (STEC) can transfer to agricultural water by cattle fecal shedding leading to pre-harvest contamination. In low -nutrient and -temperature conditions, to resist stresses, these cells can convert into culturally inactive persister cells. Persister cells have reduced metabolism and protein synthesis making them resistant to antibiotics. In a previous longitudinal study examining carriage of STEC in cattle, strains of serotype O6 were repeatedly isolated from cattle while serotypes common in human disease, O26 and O103 were isolated sporadically. We hypothesize that serotype O6 strains have a survival advantage and enter into the persister state at a greater rate than serotypes O26 and O103.

Purpose: The purpose of this study is to quantify survival and persister formation of STEC serotypes in a non-host environment, chemically simulated agricultural water.

Methods: Chemically simulated agricultural water was inoculated with six strains of STEC separately in triplicate, with an average density of 5.16 (± 0.026) log CFU/ml, and incubated at 10°C. The number of culturable cells was measured by plating onto TSA for 17 days. Persister cells were quantified by exposure to 10x MIC ciprofloxacin for 3 hours and enumeration of surviving cells. Two strains each were used from serotypes O26, O103, and O6.

Results: Over 17 days, the average log reduction of culturable cells was 0.32 ± 0.16 logCFU/ml and significant (p<0.05). The average log reduction for O103, O6, and O26 was- 0.19 (±0.03), 0.28 (±0.16), 0.51 (±0.05) respectively. The percentage of cells in the persister state from day 0 to day 17 increased significantly (p<0.05) from 0.08±0.03% to 5.6±2.85%. On day 17, the percent persister formation for O6, O26, and O103 was 3.06±2.17%, 5.93±3.54%, and 7.82±0.7%.

Significance: As their time in water increased, larger proportions of STEC populations entered into the persister state.

P1-184 Evaluation of Peroxyacetic Acid and Chlorine as Treatments for Surface Water Used in Produce Post Harvest

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❖ ***Developing Scientist Entrant***

Introduction: Produce contamination accounts for close to half of foodborne illness outbreaks, and water used both pre-harvest and post-harvest may contribute to contamination.

Purpose: Peroxyacetic acid and chlorine were evaluated as treatments for simulated surface water inoculated with generic *Escherichia coli* to determine efficacy at achieving 'no detectable generic *E. coli*' in 100 mL post-harvest agricultural water, as required by the FDA Produce Safety Rule.

Methods: Simulated surface (agricultural) water was prepared to turbidities of 2 and 100 NTU, pH was adjusted to 6.5 or 8.4, and then equilibrated to 12°C or 32°C, by following the EPA's protocol for development and registration of treatments for preharvest agricultural water, with modifications. Samples were inoculated with a generic *E. coli* cocktail (~5 log CFU/ml) and treated with 25±2 ppm free chlorine (C), 75±5 ppm peroxyacetic acid (P), or a water control (W). At 0, 5, 10, 60 min, 24 and 48hrs, samples were neutralized in Dey/Engley broth, enumerated on *E. coli*/coliform petrifilm®, and enriched in 2X Tryptic Soy Broth. Enrichments were streaked to MacConkey agar to confirm the absence of generic *E. coli*.

Results: All C and P samples were below the detection limit (0.5 CFU/ml) and negative for *E. coli* on MacConkey plates at all time points. The pH×treatment interaction was significant (P<0.0001), with W samples at pH 8.4, a mere 0.1 logs higher than 6.5. The time×temperature×treatment interaction was significant (P<0.0001), with *E. coli* increasing to 6 logs in W by 24hrs at 32°C.

Poster

Significance: Generic *E. coli* was not detected in simulated surface water following treatment with P and C, which suggests these interventions are effective at treating surface water (≤100 NTU) for post-harvest use in produce. Additional research should evaluate other turbidities and methods (enumeration and/or enrichment) to further explore the efficacy of these antimicrobials at treating surface water.

P1-185 Prevalence and Antimicrobial Resistance of *E. coli* Isolated from Residential Water Wells in South Central Virginia between 2020 and 2021

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Introduction: Approximately 20% (1.7 million) Virginians rely on wells, springs or cisterns for water supply, and water safety is of particular concern to them. Additionally, due to wide application of antibiotics for human health and agricultural practices, the emergence of antimicrobial resistant (AMR) bacteria and their presence in the environment is a potential health threat. In a previous study conducted between May 2017 and December 2019, we documented a presence of *E. coli* in 75 (2.7%) out of 2,786 samples and prevalence of multi-drug resistance in 12.5% of the tested *E. coli*.

Purpose: The aim of the present study was to evaluate the prevalence and AMR of *E. coil* in water samples obtained from residential wells in South Central Virginia between January 2020 and December 2021.

Methods: A total of 2,601 water well samples from 44 counties in the South Central region of Virginia were obtained. *E. coli* isolation and identification have been performed following AOAC-approved or performance-tested methods.

Results: *E. coli* was detected in 1.5% of total samples, which were located in nine of these counties. Of the isolates, about 37.5% showed non-susceptibility to antibiotics.

Significance: The findings of this study demonstrate the significance of well water as a source of AMR bacteria for human health and the importance of water well-owner education regarding regular water quality monitoring.

P1-186 *Escherichia coli* Levels in Pond Water Vary by Sampling Location and Depth

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Introduction: Generic *Escherichia coli* are the fecal indicator bacteria currently used by regulatory and auditing bodies to ensure produce production water is of adequate microbial quality. However, location and depth of sample collection may influence *E. coli* levels in irrigation ponds.

Purpose: The purpose of this study was to observe variation of *E. coli* populations in irrigation ponds by sample location and depth.

Methods: Water samples (0.5 L) were collected at predetermined GPS coordinates from three irrigation ponds in southern Georgia. Surface samples were collected using a grab sampler and 0.5m or 1m depth samples were collected with a peristaltic pump. Aliquots of 100 ml were processed within 6 h using IDEXX Quanti-Tray/2000 and Colilert substrate and reported as most probable number (MPN) *E. coli* per 100 ml.

Results: At Pond 1, a two-sample t-test (n=160) showed that *E. coli* levels of surface samples collected from the edge of the water (1.99 logMPN/100ml) were significantly ($p<0.05$) greater compared to those from the interior of the pond (1.54 logMPN/100ml). Surface samples from Pond 2 had significantly ($p<0.05$, n=144) greater perimeter *E. coli* levels (0.78 logMPN/100ml) compared to interior levels (0.54 logMPN/100ml). In Pond 1 (n=128), *E. coli* levels in surface samples (1.54±0.13 logMPN/100ml) were significantly lower than subsurface samples (1.68±0.18 logMPN/100ml). For Pond 2 (n=117) surface levels of *E. coli* (0.54 logMPN/100ml) were also significantly lower compared to subsurface levels (0.89 logMPN/100 ml). In Pond 3 (n=107), with no cattle access, there was not a significant difference in *E. coli* levels from water collected at different depths.

Significance: Results presented indicate that location and depth of sampling in ponds can significantly impact *E. coli* levels, which can affect how irrigation water quality is characterized.

P1-187 Comparing Agricultural Water Treatments on Total Coliforms in Irrigation Water and Soil

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Introduction: *Escherichia coli* infections have been linked to the consumption of leafy greens. Irrigation water may carry pathogens to fields and survive in soil. Safe and effective water treatments are critical.

Purpose: To compare the effect of agricultural water treatments on total coliform and *Escherichia coli* in irrigation water and irrigated soil.

Methods: Three plots of spinach, 18 rows, 1 m width x 91.4 m length (1,672 m^2) were irrigated February through May 2021, using non-treated well water. Upon spinach maturity, the water was treated with peroxyacetic acid (PAA, 8 mg/liter) or calcium hypochlorite (Cl, 2 mg/liter), while untreated water was a control. Each plot was irrigated and sampled every 5 m. Soil samples were aseptically collected from each plot after irrigation. In total, 18 water and 12 soil samples were collected per plot, and these samplings were repeated two times. Water samples were analyzed for MPN using Colilert® (Idexx), while soil samples were tested by decimal dilution and plating on Coliform/E. coli Petrifilm®.

Results: All water samples yielded undetectable levels of *E. coli*, thus total coliforms were used to compare the effects of PAA and Cl treatments on the microbial loads of the soil and agricultural water. Mean coliform counts in untreated water and water treated with PAA and Cl were 2.8, < 0.0 and 1.3 log MPN/100 ml, respectively ($p < 0.05$). Mean coliform counts on soil when irrigated with non-treated water (3.3 log CFU/g) were significantly ($p < 0.05$) higher compared to the counts on soil irrigated with PAA and Cl (2.6 log CFU/g for both treatments).

Significance: Increased reduction of coliform bacteria in irrigation water by PAA compared to Cl treatment offers growers another solution to agricultural water safety requirements while mitigating the exposure to chlorine by-products.

P1-188 Moved to Technical

P1-189 Evaluation of Sample Collection Time Periods for Improved Sensitivity of Wastewater-Based Epidemiology Surveillance

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Introduction: Wastewater-based epidemiology has been used for foodborne illness surveillance; however, the pathogen detection, communication, and application tools have grown significantly during the COVID-19 pandemic. Sample collection is integral in maintaining data integrity, particularly in highly variable samples such as human wastewater and requires additional investigation as research evolves.

Purpose: The objective of this study was to determine if wastewater could be collected for a limited time-period, allowing for improved sensitivity and expedited time to results, that could be applied to surveillance of foodborne illness-causing organisms.

Methods: Wastewater was collected from dormitories ((A)suite-, (B)apartment-, and (C)traditional-style), on days 0, 2, and 5 of this study, in 3-hour, 12-hour, and 24-hour (control) composites. Samples (n=99) were processed using modified methods from CDC's National Wastewater Surveillance System including incubation, filtration, centrifugal-ultrafiltration, and RNA extraction. SARS-CoV-2 N1/N2 target quantification was performed in triplicate using RT-qPCR. Data were analyzed using JMP16 Pro software and significant differences determined by Dunnett's Test.

Results: Overall, timeframes with increased detection were 03:00-05:00 (3-hour) and 23:00-08:00 (12-hour) composites, 33% of samples (3/9) had significantly higher ($p<0.05$) detection than controls. The remainder (6/9) of samples (3-hour and 12-hour) were not different ($p>0.05$) from controls. Notably, 24-hour composites varied significantly ($p<0.05$) by day and complex. Complex-B produced significantly greater ($p<0.05$) wastewater volumes than Complex-

es-A and -C, potentially leading to the dilution of waste and decreased pathogen detection. Complex C produced highly turbid wastewater and only one sample (9:00-11:00, day 0) was significantly different from the control over the 3 collection days.

Significance: This study provides additional framework for continued methodology development for wastewater surveillance, including epidemiological efforts of enteric viruses (*e.g.,* norovirus among others) that could be monitored during or after outbreaks. These data suggest that sampling locations should be evaluated for dilution effects using a fecal indicator (physicochemical or microbiological) to determine the optimal sampling timeframe.

P1-190 Bacterial Communities Presence in Ten Natural Mineral Water Wells

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Introduction: Natural Mineral Water (NMW) is generally extracted directly from natural (spring/resurgence) or artificial sources (water wells), via groundwater explotation, characterized by a specific contents of mineral, trace elements and other constituents. It is known that the microbiota present in NMW can vary qualitatively and quantitatively according to the source/water well, in addition to numerous variables.

Purpose: The purpose of this study was to evaluate the diversity of the bacterial communities present in NMW collected in ten different water wells in southeast region of Brazil.

Methods: After filtration and DNA extraction and quantification, the microbiota present in the 10 samples of NMW (duplicate) was evaluated through Next Generation Sequencing specifically using the V4 region of the 16S rRNA gene amplicon sequences. Taxonomy was assigned to the 16S data using a Naïve Bayes pre-trained Greengenes 13_8 99% Operational Taxonomic Unit (OTUs) classifier. Statistical analysis was performed on R using the following packages: phyloseq, Vegan for PERMANOVA analysis and mixOmics.

Results: A total of 53305 reads and 141 Amplicon Sequence Variants (ASVs) were obtained. The α-diversity ranged from 0.01 to 3.9. The significant level of changes in species richness among the NMW confirmed by the Kruskal–Wallis ($p<0.01$). It observed *Proteobacteria* (43%) and *Patescibacteria* (10%) represent more than 50% of the total phylum present in the samples. Profiles at genus level were very different among the samples. The Spearman rank correlation test indicated that some measured water parameters can influence the abundance of some generain the water.

Significance: These data suggest the occurrence of a diverse bacterial community in the different samples analyzed. It is believed that the microbiota present in the different water wells is linked with the bacterial community present in the soil where the water well is located, indicating that the pedological and geological context is determining for the diversity.

P2-01 Antimicrobial Efficacy of Pullulan Coating Incorporated with Pecan Shell Extract and Its Effect on Quality of Blueberries during Storage

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❖ ***Developing Scientist Entrant***

Introduction: Pecan shell constitutes 50% of the nut mass and is considered an agricultural waste with little economic value. However, it contains various bioactive compounds that have shown to possess inhibitory activity against microorganisms in *in vitro* conditions.

Purpose: This study evaluated the *in vivo* application of pecan shell extract-pullulan (PSE-P) coating on blueberries against foodborne pathogens and its effect on quality during post-harvest storage.

Methods: PSE-P (5% w/v aqueous PSE+5% w/v pullulan), P (5% w/v pullulan) and control (water) coating solutions were prepared. Blueberries were dip inoculated (10g berries/1mL inoculum) with nalidixic acid resistant (50µg/mL) strains of *Listeria monocytogenes* (5.94±0.07 log CFU/mL)*, Salmonella enterica* (5.05±0.26 log CFU/mL)*, or Staphylococcus aureus* (5.56±0.48 log CFU/mL), spray coated (16µL coating solution/1g berries) with PSE-P, P or control and stored at 4°C, 50±10% RH for 15 days. The effect of antimicrobial coating against pathogens and its impact on quality (color, texture,TSS, pH, weight loss and spoilage rate) was determined throughout storage.

Results: On Day 0, all treatments significantly ($P<0.05$) reduced test organisms by 0.6-2.7 log CFU/g berries. PSE-P coated berries had significantly ($P<0.05$) lower growth of *Listeria monocytogenes (*3.88±0.28–2.06±1.03 log CFU/g) in comparison to control (4.77–3.64 log CFU/g) during 15-day refrigerated storage. However, no significant effect was observed on *Salmonella enterica* (PSE-P: 3.96-3.12 log CFU/g, Control: 3.99-3.04 log CFU/g) and *Staphylococcus aureus* (PSE-P: 4.96-3.67 log CFU/g, Control: 4.77-3.64 log CFU/g)*.* PSE-P coated berries showed noticeable difference in color from control (ΔE= 1.5-3). No significant effect of PSE-P was observed on TSS and pH. PSE-P coating maintained the firmness (N) *(P*>0.05) of berries during storage, reduced ($P<0.05$) spoilage rate (21%) than control (28.5%) and pullulan (37%), and significantly decreased weight loss in PSE-P coated berries until Day 15.

Significance: Pecan shell extract has potential to be used in antimicrobial coating for maintaining safety and quality of berries.

P2-02 Performance Evaluation of 3M™ Environmental Scrub Sampler with 10mL Wide Spectrum Neutralizer for the Recovery of Microorganisms in the Presence of Sanitizers Commonly Used in the Food and Beverage Industry

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Introduction: 3M™ Environmental Scrub Sampler with 10mL Wide Spectrum Neutralizer (ESSWSN) is intended for use in the food and beverage industry for environmental microbial surface sampling. ESSWSN can neutralize commonly used sanitizers and is compatible with routinely used detection methods.

Purpose: To demonstrate the neutralizer effectiveness of the ESSWSN by assessing the quantitative and qualitative recovery of organisms in the presence of sanitizers commonly used in the food processing environment.

Methods: Neutralizer Effectiveness (NE) and Neutralizer Toxicity (NT) of the ESSWSN were conducted for *Escherichia coli* (n=5), *Salmonella* (n=5), *Listeria monocytogenes* (n=5) *Listeria* (n=5) and *Cronobacter* (n=5) species, against four classes of sanitizers: quaternary ammonium-based, chlorine/bleach-based, hydrogen/peroxyacetic acid-based and high acid sanitizers. For NE, 1mL of sanitizer was added to 10mL of WSN containing 100µL of each strain (30-100 CFU/mL). For NT, 1mL of Phosphate Buffered Saline (PBS) was used instead of sanitizer. Quantitative recovery was measured on 3M™ Petrifilm™ Plates (PF). Qualitative recovery was measured after the respective enrichment procedures for the target strains using the appropriate 3M™ Molecular Detection Assay 2 (MDS2). Testing was conducted according to ASTM E1054-08.

Results: The ESSWSN effectively neutralized (NE) the sanitizers included in this study and were shown to be non-toxic and did not affect recovery of the microorganisms studied. Results of quantitative enumeration with the respective PF plates showed a P value> 0.05 and a Mean Log Difference of < 0. 2 Log10 compared to PBS . Qualitative detection with the respective MDS2, showed no significant differences between NE and NT tests with a Chi-square (X^2) value < 3.84.

Significance: Effective neutralization of residual sanitizers and organism recovery are critical for accurate environmental surface test result integrity. ESSWSN effectively neutralizes sanitizers commonly used in the food industry, maintaining the viability of target microorganisms.

P2-03 Distinct Microbiome Signatures in Mice Treated with Commonly Used Food Preservatives

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Introduction: Diet is one of the most important factors regulating and influencing our gut microbiome, but the specific effects of commonly used antimicrobial agents, i.e., synthetic food preservatives added to our foods, are not completely understood.
Purpose: To examine how the dietary intake of the three widely used food-grade preservatives i.e., benzoic acid, potassium sorbate, and sodium nitrite, within the recommended levels, affects the gut microbiota diversity and composition in a mouse model.
Methods: Male C57BL/6J mice (age: 8 wks) were randomized into four groups (n=8/group) and received either (i) 0.3% potassium sorbate (3000 PPM), (ii) 0.1% benzoic acid (1000 PPM), or (iii) 0.05% sodium nitrate (500 PPM) supplemented in standard rodent diet, with one control group without preservative. After a 12-weeks intervention, the gut microbiome was measured by high-throughput 16S rRNA gene sequencing and analyzed using QIIME bioinformatics pipeline.
Results: The analysis of β-diversity revealed distinct microbiota signatures between mice consuming different preservatives. Alpha-diversity indices showed that the three preservatives induce specific patterns of microbial diversity, with diversity being lowest in mice consuming potassium sorbate. Each preservative demonstrated unique microbial signatures, mainly affecting the bacterial taxa belonging to Bacteroidetes, Verrucomicrobia, and Proteobacteria. Specifically, we observed increased proportion of Bacteroides, Blautia, Ruminococcus, Oscillospira, and Dorea by benzoate; increased abundance of Firmicutes, Turicibacter, and Alkaliphilus by sodium nitrate; and increased proportion of Parabacteroides and Adlercreutzia by potassium sorbate.
Significance: The findings advance our understanding of how food-grade preservatives may influence the gut microbiota and should facilitate prospective studies investigating diet-microbiome interactions in relation to intestinal and metabolic health.

P2-04 Efficacy of Homemade Electrolyzed Water Sanitizer for Inactivation of Foodborne Pathogens

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✦ ***Undergraduate Student Award Entrant***

Introduction: Electrolyzed water (EW) is gaining popularity and interest as a non-corrosive sanitizer among food industry and household users, attributed to its effectiveness, economical and ease of manufacturing. However, the antimicrobial efficacy and mode of application of homemade EW prepared using economical and easy-to-use EW generators have not been experimentally evaluated.
Purpose: The present study was to evaluate the antimicrobial efficacy of homemade EW solutions prepared and applied using different household generators and modes of application, respectively.
Methods: EW was prepared using different manufacturers' commercial generators and evaluated by spotting on agar containing bacterial lawn, spraying inoculated agar, and dipping/inoculating bacteria into EW solutions before subjected to viable cell enumeration using aerobic plate count (APC). Blood lysis/coagulation and total chlorine measurement were adopted for investigation of bleach-equivalent toxicity using animal erythrocytes and free-chlorine-testing strips, respectively. Statistical significance of EW differential antimicrobial efficacies was determined for multiple biological and technical tests using Student's t-test ($P<0.05$).
Results: Qualitative antimicrobial analysis of freshly made EW demonstrated growth inhibition zones within spotting areas pre-inoculated with test strains with reducing zone clearing as dilution increased. APC comparative analyses for two antimicrobial treatment methods revealed that the dipping method posted significantly higher microbial reduction (>5-log cfu/ml reduction, cfu/ml; $P<0.05$) than spraying ($P>0.05$), suggesting that this treatment method be used for subsequent investigations. Shelf-life and dosage analyses demonstrated that EW efficacy reduced significantly with time and dilutions (≥1/8, v/v), respectively, and its activity completely diminished at dilution 1/32. There was significant EW efficacy difference ($P<0.05$) from different generators. Comparative toxicity assays revealed that, EW (≤1 month old), unlike bleach, possessed lesser total chlorine (~16X) and did not cause blood complications.
Significance: Biological contaminant-associated food outbreaks, spoilage, and recalls may persist with present food-safety-processing regimes. Our findings suggest that EW is a non-corrosive, economic, easy-to-prepare yet effective alternative for household and food-processing-plant applications.
Keywords: Antimicrobial**,** food, foodborne pathogens, homemade electrolyzed water, household, sanitizer.

P2-05 Inactivation Efficacy of Plasma-Activated Water Against Mixed-Species Biofilms on Baby Spinach in Presence of Organic Matter

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❖ ***Developing Scientist Entrant***

Introduction: Mixed-species biofilms pose a major risk of fresh produce, and the presence of organic matter greatly impairs the efficacy of sanitizers. Plasma-activated water (PAW) is a novel sanitizer for fresh produce decontamination, however, the efficacy of PAW on mixed-species biofilms whilst the presence of organic matter is not known.
Purpose: This study aimed to determine the sanitation efficacy of PAW on baby spinach where organic matter co-exists with mixed-species biofilms.
Methods: Baby spinach leaves were inoculated with mixed strains of *E. coli* O157:H7 and *Listeria innocua* in equal proportions to grow biofilms for 24 hours at 4°C. PAW was prepared by exposing deionized (DI) water to a cold atmospheric pressure air plasma jet for 15 minutes. Organic matter was prepared by stomaching baby spinach leaves in presence of DI water followed by centrifugation. Inoculated leaves were incubated in PAW for 2 minutes (PAW15-2), or 15 minutes (PAW15-15) in presence of 450-500 ppm organic matter. The inactivation efficacy of PAW and control (150 ppm Chlorine) was evaluated in 3 independent replications and analyzed using ANOVA and student t-test at $p<0.05$.
Results: In the presence of organic matter, treatment of PAW15-2 reduced biofilms by 1.0 ± 0.2 log CFU/leaf while chlorine-2 achieved 1.4 ± 0.4 log CFU/leaf reduction. For incubation time of 15 min, reductions of 1.9 ± 0.1 log CFU/leaf and 2.5 ± 0.1 log CFU/leaf were observed for PAW15 and chlorine, respectively. Survival bacteria were not detected (<1 log CFU/leaf) from spent wash solutions of chlorine and PAW15 for both incubation times.
Significance: PAW is a promising sanitizer against mixed-species biofilms on fresh produce in challenging conditions. PAW has the potential to be an alternative to traditional sanitizer after further optimization.

P2-06 Efficacy of Common Antimicrobial Interventions at and Above Regulatory Allowable Pick-Up Levels

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Introduction: Antimicrobial interventions with organic acids such as lactic and peracetic acid are being used as processing aids in food plants, though the retained water cannot exceed 0.49% addition to product weight, these agents are applied to reduce foodborne pathogens like *E. coli* and *Salmonella* which are the leading cause of illnesses worldwide.
Purpose: The objective of this study was to evaluate the food safety efficacy of common antimicrobial interventions at and above required uptake levels for processing aids.
Methods: Beef trim was inoculated with *E. coli* (ECRC 0.1302, ECRC 97.1377, ECRC 2.0164, ECRC 3.1009, ECRC 3.1064, ECRC 9.0538, and ATCC 51657) or *Salmonella* (ATCC 14028, ATCC 6962, and ATCC 31194). Trim weight was recorded prior to intervention (peracetic acid; 400ppm or lactic acid solution; 4.5%) through spray or dip application to obtain a broad uptake level. Afterwards, 2 lb intervened trim bags (n = 480) were weighed to measure pick up level.

Meat rinses were serially diluted and plated following the drop dilution method and an enumerable range of 2-30 colonies was used to report results after log transformation.

Results: *E. coli* and *Salmonella* have an average reduction of 0.16 LogCFU/g, suggesting that for every 1% increase in uptake there is an increase of 0.16 LogCFU/g of reduction. There is statistical significance in the reduction of *Escherichia coli* in relation to the uptake percentage ($P < 0.01$). The addition of explanatory variables increases the adjusted R^2 of the regression for *E. coli*, where all the additional explanatory variables are statistically significant for reduction ($P < 0.01$). The same can be observed for *Salmonella*, but only trim type is significant for reduction ($P < 0.01$).

Significance: This study suggests that an increase in uptake percentage of antimicrobial agents on beef trim causes an increase of reduction of pathogens, thus, improving the microbial safety of meat products and assuring safety of consumers.

P2-07 Sorbate Replacement in Cultured Dairy with Plant Extract and Fermentate Combination

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Introduction: Greater consumer label awareness has resulted in food manufacturers to seek clean-label alternatives for preserving or extending product shelf-life. Cultured dairy products are susceptible to yeast and mold spoilage, with few clean-label options to sorbate available.

Purpose: To evaluate the performance of a plant extract + ferment-based antimicrobial (C1) on yeast and mold growth at 4°C in a cultured fresh cheese while maintaining live culture survival and pH stability within the product.

Methods: A commercial cultured fresh cheese (pH 4.4, 76.51% moisture) containing no preservatives was supplemented with 0.04% sorbate or 0.275% C1 prior to inoculation with 3-log CFU/g yeast (*Debaromyces hansenii, Candida parapsilosis)* or mold (*Mucor jansenii, Eupenicillium javinivum, Penicillium roqueforti, P. camemberti*) cocktails. Yeast samples were assayed weekly by pour-plating with PDA for 49 days, while mold samples were visually inspected every 2-3 days. Uninoculated samples were assayed for pH and live cultures (*Lactobacillus bulgaricus, Streptococcus thermophilus)* by plating weekly on acidified MRSA and LM-17 for 49 days. Additionally, growth curves of *Zygosaccharomyces rouxii, Z. bailii* and *Saccharomyces cerevisiae* were generated in PDB (pH 4.5) with 0.275% C1, sorbate (0.04%, 0.1%) or benzoate (0.1%) (BioTek LogPhase600, 48 h, 25°C).

Results: No-antimicrobial fresh cheese supported >2-log CFU/g yeast growth by day 35, while 0.275% C1 inhibited yeast growth (<1-log) for up to 49 days. Time to mold was increased 35% with 0.275% C1 compared to the no-antimicrobial control (from 21 to 28 days). The addition of 0.275% C1 did not impact pH (pH 4.40±0.05) nor live culture survival over 49 days, with cultures at >8-log CFU/g throughout the test period. 0.275% C1 in PDB inhibited growth of *Z. rouxii, Z. bailii* and *S. cerevisiae*.

Significance: This work supports the use of C1 as a suitable clean-label antimicrobial against yeast and mold in cultured dairy while maintaining product pH and active cultures populations.

P2-08 Impact of Environmental Conditions and Cumulative Soiling on Antimicrobial Efficacy of Powdered Sanitizers over Time

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Introduction: Use of powdered sanitizers in facility entryways plays a crucial role in controlling microbial transfer, however infrequent replenishment or exposure to harsh environmental conditions may limit effectiveness of the intervention.

Purpose: This study assessed the longevity of active components and antimicrobial efficacy of selected EPA-registered and nonregistered powdered sanitizers exposed to elevated temperature, humidity, and soiling.

Methods: Four powder sanitizers with differing biocidal actives were evaluated: 1) Sterilex Ultra Step (Product A), 2) Provox (Product B), 3) ProvaStride (Product C) and 4) Traffic C.O.P. (Product D). 2000-gram samples of each were dispensed into foot pans in triplicate and subjected to environmental conditions in an enclosed outdoor shelter for 3 weeks. Temperature and relative humidity were recorded. Autoclaved composted manure was added daily with a resulting cumulative soil concentration of 1% by weight of the powder at the end of three weeks. Sanitizer was sampled weekly and analyzed for active ingredient concentrations by test strips, as well as subjected to ASTM 1153 Non-food Contact assay against *S*. enteritidis ATCC 13706 at a dose rate of 37 g/L.

Results: The average maximum temperature and relative humidity throughout the study was 82 ±11°F and 84±6 %, respectively. Product D's active chlorine rapidly degraded after 1 week from 500 to 0 ppm and failed to achieve a required 3-log reduction (Initial=4.82, Week 1=0.45, Week 2=0.09). Product B also failed to achieve a 3-log reduction after 1 week (Initial=5.38, Week 1=2.14, Week 2=2.04, Week 3=1.04). Product A achieved a 3-log reduction after 1 week (Initial=5.38, Week 1=3.19, Week 2=2.37, Week 3=2.12). Product C achieved a 3-log reduction for 2 weeks (Initial=5.38, Week 1=4.30, Week 2=3.64, Week 3=2.95).

Significance: The results suggest that EPA-registered products that undergo rigorous and validated testing maintain active ingredient integrity, and as a result, efficacy against *S*. enteritidis despite exposure to high temperature and humidity.

P2-09 Changes of Antimicrobial Activities of UV-C Irradiation as Affected by Types of Microorganisms and Abiotic Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Ultraviolet (UV) irradiation has been known as an effective surface decontamination method, but the changes of antimicrobial activities of UV-C irradiation as affected by the types of microorganisms and the types of abiotic surfaces have not been intensively studied.

Purpose: This study was done to determine the changes of antimicrobial activities of UV-C irradiation against different types of microorganisms attached on various types of abiotic surfaces.

Methods: ***Escherichia coli*** O157:H7, *Staphylococcus aureus*, *Bacillus cereus* spores, *Bacillus atrophaeus* spores, *Cladosporium cladosporioides* spores were inoculated on 3 types of abiotic coupons (glass, plastic and wood; 2cm×5cm; ca. 7 logCFU/coupon). The coupons were exposed to UV-C(272nm, 27mW/cm^2) light which had been generated from light-emitting diodes (LED) at 5cm distance for 1 minute. The population of microorganisms were determined by direct plating method.

Results: It was observed that bacterial and fungal spores were more resistant to UV-C irradiation compared to *E. coli* O157:H7 or *S. aureus* cells. Among the spores, *C. cladosporioides* spores showed the strongest UV-C resistance. For example, when microorganisms were inoculated on glass surface and irradiated with UV-C, *E. coli* O157:H7 and *S. aureus* were completely inactivated within 1 minute, but 1.8±0.2 logCFU/coupon of *C. cladosporioides* spores were survived. The resistance of microorganisms was significantly affected by the types of abiotic surfaces; microorganisms inoculated on wood showed strongest resistance compared to microorganisms on glass or plastic. For example, after 1 min of UV-C irradiation, on plastic, *E. coli* O157:H7 and *S. aureus* were completely inactivated, and the survived populations of *B. cereus* spores, *B. atrophaeus* spores, *C. cladosporioides* spores were 1.6±0.1, 0.5±0.9, 2.0±0.3 logCFU/coupon, respectively, but, on wood, the survived populations of *E. coli* O157:H7, *S. aureus*, *B. cereus* spores, *B. atrophaeus* spores, and *C. cladosporioides* spores were 2.7±0.3, 3.2±0.1, 3.1±0.2, 4.2±0.1 and 2.3±0.2 logCFU/coupon, respectively.

Significance: The results of this study provide basic information for the development of methods to inactivate hazardous microorganisms on abiotic surfaces using UV-C light.

P2-10 Reduction of Microbial Indicator Bacteria in Beef Trimmings after Immersion in Lactic Acid (1-2%) vs. Citrilow (pH 1.2) as a Food Safety Intervention and Sequential Spraying with Ozonated Water (BioSafe)

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❖ ***Developing Scientist Entrant***

Introduction: The meat industry needs to explore alternatives for trimming interventions to reduce microorganisms and comply with performance standards; Citrilow and Lactic Acid (LA) have shown efficacy in reducing microbial indicators in beef products.

Purpose: To evaluate the antimicrobial performance of Citrilow vs LA after application in beef trimmings (chuck and shank) followed with sequential application of ozonated water.

Methods: Treatments consisted of immersion of beef trimmings on Citrilow or LA during 12s at a temperature range of 72-76°F. Subsequently BioSafe (700-900mV ORP) was sprayed onto the trimmings at 50-75°F (concentration 1.5-2.3 ppm and ≥20psi pressure). Samples were collected using sterile pre-hydrated sponges with 25 mL of neutralizing buffer. A 200 cm^2 area was swabbed on each piece of trim. For LA 210 samples were taken (105 chuck, 105 shank) in duplicate before and after treatment. For Citrilow, 180 samples (90 chuck, 90 shank) were evaluated in six repetitions. Total aerobic plate (AC), Enterobacteriaceae (EB), and generic *Escherichia coli* (EC) counts were determined using the TEMPO® system.

Results: After LA treatment, AC were reduced on average by 2.06 and 1.03 LogCFU/cm^2 (P<0.001), EB counts were reduced on average by 2.16 and 1.33 Log CFU/sample (P<0.001) and EC counts were also significantly reduced on average by 1.59 and 0.78 LogCFU/sample (P<0.001), on chuck and shank respectively. After Citrilow treatment, AC was reduced on average by 1.35 and 1.74 LogCFU/cm^2 (P<0.001). EB counts were reduced on average by 1.42 and 1.63 LogCFU/sample (P<0.001) and EC counts were also significantly reduced on average by 0.91 and 0.56 LogCFU/sample (P<0.001). After BioSafe intervention there was no additional statistical difference in AC, EB and EC counts.

Significance: Evaluating the efficacy of alternative interventions for beef trimmings will assist the meat industry in increasing the number of tools for decision-making on intervention schemes that are more efficient to protect public health.

P2-11 Silk Polymeric Carriers Designed for Encapsulation of Essential Oils

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Introduction: The antimicrobial properties of essential oils have been observed in numerous studies. However, their direct use is limited because of their volatility, strong flavor, and low water solubility. Encapsulation of essential oils can provide many benefits such as controlled release, improved water solubility, prevention of volatile component evaporation, and protecting them from environmental effects. Silk fibroin, a natural protein polymer derived from *Bombyx mori* cocoons, has been a widely used protein polymer for biomaterial applications. In this study, silk fibroin was used due to unique properties of silk as an encapsulating material applied to various essential oils.

Purpose: The purpose of this study was to synthesize essential oil-loaded silk nanoparticles with enhanced antibacterial properties.

Methods: Essential oil-loaded silk nanoparticles were characterized for size, encapsulation efficiency, storage stability, and optical density (OD) measurements for *Escherichia coli* growth. Antimicrobial silk particles were produced by sonication. Dynamic light scattering (DLS) was used to analyze the size distribution of the particles. Drug release was measured by UV/Visible scanning spectrophotometer.

Results: Size of the silk nanoparticles (sonicated for 2 minutes with the formulation of 1% oil: 0.2% 30 minute-boiled silk) were 312.6 ± 6.5 nm for thyme oil, 324.8 ± 2.458 nm for oregano oil, 271.0 ± 4.063 nm for ginger oil, 248.5 ± 3.102 nm for rosemary oil. Various parameters such as silk and oil concentrations, sonication parameters, and silk boiling times effected the size of silk nanoparticles. Silk encapsulated essential oils (thyme, oregano, ginger, rosemary) showed a sustained drug release by maintaining their antibacterial properties over time. Silk encapsulated essential oils showed better antibacterial activity compared to their non-encapsulated forms.

Significance: Our study demonstrated that silk can be used as an encapsulating material for various essential oils without the need of any solvent and surfactant. Our silk encapsulated essential oils are better alternatives to chemical additives for surface sanitization.

P2-12 Inhibition of *Listeria monocytogenes* and *Clostridium botulinum* in Cooked, Uncured Meat Products Formulated with Citric Acid (CA) and Cultured Dextrose-Buffered Vinegar (CDV)

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Introduction: Cooked, uncured meat products under reduced oxygen packaging conditions require the control of *Clostridium botulinum* and *Listeria monocytogenes*, especially if they become re-contaminated post cook. Antimicrobials added to a formulation to control these pathogens must be validated.

Purpose: To determine the inhibition of *L. monocytogenes* and *C. botulinum* in cooked, uncured turkey and pork with citric acid (CA)-cultured dextrose vinegar (CDV) antimicrobial system under mild or moderate temperature abuse.

Methods: Three treatments of shredded meat products were prepared using turkey breast (~61% moisture; 1.3% salt) and pork butt (~46% moisture; 1.5% salt) cooked to 90.6°C, shredded and chilled. Treatments included TRT-A, tumbled pre-cook with 0.5% CA and 2% CDV (turkey final pH 5.3; pork pH 5.1), TRT-B antimicrobials added during shredding post-cook (turkey pH 5.5; pork pH 5.4), or TRT-C control with no antimicrobials (turkey pH 6.1; pork pH 5.9). Each formulation was inoculated with 3-log CFU/g of *L. monocytogenes* (5-strains) or *C. botulinum* spores (7 proteolytic, 3 nonproteolytic strains), vacuum-sealed, incubated at 7.2˚C (*L. monocytogenes*) or 12.8˚C (*C. botulinum*) and assayed biweekly for up to 12 weeks. *L. monocytogenes* was enumerated by plating onto Modified Oxford agar and botulinum toxin was detected using the mouse bioassay.

Results: Populations of *L. monocytogenes* increased 4-log in 2 and 4 weeks at 7.2˚C for turkey and pork TRT-C formulations, respectively, but no growth was observed in 12 weeks for any antimicrobial treatment. Similarly, botulinum toxin was detected in both controls at week 2 at 12.8˚C, however no toxicity was observed in any antimicrobial treatments through 12 weeks.

Significance: These data suggest that a combination of 0.5% citric acid and 2% cultured dextrose-vinegar inhibits the growth of *L. monocytogenes* and toxin production of *C. botulinum* in uncured shredded turkey and pork products stored under mild temperature abuse conditions for up to 12 weeks in reduced oxygen packaging.

P2-13 *Listeria* Reduction and Elimination: Testing Results of Extreme Microbial Technologies Microbial Area Kleener (MAK9)

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Introduction: Non-toxic and chemical free continuous purification solutions have been proven to reduce or eliminate 99.9% of viruses and bacteria, both in the air and on/under surfaces. This ensures bacteria and microbial protection for food products, equipment, and staff continuously on a 24/7 basis, eliminating the need for shut downs and recalls.

Purpose: The purpose of this study was to evaluate Ionized Hydrogen Peroxide Technology on the reduction of Listeria monocytogenes in general room settings.

Methods: Modified EPA DIS/TSS-10 Organisms: Listeria monocytogenes ATCC 13932 using the MAK9 Ionized Hydrogen Peroxide Technology with an exposure time: O hrs., 6 hrs. and 24 hrs. Inoculum concentration: 1.5 x 106 cfu/ml (McFarland #0.5 standard) amount of inoculum used: 0.1 ml Volume of TSB: 5 ml Volume plated: 0.1, 0.01, and 0.001 ml. Incubation time: 24 hrs. @ 35°c ±1.

Results: The effect of MAK9 Ionized Hydrogen Peroxide Technology on the reduction of Listeria monocytogenes in general room settings resulted in percent reduction exposed 6 hrs./CFU and 24 hrs. 99.9%.

Significance: This technology greatly reduces food safety risk by eradicating listeria and other pathogens from food processing, packaging, storage, and transporting areas. This provides scientifically proven methods that proactively seek out contaminants in the air and on surfaces to deactivate and decontaminate harmful pathogens, and then works to continually disarm these contaminants as they're reintroduced into the air and on surfaces.

P2-14 Broad-Spectrum Antimicrobials with Potential Food Applications Coproduced by *Bacillus velezensis* Osy-GA1

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❖ ***Developing Scientist Entrant***

Introduction: The genome of *Bacillus velezensis* OSY-GA1 encodes at least seventeen biosynthetic gene clusters, many of which appear to be for novel antimicrobials or variants of known antimicrobials that are potentially useful as natural food preservatives.

Purpose: Characterizing the antimicrobials expressed by *B. velezensis* OSY-GA1 and determining if any are novel antimicrobials or more powerful variants of known antimicrobials.

Methods: *B. velezensis* OSY-GA1 was grown in optimized conditions, and putative antimicrobials were extracted from the culture using beads of Amberlite XAD7 polymeric resin. Compounds eluted from the beads, via 75% acetonitrile, were purified using high-performance liquid chromatography (HPLC). HPLC fractions were screened for antimicrobial activity, and active fractions were further purified via additional HPLC separation. The new HPLC fractions were then analyzed via matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometry to identify possible compounds contained in the HPLC fractions, and the masses were matched to the predicted compounds identified in the genome.

Results: *B. velezensis* OSY-GA1 produced compounds active against *Listeria innocua* ATCC 33090 during the producer's late exponential and stationary phases of growth (10h of incubation and beyond) and compounds active against *E. coli* K12 during its stationary phase (20h and beyond). Anti-fungal activity against *Candida albicans* SC5324 was also observed during both late exponential and stationary phases. MALDI-TOF-MS analysis of HPLC fractions suggests the production of macrolactin H, fengycin, iturin A, surfactin, plantazolicin, difficidin, amylocyclicin, bacillibactin, and a thiopeptide, which is consistent with the whole genome sequencing data for *B. velezensis* OSY-GA1.

Significance: *B. velezensis* OSY-GA1 produces multiple antimicrobial compounds that are effective against a broad range of organisms and may be suitable for food preservation applications.

P2-15 The Impact of Florfenicol Treatment on the Microbial Populations Associated with Live Catfish

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Introduction: For the last 30 years, the consumption of freshwater fish and seafood per capita has been increasing, and catfish is the leading aquaculture finfish species. Florfenicol has been approved by the Food and Drug Administration (FDA) to treat the enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri*. However, there is limited information about the impact of such treatment on the native microbiota associated with catfish.

Purpose: The objective of this study was to investigate the impact of therapeutical florfenicol treatment on the microbial populations present on the skin and gill, and in the intestine of catfish.

Methods: Eight 35-gallon tanks were set up for this study with 25 fish per tank at 25 °C (a temperature at which ESC typically occurs). Five fish were taken from each tank before and after the treatment as well as at the end of withdrawal period. Microorganisms present on fish gill and skin, and in the intestine were collected using FLOQSwabs. Their abundance and diversity were analyzed by direct plating and 16S rDNA sequencing.

Results: The total anaerobic count (AnPC) in the intestine significantly ($P > 0.05$) increased by 3.02-log at the end of treatment and continued increasing by another 1.6-log at the end of withdrawal period. In comparison, < 1-log increase of AnPC was observed from gill and skin. Similar trend was observed for total aerobic counts. Currently available sequencing results indicated that the abundance of bacterial phyla, such as *Firmicutes*, *Fibrobacterota*, and *Proteobacteria*, present in the intestine of catfish was significantly altered by the florfenicol treatment.

Significance: Considering the microbial composition can influence the catfish health and growth. This study provides valuable information on the impact of florfenicol treatment on catfish microbiome during catfish production, which is needed for ensuring the sustainability of aquaculture production and for improving food safety.

P2-16 Survival of *Listeria monocytogenes* and *Salmonella* in Citrus Storage Waxes

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Introduction: To better match market demand, lemons are sprayed with storage waxes after washing and stored wet for up to 6 months at 12 to 14°C. The storage waxes are stored at ambient temperature in citrus packinghouses for up to 3 months at full strength or up to 7 days in diluted form. The fate of common foodborne pathogens in storage waxes is unknown.

Purpose: The purpose of this study was to evaluate the survival of *Listeria monocytogenes* (*Lm*) and *Salmonella* in citrus storage waxes under simulated packinghouse storage conditions.

Methods: Four commercial citrus storage waxes at full strength or dilute application rates were each separately inoculated with five-strain cocktails of *Lm* or *Salmonella* at 6.0 log CFU/mL and stored at 4 and 22°C to mimic ambient temperatures of packinghouses (n=6). Populations of *Lm* and *Salmonella* were monitored in full strength waxes for 24 h, and populations of *Lm* in full strength and diluted waxes for 135 and 10 days, respectively.

Results: Significantly greater reductions were observed for *Salmonella* than for *Lm* during 24 h storage of full-strength waxes regardless of storage temperature. During storage waxes, *Lm* survived better at 4°C than at 22°C. Populations of *Lm* in the four full-strength waxes ranged from < 1 to 2.9 log CFU/mL after 135 days of storage at 4°C; *Lm* was not detected by enrichment of 0.5 mL after 105 days of storage at 22 °C. Populations of *Lm* in diluted waxes did not significantly decline in any wax held at 4°C for 10 days; significant declines were observed with some waxes at 22°C. Within each wax product, smaller population declines were observed as dilution rates increased.

Significance: Common foodborne pathogens can survive during storage and handling of commercial citrus storage waxes which should be considered in packinghouse hazard assessments.

P2-17 Genus-Level Microbial Community Profiling during Shelf Life of Raw Plant-Based Patties

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Introduction: Natural, plant-based foods are increasingly popular among customers but present novel shelf-life challenges due to clean-label demands.

Purpose: This study investigated the proliferation of microbial spoilage organisms in raw, plant-based patties across different treatments and packaging methods.

Methods: Commercially produced plant-based patties were treated with Natural Preservative A (NP-A), Natural Preservative B (NP-B) or no preservative (Control), packed in non-MAP trays or sealed in vacuum bags, and stored at 4C. At each timepoint, samples from each treatment and package type were analyzed in duplicate for Aerobic Plate Count (APC), Lactic Acid Bacteria (LAB), Yeast and Mold (YM) and 16S rRNA Illumina microbiome sequencing.

Results: APC and LAB counts quickly rose in tray Control samples, reaching 10^5 CFU/g at day 14. APC and LAB counts in NP-A and NP-B samples remained at 10^3 through day 14. All tray samples showed visible mold growth at day 21. Vacuum bag samples reached 10^8 CFU/g APC and 10^5 CFU/g LAB by day 21 in Control samples, compared to $<10^3$ in NP-A and NP-B samples. Visible mold was present only on the Control sample at day 21 but yeast growth was countable on all three vacuum-packed treatments. Microbiome analysis showed bacterial spoilage organisms were initially similar in non-MAP tray and vacuum-packed storage, but eventually favored different populations. Day 14 Control tray sample identified 89% *Leuconostoc* and day 14 and 21 vacuum bag sample was >97% *Pseudomonas*. Both NP-A and NP-B sample communities remained diverse with one spoilage genus not exceeding 60% of the population throughout the study.

Significance: Both natural preservative treatments improved shelf-life of raw, plant-based patties by maintaining lower microbial levels and higher diversity compared to the control patties under the same packaging conditions.

P2-18 Inhibition of *Clostridium perfringens* by Clean-Label Antimicrobials in a Model Meat System during Extended Cooling

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Introduction: USDA-FSIS Appendix B Guidelines define cooling limits for meat and poultry products to inhibit outgrowth of *Clostridium perfringens*, with longer cooling options for products using validated antimicrobials systems, such as nitrite. In response to consumer interest in uncured products, a novel cultured sugar and vinegar antimicrobial system (CSV; Verdad® Avanta CY2) was developed to extend cooling limits and shelf-life as an alternative to use of sodium nitrite.

Purpose: To validate a novel CSV to inhibit *C. perfringens* outgrowth in a model ham system during 15 h extended cooling used for cured meat products.

Methods: Five treatments of ground ham muscle (72% moisture, 2% salt, pH 6.3) including no antimicrobial, 100 ppm sodium nitrite plus 250 ppm sodium erythrobate, or 1.9, 2.2, or 2.5% CSV, were inoculated with ~3 log CFU/g *C. perfringens* spores (3-strain mixture). Samples (25 g) were vacuum packaged, flattened to uniform thickness, cooked to 73°C (internal temperature) and immediately transferred to a programmable incubator for cooling. Samples were cooled from 54.4°C to 26.7°C in either 2.5 h or 5 h, then from 26.7°C to 7.2°C in 10 h (P1, 12.5 total h; P2, 15 total h). Triplicate samples were removed pre- and post-cook and during cooling, serially diluted, and enumerated on tryptose-sulfite-cycloserine agar. Data is reported as average log change at each testing interval. Two trials were performed.

Results: *C. perfringens* populations increased by 1.4 and 4.5 log in meat without antimicrobials during P1 and P2 cooling, respectively. In contrast, *C. perfringens* outgrowth was inhibited in the nitrite and ≥1.9% CSV treatments in both the 12.5 and 15 h cooling, with populations decreasing ~0.9-1.3 log in all antimicrobial treatments.

Significance: Application of ≥1.9% CSV inhibited outgrowth (<1 log CFU/g increase) of *C. perfringens* similar to nitrite in a model meat system during extended (15 h) cooling.

P2-19 Chemical Sanitizer's Effectiveness to Eliminate Planktonic and Sessile STEC, Spoilage and Lactic Acid Bacteria on Food Contact Surfaces

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Introduction: Shiga toxin-producing *Escherichia coli* are a public health concern worldwide. In addition, lactic acid bacteria and spoilage bacteria can add to the contamination problem and affect food quality and shelf life.

Purpose: To investigate chemical sanitizer's effectiveness on planktonic cells and single-species biofilms formed by STEC, LAB and SP at 10 and 25º C.

Methods: The susceptibility of the STEC, SP and LAB in the planktonic and sessile stage were tested against regular sanitizers often used in food processing facilities (quats, chlorine, sodium hydroxide, hydrogen peroxide, peroxyacetic acid) and one sanitizer specifically designed to eliminate biofilms (BioDestroy®-organic peroxy acid). Sanitizer's concentrations were prepared following the manufacture's recommendations and tested at 10 and 25 °C. STEC, SP and LAB were allowed to form mature biofilms in polystyrene 96-wells microplates. To test planktonic cells, STEC, SP and LAB were grown and the culture concentration adjusted to 10^6 CFU/ml; cultures were then transferred to 96 well-microplates. Biofilms and planktonic cells were treated with different sanitizers. Minimum bactericidal concentration and biofilm eradication concentration was calculated. Experiments were carried out in triplicates (n=1440).

Results: As expected, Planktonic cells were more susceptible to all tested sanitizers at 25°C ($p<0.0001$). Biofilms required higher sanitizer concentrations at 25°C to inhibit their growth than at 10°C ($p<0.0001$). When evaluating each disinfectant individually, LAB was significantly more reduced than STEC and SP at 10 and 25°C. The STEC biofilms O111, O121, O157, O45 were 100% resistant to chlorine (25%; $P<0.01$) at 25°C than the other STEC strains. The most effective sanitizers were organic peroxy acid followed by quats, hydrogen peroxide, sodium hydroxide and chlorine.

Significance: STEC biofilms grown at 25°C were resistant to chlorine sanitizer ($p<0.0001$), while organic peroxy acid and quats were more effective sanitizers for biofilm eradication. Food producers can use this information to choose sanitizers based on their needs.

P2-20 Combined Effects of Gaseous Organic Acid and Essential Oil in Inhibiting the Growth of *Bacillus cereus*

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❖ ***Developing Scientist Entrant***

Introduction: Organic acids (OAs) and essential oils (EOs) are known as natural antibacterial agents, but the synergistic antibacterial effects between gaseous OAs and EOs against *Bacillus cereus* have not been intensively studied.

Purpose: The aim of study was to determine the minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) of gaseous OAs and EOs against *Bacillus cereus* on a laboratory medium, and to identify the combinations of gaseous OAs and EOs that exert synergistic antibacterial effects against *B. cereus* on a laboratory medium.

Methods: Vapor diffusion assay was used to screen gaseous OAs and EOs which have relatively strong antibacterial activities against *B. cereus.* MICs and MLCs of selected gaseous OAs and EOs against *B. cereus* at the optimal growth temperature(30℃) and room temperature(22℃) were determined in 1.8 L airtight containers. Finally, the combinations of gaseous OAs and EOs showing synergistic antibacterial effects against *B. cereus* were determined using a modified checkerboard assay.

Results: At 30℃, MICs of gaseous acetic, formic, and propionic acid against *B. cereus* were 50, 25, and 50 mg/L, respectively, and MLCs were higher than 400 mg/L regardless of types of OAs. At 22℃, MICs were 50, 25, and 50 mg/L for gaseous acetic, formic, and propionic acid, respectively, and MLCs > 400 mg/L regardless of types of OAs. Among tested gaseous EOs, cinnamon bark EO showed relatively stronger antimicrobial activities against *B. cereus*. MIC of gaseous cinnamon bark EO were 50 mg/L at 30℃ and 25 mg/L at 22℃, and MLCs was > 400 mg/L regardless incubation temperature. At 30℃, the combination of gaseous propionic acid and cinnamon bark EO showed a partial synergistic effect(FIC=0.625), and at 22℃, the combination of gaseous acetic acid and cinnamon bark EO and the combination of gaseous propionic acid and cinnamon bark EO showed a partial synergistic effect(FIC=0.75).

Poster

Significance: This study provides the useful information in developing a decontamination method against *B. cereus* using gaseous OAs and EOs.

P2-21 Antimicrobial Effect of *Ohelo* Berry (*Vaccinium calycinum*) on Pathogenic Bacteria in Whole Milk Compared with Cranberry *(Vaccinium macrocarpon)*

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❖ ***Developing Scientist Entrant***

Introduction: *Ohelo* berry (*Vaccinium calycinum*) is a Hawaiian wild relative of cranberry (*Vaccinium macrocarpon*). In our previous studies, pathogenic *Listeria monocytogenes* was significantly inhibited in whole and skim milk supplemented with *ohelo* berry juice.
Purpose: This study aimed to evaluate the antimicrobial activity of *ohelo* berry extract against *L. monocytogenes* and *Escherichia coli* O157:H7 in culture medium and whole milk in comparison with cranberry extract.
Methods: Growth potential, physicochemical properties (auto-aggregation, hydrophobicity, and motility), and biofilm formation capability of *L. monocytogenes* and *E. coli* O157:H7 were evaluated in the presence of *ohelo* berry or cranberry extracts. Additionally, the antimicrobial properties of *ohelo* berry and cranberry extracts were compared in artificially inoculated whole milk through 7-day incubation at 7°C.
Results: *E. coli* O157:H7 was more sensitive to cranberry extract than *ohelo* berry extract, as indicated by lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). *Ohelo* berry extract showed lower MIC and MBC against *L. monocytogenes* than cranberry extract. The sub-inhibitory concentrations (1/2 MIC) of both extracts significantly increased the auto-aggregation and hydrophobicity, and decreased the swimming motility, swarming motility, and biofilm formation capability of *L. monocytogenes* and *E. coli* O157:H7 in varying degrees. The *E. coli* O157:H7 was eliminated by both extracts at 4× MBC in whole milk within 2 to 4 h. The *ohelo* berry extract at 4× MBC could inhibit the growth of *L. monocytogenes* in whole milk, while cranberry extract at 4× MBC was able to kill *L. monocytogenes* within 12 to 24 h.
Significance: These findings highlight the potential of *ohelo* berry as a natural preservative and a functional food to prevent pathogenic bacterial infection.

P2-22 *Limosilactobacillus reuteri* Amoxicillin Resistance Differs as a Function of Strain Host

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Introduction: *Limosilactobacillus reuteri* is commonly found in the vertebrate intestinal tract. Select strains are deployed as probiotics in humans and agriculture. We hypothesized *L. reuteri* amoxicillin resistance levels are dependent on host origin potentially as a function of the degree to which each host was exposed to amoxicillin treatments.
Purpose: This study estimated and compared the minimum inhibitory concentrations (MIC) of amoxicillin for *L. reuteri* strains isolated from different hosts including human, chicken, rat, murine, and porcine.
Methods: Three *L. reuteri* strains per host species were used to make glycerol stocks (3:7 v/v, 50% glycerol:cultured strains). Subcultures with an optical density of 1 were centrifuged with pellets suspended in phosphate-buffered saline (PHS). The re-suspended culture was mixed with PHS (1:1), with 100µl of the solution plated onto an MRS plate. Plates were incubated overnight in a hypoxic chamber (5% CO_2, 2% O_2) with a gradient amoxicillin test strip (Liofilchem™, Italy) in the center of each plate. The MIC was recorded at the intersection of the line of the zone of inhibition and the strip on the plate. The effect of host source on MIC values was evaluated using ANOVA ($p<0.05$) and means were compared using Tukey pairwise comparisons.
Results: The average MIC of *L. reuteri* strains isolated from a human host was significantly higher than the four animal sources (MIC of 3.6 µg/mL compared to 1.6, 0.44, 0.43, 0.30 ug/ml and $P < 0.05$). Mean amoxicillin MICs of strains isolated from chicken, porcine, rat, and murine hosts were not different from each other.
Significance: Our preliminary findings provided insight in the putative impact of host-specific antibiotic exposure on antibiotic resistance.

P2-23 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 mixture of foodborne pathogens (*Bacillus cereus strains* NCCP14043, NCCP14796, NCCP15909, NCCP15910, NCCP16296, *Escherichia coli* strains NCCP14043, NCCP14796, NCCP15909, NCCP15910, 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 *E. coli* strains. The sizes of the clear zones were finally 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 acitivties, and the antimicrobial activities can be induced by bacteriocin productions.

P2-24 Targeted Genome Mining Reveals the Psychrophilic *Clostridium estertheticum* Complex as a Potential Source for Novel Bacteriocins, Including Cesin A and Estercticin A

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial resistance in pathogenic bacteria is considered a major public health issue necessitating the discovery of alternative antimicrobial compounds. In this regard, targeted genome mining in bacteria occupying under-explored ecological niches has the potential to reveal such compounds, including bacteriocins.

Poster

Purpose: Determine the bacteriocin biosynthetic potential of the psychrophilic *Clostridium estertheticum* complex (CEC) through a combination of genome mining, phenotypic screening and biochemical analysis.

Methods: The genome mining was performed in 40 CEC genomes using antiSMASH. The production of bacteriocin-like compounds was phenotypically validated through agar well (primary screening) and disk diffusion (secondary screening) assays using cell free supernatants (CFS) and partially purified extracts, respectively. Stability of four selected CFS against proteolytic enzymes, temperature and pH was determined while one CFS was analyzed by HRMS and MS/MS to identify potential bacteriocins.

Results: Twenty novel bacteriocin biosynthetic gene clusters (BBGC), which were classified into eight (six lantibiotics and two sactipeptides) distinct groups, were discovered in 18 genomes belonging to *C. estertheticum* (*n* = 12), *C. tagluense* (*n* = 3) and genomospecies2 (*n* = 3). Primary screening linked six BBGC with narrow antimicrobial activity against closely related clostridia species. All four preselected CFS retained activity after exposure to different proteolytic, temperature and pH conditions. Secondary screening linked BBGC1 and BBGC7 encoding a lantibiotic and sactipeptide, respectively, with activity against *Bacillus cereus* while lantibiotic-encoding BBGC2 and BBGC3 were linked with activity against *B. cereus*, *Staphylococcus aureus* (methicillin-resistant), *Escherichia coli* and *Pseudomonas aeruginosa*. MS/MS analysis revealed that *C. estertheticum* CF004 produces cesin A, a short natural variant of nisin, and HRMS indicated the production of a novel sactipeptide named estercticin A.

Significance: We have shown the CEC, in particular *C. estertheticum*, is a source of novel and stable bacteriocins that have activities against clinically relevant pathogens.

P2-25 Polyphenolic Compounds Isolated from Tea (*Camellia sinensis* (*L.*) O. Kuntze) Showed Antibacterial and Inhibitory Potential Against Cell Division Protein FtsZ of *Bacillus cereus*

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Introduction: *Bacillus cereus* is a pathogen that contaminates a wide range of food products, thereby causing intestinal illness. The study of natural compounds to control bacterial pathogens is a current trend. Studies on phytopolyphenolics using computational and *in vitro* approaches offer an opportunity to discover bioactive compounds with antibacterial potential.

Purpose: To evaluate 21 Tea polyphenolic compounds as antibacterials, and as inhibitors of the cell division protein FtsZ of *B. cereus*, *in silico* via molecular docking.

Methods: For the antibacterial assay, minimum bactericidal and inhibitory concentrations (MBCs and MICs) of the polyphenolic compounds were determined at 37°C by the microdilution method. FtsZ inhibitory potential of polyphenolics was determined via molecular docking using PyRx and Discovery Studio software.

Results: Eleven out of 21 polyphenolic compounds showed antibacterial activity against *B. cereus*. MICs and MBCs of the compounds ranged from 0.5 to 1.85 mg/ml and 0.53 to 1.9 mg/ml, respectively. Olonghomobisflavan A, oolonghomobisflavan B, and theaflavin 3,3′-digallate exhibited the lowest MICs <0.52 mg/ml, hence, they are the most effective of polyphenolics to inhibit the growth of *B. cereus* ($p \leq 0.05$). The lowest MBC was exhibited by theaflavin 3,3′-digallate (0.53 mg/ml), whereas the highest was for theasinensin B (1.9 mg/ml). Molecular docking results revealed that theaflavin-3, 3′-digallate and olonghomobisflavan A as the best potential inhibitors of FtsZ, based on their strong hydrogen bond interactions with the key residues of FtsZ interdomain cleft (IDC) at binding affinity energies of -10.1 kcal/mol and -8.6 kcal/mol, respectively in comparison with PC190723 (control inhibitor). The correlation between the antibacterial and *in silico* studies suggests FtsZ inhibition as one of the mechanisms of actions of the polyphenolics. However, further experimental studies are required to establish the FtsZ inhibitory potentials of theaflavin-3,3′-digallate and olonghomobisflavan A.

Significance: The information generated in this study will expand the existing data on these polyphenolic compounds as natural antimicrobials and their potential use in food particularly to control *B. cereus*.

P2-26 Characterization of a Novel Anti-*Listeria* and Anti-*Campylobacter* Bactericidal Protein

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Introduction: A species of sporulating bacteria isolated from poultry fecal material exhibited bactericidal control of *Listeria monocytogenes* and *Campylobacter jejuni*.

Purpose: The purpose of this study was to characterize the bactericidal substance produced by the *Listeria*- and *Campylobacter*-inhibitory bacterial isolate (strain NH).

Methods: The bacterium was isolated on starch agar from a culture of rooster cecal droppings. The 16s ribosomal gene V4 region was sequenced in both directions. Six biological replicates of competitive exclusion cultures were run in parallel, and differences in means were evaluated using a 2-tailed Student's t-test. The inhibitory substance was partially purified from cell-free culture supernatant using a combination of ammonium sulfate precipitation, cation exchange chromatography, and ultrafiltration. The substance was further characterized by isoelectric focusing and SDS-PAGE.

Results: *Listeria monocytogenes* and *Campylobacter jejuni* were significantly reduced to undetectable levels (<1.0 log CFU/ml) in mixed cultures with strain NH by 24 hours post-inoculation, compared to growth of >9.1 log CFU/mL in the absence of strain NH ($P < 0.01$). Sequencing indicated isolate NH is a member of the Paenibacillaceae family. Size determination by gel filtration chromatography indicated a size of ca. 50 kDal for the active substance. Only minimal activity passed through 30 kDal MWCO, and was fully retained by, 10 kDal MWCO ultrafiltration devices. The bactericidal activity bound strongly to a cation exchange resin but only minimally to anion exchange resin in the pH range 4.5 – 8.5. Isoelectric focusing indicated an isoelectric point of 10.6 for the bactericidal substance. SDS-PAGE indicated that an ~ 11 kDal protein was enriched in the active fraction from the cation exchange column.

Significance: Both *Listeria monocytogenes* and *Campylobacter jejuni* populations were reduced significantly in the presence of strain NH. The bactericidal protein characterized in this research may have potential as a novel post-harvest biopreservative of foods to control *Listeria* or *Campylobacter*.

P2-27 Inhibitory Effect of Aqueous Extracts of Commercial Pomegranate Products Against Enterohemorrhagic *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: Several phytochemicals including polyphenols are found in both edible and inedible part of the pomegranate fruit. Pomegranate peels, a byproduct of pomegranate juice extraction, contain high concentrations of ellagitannins which is a type of polyphenol with antimicrobial activities.

Purpose: This study compared the efficacy of aqueous extracts of selected pomegranate peel and juice products in inhibiting the growth of enterohemorrhagic *Escherichia coli* (EHEC).

Methods: Ten aqueous extracts of five commercial pomegranate products were obtained by two different methods (concentrated/ unconcentrated). Cell suspension of each of two tested EHEC strains (*E. coli* O157:H7 4546 and *E. coli* O157:H7 4492) at 10^5 CFU/ml was added into tryptic soy broth (TSB) or phosphate buffered saline (PBS) amended with 9% or 23% of each extract. After treatment for 5 h, 10 h, 24 h at 25 °C, reduction of EHEC population in relation to the controls were determined using standard plate count assay. Results were analyzed using Fishers Least Significance Test of the Statistical Analysis Software.

Poster

Results: Results showed that the reduction of EHEC population in TSB was significantly higher ($P \leq 0.05$) than that in PBS. *E. coli* O157:H7 4546 had a significantly higher ($P \leq 0.05$) population reduction than *E. coli* O157:H7 4492 in PBS and a numerically lower ($P > 0.05$) reduction in TSB. The extracts from the three powdered peels had significantly greater activity ($P \leq 0.05$) in reducing EHEC population (1.38–1.98 Log CFU/ml) than the extract from the whole peel (0.92 Log CFU/ml) and juice powder (0.73 Log CFU/ml). Higher dose of extracts resulted in a greater reduction in EHEC population in TSB (2.48 Log CFU/ml) or PBS (0.24 Log CFU/ml) than system with lower dose of the extracts. The level of EHEC population reduction correlated positively with the polyphenolic content (R^2=0.56–0.83) and titratable acidity (R^2=0.65–0.89) in the treatment systems.

Significance: Study suggests that pomegranate peels contain promising natural phytochemicals with antimicrobial properties that can help to control pathogens like EHEC.

P2-28 Green Synthesis of Silver Nanoparticles Using *Terrabacter humi* sp. Nov. and Their Antimicrobial Activity and Mechanisms Investigation Against Foodborne Pathogens

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Introduction: Food-borne pathogens are a major threat for food safety and human health and it is essential to develop and discover alternative eco-friendly antibacterial agents due to the emergence of multi-drug-resistant pathogens.

Purpose: In this study, we isolated and characterized a novel bacterium named *Terrabacter humi* MAHUQ-38^T, utilized for the rapid, facile and eco-friendly synthesis of silver nanoparticles (AgNPs) and the synthesized AgNPs were used to control multi-drug-resistant food-borne pathogens.

Methods: The culture supernatant of strain MAHUQ-38^T was used for biosynthesis of AgNPs. The biosynthesized AgNPs were characterized by FE-TEM, SAED, EDS, XRD, DLS and FT-IR spectroscopy. Disc diffusion assay, broth dilution assay and FE-TEM were used to investigate the antibacterial activity and mechanisms against food-borne pathogens.

Results: The novel bacteria *Terrabacter humi* MAHUQ-38^T has a genome of 5,156,829 bp long (19 contigs) with 4555 protein-coding genes, 48 tRNA and 5 rRNA genes. AgNPs synthesized by *Terrabacter humi* exhibited highest absorbance at 413 nm and the TEM image revealed spherical shape with 6 to 24 nm in size. The green synthesized AgNPs showed strong anti-microbial activity against all tested pathogenic microbes. Minimal inhibitory/bactericidal concentrations against *Escherichia coli* and *Pseudomonas aeruginosa* were 6.25/50 and 12.5/50 µg/mL, respectively. Our data revealed that synthesized AgNPs created morphological changes and harmed the membrane integrity of *P. aeruginosa* and *E. coli*.

Significance: This study encourages the use of *Terrabacter humi* for the ecofriendly synthesis of AgNPs to control multi-drug-resistant food-borne pathogenic bacteria.

P2-29 Distribution of Antibiotic-Resistant *Escherichia coli* in the Brazilian Dairy Production Chain

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Introduction: Antibiotics are widely used on dairy farms to control mastitis and other diseases. Although their impacts are unclear, abusive antibiotics use can select resistant bacteria, a worldwide concern. Thus, surveillance of antibiotic resistance in the dairy production is important to control this hazard. *Escherichia coli* are good indicators of the selective pressure imposed by antibiotics in animals.

Purpose: We aimed to characterize the antibiotic resistance of *E. coli* obtained from different points of a Brazilian dairy production chain.

Methods: *E. coli* isolates (n = 1,152) were obtained from raw milk (n = 34), water (n = 2), feces from lacting cows (n = 665), calves (n = 325) and humans (n = 120) and dairy products (n = 6), on 20 dairy farms and in 2 dairy industries located in Southern and Southeastern Brazil. Isolates were evaluated for resistance to six antibiotics (amoxicillin, ceftiofur, ciprofloxacin, chloramphenicol, sulfatriaxone and tetracycline) by the agar dilution method at each antibiotic breakpoint, based on CLSI. *E. coli* ATCC 25922 was included as a pansusceptible control.

Results: Multidrug resistance was identified in 74 (6.4%) isolates, while 155 (13.5%) were resistant to amoxicillin, 35 (3.0%) to ceftiofur, 26 (2.3%) to ciprofloxacin, 51 (4.4%) to chloramphenicol, 89 (7.7%) to sulfatriaxone and 205 (17.8%) to tetracycline. Calves and human feces presented the highest rates of resistance, especially to tetracycline (n = 94, 28.9%, and n = 18, 15.0%, respectively) and amoxicillin (n = 70, 21.5%, and n = 19, 15.8%, respectively). Also, 89 isolates (13.4%) from cow feces were resistant to tetracycline.

Significance: The distribution and relatively high frequencies of resistance to amoxicillin and tetracycline highlight the need for surveillance of this hazard in the dairy production chain. Acknowledgments: CAPES, CNPq, FAPEMIG, 3M.

P2-30 Using P100-Like Phage CKA15 to Degrade *Listeria monocytogenes* Mono-Species Biofilm Grown under Simulated Food Processing Conditions

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Introduction: Biofilms containing *Listeria monocytogenes* in food processing environments represent a public health risk and are costly to the food industry. Biofilm bacteria are more resistant to industrially used sanitizers and can be a source of contamination of finished food products. A sanitation strategy complementary to current practices is to exploit bacteriophages to kill specific pathogenic bacteria.

Purpose: The purpose of this study was to evaluate the use of phage CKA15 to degrade *L. monocytogenes* biofilm in simulated food processing conditions.

Methods: *L. monocytogenes* (ATCC19111) cells were grown on stainless steel (ss) coupons in a CDC bioreactor for five days. The growth medium (dilute ultra-high temperature treated milk), incubation temperature (15°C), as well as cycles of feeding, washing, and desiccation were selected to mimic the conditions in a small-scale dairy plant. Phage was added once to five-day old biofilms at a titer of log8 PFU/ml and a second time at the same titer after a 48-hour period of desiccation corresponding to a weekend shutdown.

Results: The mean CFU/cm^2 counts on the phage-treated coupons were 55% of the counts on the untreated coupons 168 hours post-treatment (p value 0.0078). The low degree of efficacy observed compared to previous 96-well plate crystal violet and viability assays led us to investigate possible reasons for the discrepancy. Kinetic adsorption assays conducted with planktonic and resuspended sessile bacteria allowed us to rule out lower adsorption rates for sessile bacteria. Similarly, adsorption assays conducted with dilute milk medium showed that macromolecules present in the medium were not interfering with adsorption. However, a follow-up experiment showed that addition of solution to biofilms grown on ss coupons can resuspend bacterial biomass. This would lead to preferential phage adsorption to resuspended rather than attached bacteria.

Significance: We suggest wash steps be included before phage addition to ensure that phage is targeted at attached bacteria.

P2-31 Antimicrobial Activity of Citral Nanoemulsions Against *Listeria monocytogenes* in Fresh-Cut Papaya Stored at Different Temperatures

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Introduction: Fresh-cut papaya has been linked to listeriosis outbreaks worldwide. Citral has anti-listeria activity, but its use in ready-to-eat fruits is often limited due to its high volatility, intense flavor, and low solubility. The use of citral nanoemulsions can overcome some limitations, but its anti-listerial effects on these products remain unclear.

Purpose: To determine the antimicrobial effects of citral nanoemulsions (CNE) against *L. monocytogenes* C1-387 on fresh-cut papaya during storage at different temperatures.

Methods: Minimum inhibitory concentration of CNE obtained by emulsion phase inversion was 0.6 µL/mL. Sanitized papaya where peeled, cut in 1x1 cm pieces and spot inoculated with *L. monocytogenes* (5 log cfu/g). After spots have dried, samples were immersed in CNE (0.3 and 0.15 µL/mL) for 1 min and stored at 4, 8, 12, and 16 °C for 7 days. *L. monocytogenes* was enumerated by plating on *Listeria* Selective Agar every 24 h (1.5 log cfu/g detection limit).

Results: After 24 h, *L. monocytogenes* counts decreased ~3 log cfu/g in fresh-cut papaya treated with 0.3 µL/mL CNE stored at 4 and 8 °C. Lower reduction ($p<0.05$) was observed in samples stored at 12 and 16 °C. No viable cells were found in samples stored at 4 and 8 °C after 3 days, or at 12 and 16 °C after 5 days. When papaya was treated with 0.15 µL/mL CNE, longer time was needed to achieve the same *L. monocytogenes* reductions, regardless of storage temperature. After 5 days, no viable counts were found in fresh-cut papaya treated with 0.15 µL/mL CNE stored at 4, 8, and 12 °C, but for samples stored at 16 °C, *L. monocytogenes* counts were ~1.9 log cfu/g.

Significance: Citral nanoemulsions might reduce *L. monocytogenes* in fresh-cut papaya during storage and could be further explored for its preservation. The concentration to be used should consider the storage temperature.

P2-32 A Novel Cultured Sugar Antimicrobial Controls *Listeria monocytogenes* in Deli-Style Turkey

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Introduction: The Canadian Food Inspection Agency has promulgated rules that buffered vinegar-containing products need to be labeled with the associated buffering system beginning November 2022. A novel cultured sugar antimicrobial ("NCS") has been developed to control *Listeria* in ready-to-eat (RTE) food products as an alternative to buffered vinegar.

Purpose: To validate the control of *L. monocytogenes* in vacuum-packed uncured RTE deli-style turkey containing NCS.

Methods: Uncured, deli-style turkey was prepared without antimicrobial (negative control; "NC"), or containing 1.3% NCS ("NCS"). Samples were inoculated with a 5-strain cocktail of *L. monocytogenes* (ca. 2.5 log CFU/g) or left uninoculated, vacuum packaged, and stored at 3.3°C until enumeration. Upon enumeration, samples were transferred to a sterile stomacher bag to which a 1:2 weight of buffered peptone water was added. The sample-diluent mixture was stomached and serial dilutions were performed using Butterfield's buffer. Inoculated sample-diluent mixtures were spread plated on Modified Oxford agar (35°C for 48 h) to enumerate *L. monocytogenes* and non-inoculated product was spread plated on de Man, Rogosa, and Sharpe agar (30°C for 48 h) to enumerate lactic acid bacteria (LAB).

Results: Samples were analyzed for 145 d. Both treatments had similar starting *L. monocytogenes* populations of ca. 2.5 log CFU/g. *L. monocytogenes* reached 1-log outgrowth in ca. 9 d and 2-log outgrowth in ca. 11 d in NC. When applying NCS, time to 1-log outgrowth was ca. 105 d, and time to 2-log outgrowth was ca. 145 d. Populations were significantly lower ($P < 0.05$) when applying treatment compared to NC beginning at day 7. LAB were controlled (no outgrowth) throughout the entire shelf-life.

Significance: Application of NCS can serve as an alternative to buffered vinegar and control *L. monocytogenes* in RTE products such as uncured deli-style turkey.

P2-33 Powdered Vinegar Inhibits Growth of *Listeria monocytogenes* in Fully-Cooked Pork Sausage Stored Under Vacuum

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Introduction: *Listeria monocytogenes* remains a risk in refrigerated food products. Verdad® Powder N6 (VP) is a vinegar powder antimicrobial that has been shown to extend the shelf life of refrigerated foods by inhibiting *L. monocytogenes* growth.

Purpose: To validate the control of *L. monocytogenes* in vacuum-packed sausage patties containing vinegar powder.

Methods: Pork sausage patties were prepared without antimicrobial (negative control; "NC"), 0.55% VP ("VP-1"), or 0.80% VP ("VP-2"). Sausage was inoculated with a 5-strain cocktail of *L. monocytogenes* (target ca. 2.5 log CFU/g) or left uninoculated, vacuum packaged, and stored at 4.4°C. Upon enumeration, samples were transferred to a sterile stomacher bag to which a 1:2 weight of buffered peptone water was added. The sample-diluent mixture was stomached and serial dilutions were performed as necessary. Inoculated sample-diluent mixtures were spread plated on Modified Oxford agar (35°C for 48 h) to enumerate *Listeria* and non-inoculated product was spread plated on de Man, Rogosa, and Sharpe agar (30°C for 48 h) to enumerate innate lactic acid bacteria (LAB).

Results: Samples were analyzed for 49 d. All treatments had similar starting *L. monocytogenes* populations of ca. 2.7 log CFU/g. NC realized 1-log outgrowth of *L. monocytogenes* in 15 d and 2-log outgrowth in 22 d. Both VP-1 and VP-2 inhibited outgrowth of *L. monocytogenes* (<1-log outgrowth) for the study duration. Populations were significantly lower ($P < 0.05$) in treatments compared to control by day 12. No LAB outgrowth was observed for all treatments.

Significance: Control of *L. monocytogenes* can be achieved in pork sausage patties by application of VP (Verdad® Powder N6).

P2-34 Withdrawn

P2-35 The Effects of *Ohelo* Berry (*Vaccinium calycinum*) Fractions on Growth Potential, Physicochemical Properties and Biofilm Formation of *Escherichia coli* O157:H7

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❖ ***Developing Scientist Entrant***

Introduction: The health benefits of berries have been attributed to various active ingredients, such as polysaccharides, phenolic acids, flavonoids, and anthocyanins. Previous studies have revealed that *ohelo* berry (*Vaccinium calycinum*), a Hawaiian wild relative of cranberry (*Vaccinium macrocarpon*), is rich in bioactive compounds.

Purpose: This study aimed to evaluate the antibacterial effects of *ohelo* berry extract and its fractions on *Escherichia coli* O157:H7.

Methods: *Ohelo* berry crude extract (F0) was fractionated into sugar plus organic acids (F1), non-anthocyanin phenolics (F2), and anthocyanins plus proanthocyanidins (F3). The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of each fractional component against *E. coli* O157:H7 were determined using a two-fold-serial dilution method. Growth potential, physicochemical properties (auto-aggregation, hydrophobicity, and motility), and biofilm formation capability of *E. coli* O157:H7 treated with each fraction at a sub-inhibitory concentration of 1/2 MIC were investigated.

Results: F0 was found to exhibit the greatest antimicrobial activity against *E. coli* O157:H7, with MIC and MBC being 3.12% and 12.5%, respectively. The F1 and F2 in *ohelo* berry were the major components that contribute to its antimicrobial properties. The sub-inhibitory concentrations of all fractions were able to increase the auto-aggregation and hydrophobicity, and decrease the swimming motility, swarming motility, and biofilm formation capability of *E. coli* O157:H7 in varying degrees. F0 caused the most significant decrease in auto-aggregation and hydrophobicity by 3.96% and 76.15%, respectively, and the most significant increase in swimming and swarming motility by 95.23% and 80.63%, respectively. F2 led to the most significant reduction of biofilm formation by 95.63%.

Significance: These findings highlight the potential of *ohelo* berry extract and fractions as nutritional supplements for enhancing food safety and human health.

P2-36 High-Throughput Screening of Essential Oils Against *Listeria monocytogenes* on Deli Ham

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❖ ***Developing Scientist Entrant***

Introduction: Novel plant essential oils are continually proposed as potential clean-label strategies to control *L. monocytogenes* in deli-ham. However, there are not modern methods to screen large numbers (>5) of antimicrobials directly on food surfaces.

Purpose: This study implements a high-throughput, bioluminescence screening tool to identify antimicrobial treatments to control growth of *L. monocytogenes* on deli-ham.

Methods: Ham was made, sliced, punched with a circular die, and placed into microtiter plates. Bioluminescent *L. monocytogenes* Xen19 was inoculated on the surface of the hams at ~6 log(CFU/g). A library of 33 essential oils was tested directly on the surface of the ham at 500 ppm. Oils were emulsified in peptone buffered water by sonication before each treatment. Oils were applied individually to the hams, in triplicate. On day 12, light emission (Relative Light Units [RLUs] at 400-750 nm) was read directly from the surface of the ham disks as indirect measurement of *L. monocytogenes* growth. Oils that limited *L. monocytogenes* growth as measured by less light emission than non-treated hams were considered promising antimicrobial compounds.

Results: Only black pepper, cassia, clove bud, cinnamon, cumin seed, cognac, coumarin, eucalyptus, garlic, ginger, and peru oil each significantly reduced light emission of *L. monocytogenes* Xen 19 compared to no-treatment controls (controls increased 1.8±0.3 log RLU after 12 days at 4ºC). The remaining 23 oils had RLU levels indistinguishable from the no-treatment control and, therefore, were considered as non-effective antimicrobial compounds.

Significance: This method is a screening tool to distinguish between effective and non-effective antimicrobial compounds and could be implemented in other food matrices in which *L. monocytogenes* growth is relevant. The oils that were identified as effective will be further studied for possible synergistic interactions that may potentiate their antimicrobial activity on deli-ham.

P2-37 B-Cyclodextrins and Lipidic Dispersions as Encapsulating Vectors of Oregano (*Origanum vulgare* L.) Essential Oil: Characterization and Evaluation of Their Antimicrobial Activity Against *Listeria monocytogenes* in Broth and Model Food

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Introduction: The hydrophobicity, strong flavor and high volatility of essential oils impede their commercial application in foods.

Purpose: To study the encapsulation of Oregano essential oil (OEO) in β-cyclodextrin (β-CD) and lipidic dispersions to enhance dispersion and controlled release of OEO and evaluate their effect on the survival of *Listeria monocytogenes* in Tryptic Soy Broth (TSB) and cheese broth.

Methods: The encapsulation of OEO in β-CD was performed with the co-precipitation method, whereas for the incorporation of OEO into lipidic dispersion the thin-film hydration method was applied. Size distribution(PDI) and zeta-potential(ζ) of the formed inclusion complexes(IC) were assessed by Dynamic Light Scattering(DLS). Determination of encapsulation efficiency (EE%) and release rate of the formed ICs was performed by UV-Vis spectroscopy. The effect of ICs and free OEO at MIC, ½ MIC and 2xMIC against *L. monocytogenes* (3-strain cocktail: 10^6 CFU/ml), was determined in TSB (pH 7.0, 4.3) and "katiki" cheese broth (pH 4.3) at 7ºC.

Results: The complexes presented nanoscale size (<1000nm), 127.24±25.83 nm with an intensity of >90% for OEO-β-CD and 106.84 ±42.81 and 948.05 ±409.95nm with intensity of 47% and 51.9%, respectively for OEO-lipidic dispersion. The size dispersion was 0.433 ±0.064 nm for OEO-β-CD and 0.872 ±0.070 nm for OEO-lipidic dispersion. An acceptable stability was observed (>20 mV) with ζ-potential -23.57±6.76 mV for OEO-β-CD and -49.5±6.18 mV for lipidic dispersion. EE% was 80.3%±4.0 and 87.8±3.7 for OEO-β-CD and OEO-lipidic dispersion, respectively. The OEO release from both complexes occurred continuously for 18 days. The dose-dependent inhibitory effect of encapsulated OEO against *L. monocytogenes* was higher in TSB than in cheese-broth and increased at lower pH. Lipidic dispersion was more effective than OEO-β-CD at both pH values. The antimicrobial effect of encapsulated OEO was not as rapid as the effect of free EO, confirming the slow release of EO due to encapsulation.

Significance: The results are indicative for the application of encapsulated OEO to control of *L. monocytogenes* in foods.

P2-38 Biofilm Formation of Pathogenic *Listeria monocytogenes* and *Salmonella* Serovars and Non-Pathogenic *L. innocua* and *Salmonella* LT2 and Their Inactivation Using Industrial Antimicrobials

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Introduction: Various epidemiologically significant serovars of non-typhoidal *Salmonella enterica* and public health significant serotypes of *Listeria monocytogenes* continue to be important concerns in primary and further processing of an array of food commodities.

Purpose: Current study investigated the effects of sodium hypochlorite, 5% acetic acid, and quaternary ammonium compounds against planktonic cells and biofilms of pathogenic *Listeria monocytogenes* and *Salmonella* serovars as well as non-pathogenic *L. innocua* and *Salmonella* LT2.

Methods: This study examined the effects of the above-mentioned sanitizers (one-minute submersion) on day 0 (planktonic cells) and against one- and two-week mature biofilms of the pathogens. Two separate four-strain mixtures of wild-type *Listeria monocytogenes* and non-typhoidal *Salmonella* serovars were used in this study in addition to two separate single strains of *L. innocua* and *Salmonella* LT2. Trials were randomized complete block designs and were statistically analyzed using ANOVA followed by Tukey-adjusted means separation.

Results: Our results indicate that tested antimicrobials are very efficacious to eliminate the pathogen at the planktonic state from steel surfaces, exhibiting >3 logs CFU/cm^2 reductions ($P < 0.05$) of the inocula. However, overall, 5% acetic acid was less effective ($P < 0.05$) compared to sodium hypochlorite and quaternary ammonium compounds. In contrast, all three commercial antimicrobials exhibited low decontamination efficacy against sessile cells of pathogenic and non-pathogenic bacteria. Additionally, we observed that the non-pathogenic surrogates for *Listeria monocytogenes* and non-typhoidal *Salmonella* had comparable biofilm formation capability and sensitivity to sanitizers and thus could be used interchangeably in public health microbiological hurdle validation studies.

Significance: Our study indicates that the treatments that are validated against planktonic cells of microbial pathogens do not have the same efficiency when tested against microbial biofilms. Thus, bacterial biofilms have to be considered carefully for validated SSOP's in food manufacturing and healthcare facilities to ensure the public's health.

P2-39 Emergence of the Plasmid-Borne Mobile Colistin-Resistance Gene, *Mcr-1*, in *Escherichia coli* Isolated from Lebanese River Water Used in Irrigation

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❖ ***Developing Scientist Entrant***

Introduction: Surface water used for irrigation plays an important role in the safety of produce. Fecally contaminated waters can be a reservoir for pathogens and contribute to the emergence and dissemination of antibiotic-resistant (ABR) bacteria and genes. Resistance to colistin, a last-resort antibiotic, has been increasingly detected in environmental samples. However, reports on the detection of the plasmid-borne mobile colistin resistance gene (*mcr*) in surface water are limited

Purpose: This study aimed to detect and characterize *mcr*-carrying *Escherichia coli* in water used for irrigation in Lebanon.

Methods: Freshwater samples (n=135) were aseptically collected from the 14 major rivers across Lebanon. Samples were filtered and incubated on RAPID' *E. coli* 2 agar supplemented with 4 µg/ml of colistin. A total of 116 colonies (1-3 colonies per sample) were collected and tested for (*mcr-1* to *mcr-8*) and other ABR genes by PCR. Disc diffusion and broth microdilution (MIC) assays were used to assess resistance profiles against eighteen important antibiotics and determine colistin MIC, respectively. Plasmid types were determine using a PBRT Kit. Transformation, survivability, and BOX-PCR were used to further characterize the *mcr*-carrying isolates.

Results: Colistin-resistant *E. coli* was detected in 95% of freshwater samples (n=128). All isolates (n=116) were *mcr-1*-positive and negative to other tested *mcr* genes. *mcr-1* was identified in 13 of the rivers. The majority of the *E. coli* (91%) were classified as multi-drug resistant and the colistin MIC ranged between 4-64 µg/ml. Various plasmid Inc types (IncI2α, IncX4, IncFII) were identified, and *mcr-1*-carrying plasmids were successfully transferred to naïve *E. coli*. The *mcr-1*-positive isolates were genotypically diverse.

Significance: The high prevalence of MDR *mcr-1*-positive *E. coli* in Lebanese river water poses a significant threat to human health and produce safety. Actions and policies addressing antibiotic use are required to mitigate the potential spread of antibiotic resistance in the food chain in Lebanon.

P2-40 Stress Response and Survivability of *Listeria monocytogenes* Affected by Sublethal Concentrations of Bactericidal Antibiotics

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is ubiquitous and gram-positive bacteria found in various environmental niches that cause listeriosis and has excellent adaptability to external changes and attack. Impact and stress response of *L. monocytogenes* by environmental stress attracts attention because it confers resistance against food processing environments and eventually make it survive in food processing condition.

Purpose: The goal of this study was to evaluate stress response of *L. monocytogenes* affected by bactericidal antibiotics.

Methods: Two bactericidal antibiotics (gentamicin and vancomycin) were used as an external stimulus factor. *L. monocytogenes* was grown together with sublethal concentrations of each antibiotic for 24 h and cell pellet was obtained. Mutation frequency changes, biofilm forming ability, extracellular polymeric substances [EPS] production, and viability under food-associated stress of L. monocytogenes was tested.

Results: Sublethal concentration of bactericidal antibiotics (gentamicin and vancomycin) increased mutation frequency of *L. monocytogenes*. Antibiotic-induced-mutated (AIM) *L. monocytogenes* showed slow growth than non-mutated *L. monocytogenes*, while biofilm forming ability was higher than non-mutated *L. monocytogenes*. The production of EPS (protein and polysaccharide) was also higher in AIM *L. monocytogenes*. When AIM *L. monocytogenes* was exposed to food-associated stress, it showed high survivability in thermal, acidic, and osmotic stress than non-mutated *L. monocytogenes*.

Significance: These results demonstrate that AIM *L. monocytogenes* has superior adaptability to environment even in food-associated stress and can threat food safety.

P2-41 *Escherichia coli* O157:H7 and *Listeria monocytogenes* Control on Blueberries by Chlorine Dioxide and Muscadine Grape Extract

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Introduction: Berries have been linked to 6% of foodborne produce outbreaks where *Escherichia coli* 0157:H7 and *Listeria monocytogenes* are among the leading cause. Previous work showed that chlorine dioxide (ClO_2) and muscadine grape extract (MGE) were effective at reducing these pathogens in broth and on spinach (1 to >4 log reductions). Therefore, they could be suitable antimicrobial alternatives for the produce industry.

Purpose: To determine the antimicrobial activity of MGE and ClO_2 against *Escherichia coli* 0157:H7 and *Listeria monocytogenes* on inoculated blueberries.

Methods: Blueberries were inoculated with a 3-strain cocktail per pathogen to reach an initial concentration of 5 log CFU/g. MGE was diluted in water or coating solution. The pathogen reduction was evaluated after exposure to treatments (water, water + coating, 3 ppm ClO_2, 300 mg/ml MGE, 3 ppm ClO_2 + 300 mg/ml MGE diluted in coating, and water + 300 mg/ml MGE diluted in coating) for 10 min + 30 min dry time + storage at 8°C for up to 6 days. Microbial enumeration was done using overlaid (CT-SMAC or MOX) plates incubated at 37°C for 24 h.

Results: On average, water and water + coating (controls) treatments had the highest ($P \leq 0.05$) pathogen counts throughout the study. For *E. coli* 0157:H7, 3 ppm ClO_2 + 300 mg/ml MGE reported similar counts to MGE alone but lower ($P \leq 0.05$) than ClO_2 alone at day 0, achieving a 4-log reduction. No further differences existed among the treatments. However, reductions persisted >3 log CFU/g for the remaining days. For *L. monocytogenes*, 3 ppm ClO_2 + 300 mg/ml MGE reported the lowest ($P \leq 0.05$) counts on days 1, 4, and 6, leading to surface reductions >4.1 log CFU/g.

Significance: MGE, alone or combined, with 3 ppm ClO_2 could be a good candidate as postharvest treatment for blueberries to reduce the presence of foodborne pathogens.

P2-42 Antibiotic Resistance Could Influence Lactic Acid Tolerance in Shiga-Toxin Producing *E. coli*

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Introduction: Antibiotic resistance in pathogenic bacteria is a global public health threat. Bacterial resistance mechanisms to antibiotics could result in cross-tolerance to sanitizers.

Purpose: Differences in cross-tolerance among antibiotic-resistant *E. coli* O157: H7 H1730 to lactic acid were evaluated and compared.

Methods: Antibiotic resistance to ampicillin, streptomycin or both was developed in *E. coli* O157: H7 H1730 either by incremental exposure to the antibiotics (amp C, strep C, amp C strep C) or through transformation with a GFP plasmid (pGFPuv) encoding for ampicillin resistance (amp P). Each strain was exposed to 2.5 % or 5 % lactic acid for 30 s and 300 s and surviving colonies were enumerated on TSA. reductions in bacterial population were analyzed using ANOVA and compared with appropriate tests. Experiments were conducted in triplicates.

Results: The parent strain had the highest population decrease of 2.74±0.61 log CFU/ml post exposure to 2.5 % v/v lactic acid ($p<0.05$). *E. coli* O157: H7 strains- amp C and amp P strep C had the least population decrease of 0.50±0.20 log CFU/ml and 0.17±0.16 log CFU/ml, respectively ($p<0.05$) after exposure to 2.5 % v/v lactic acid. Post exposure to 5 % v/v lactic acid, strains-strep C, NR and amp P had the highest decreases in population of 6.22±0.04 log CFU/ml, 5.92±0.07 log CFU/ml and 5.80±0.09 log CFU/ml, The highest tolerance to the 5% v/v lactic acid was observed in *E. coli* O157: H7 strain amp C followed by amp P strep C and amp C strep C with population decreases of 2.56±0.21 log CFU/ml ($P<0.05$) , 2.86±0.08 and 3.54±0.82 respectively.

Significance: The development of antibiotic resistance in *E. coli* O157: H7 H1730 could result in cross tolerance to lactic acid, a commonly used sanitizer on beef carcasses.

P2-43 Tolerance of Antibiotic-Resistant and Non-Resistant *Salmonella* Newport to Lactic Acid

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Introduction: *Salmonella* Newport is the third-most common serotype of *Salmonella* and about 25 percent of *S. Newport* submitted to NARMS is reported to be multi-drug resistant. Cross-tolerance occurs when resistance to one group or class of antimicrobials results in tolerance to other groups.

Purpose: In this study, the tolerance of *Salmonella* Newport and an ampicillin-resistant variant to lactic acid was evaluated.

Methods: Resistance to ampicillin (amp P) was developed in *S. Newport* through transformation with a GFP plasmid (pGFPuv) encoding for ampicillin resistance. Non-antibiotic resistant *S. Newport* (S.NP NR; 5 log CFU/ml) and antibiotic-resistant variant (S.NP GFP; 5 log CFU/ml) were exposed to 2.5 % or 5 % lactic acid for 30 s and 300 s. Surviving colonies were enumerated on Tryptic soy agar (TSA) after incubation at 37°C for 24 h. The MIC of lactic acid at concentrations ranging from 0.03 % to 2 % was also determined using the dilution broth method in microtiter plates. Plate counts from enumeration were converted to log CFU/ml and differences in log reductions were analyzed using ANOVA. Experiments were conducted in Triplicates.

Results: MIC of 0.3 % lactic acid was the same for all bacterial strains evaluated. Exposure to 5 % lactic acid at both exposure times reduced the bacterial population to below the limit of detection (10 cfu) but both bacterial strains survived exposure to 2.5 % lactic acid ($P<0.05$). Population reductions in 2.5% lactic acid after 30 s were 1.47±1.81 log CFU/ml for S.NP NR and 2.36±1.81 log CFU/ml for S.NP GFP. After 5 min exposure, population reductions were 2.32±0.92 log CFU/ml for S.NP and 2.64±1.09 log CFU/ml for S.NP GFP.

Significance: Understanding antimicrobial tolerance in a common pathogen like *Salmonella* Newport might be essential in understanding risk factors that could lead to the pathogen's survival.

P2-44 Comparative Genomic Analysis of Virulence, Antimicrobial Resistance, and Plasmid Profiles of *Salmonella* Enteritidis Isolated from Humans in China

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Introduction: Multidrug resistant (MDR) *Salmonella* has been a long-standing challenge in public health and food safety and MDR *Salmonella* vary by serotypes, sources, and geographical locations of isolation.

Purpose: The prevalence of MDR *S.* Enteritidis in China is significantly higher than those from the U.S. and European countries and we aim to investigate the underlying mechanisms.

Methods: A dataset of 197 *S*. Enteritidis genomes, including 16 sequenced clinical isolates from China and 181 downloaded genomes of human isolates from the U.S., Europe, and Africa, was analyzed to infer genomic diversity, virulence potential, and antimicrobial resistance.

Results: Phylogenomic analyses identified four major well-supported clades (I-IV) and the 16 China isolates resided in clade II, a geographically diverse clade, forming a localized subclade IIA. While AMR genotype profiles in the majority of clade I and IV isolates displayed pan-susceptible, 81.8% (9/11) and 22.0% (13/59) MDR prevalence was observed in clades III and II, respectively. It is noted that 77% (10/13) MDR isolates in clade II were from China. The most common AMR genes from Chinese isolates carried were *aph(3')-IIa*, $bla_{\text{CTX-M-55}}$, and $bla_{\text{TEM-1B}}$, whereas $bla_{\text{TEM-1B}}$, *sul1, sul2, drfA7, aph(3")-Ib*/*strA*, and *aph(6)-Id*/*strB* were most identified from Africa isolates (clade IV). Among the 14 plasmid types identified, IncX1 and IncFII(pHN7A8) were exclusively found in Chinese MDR isolates, while IncQ1 was highly associated with MDR isolates from Africa. All Chinese isolates carried 17 *Salmonella* pathogenicity islands (SPIs), including SPIs-1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 16, 17, 19, 23, and 24. The *spvRABCD* virulence operon was presented in 94.9% isolates (187/197) and was highly associated with IncF (IncFII and IncF1B) plasmid. Phylogenetic difference in distribution of prophage and other accessory genes was also noted.

Significance: Together these findings reinforced adaptive diversification of *S*. Enteritidis and provided new insights into the molecular mechanisms underlying diversification of the MDR *S.* Enteritidis in China.

P2-45 Investigation into Mechanism of Oil-Based Antimicrobial Compounds Against Desiccated *Salmonella* sp.

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❖ ***Developing Scientist Entrant***

Introduction: Our lab has previously demonstrated using plate-count assay that peanut oil acidified with acids such as acetic acid was highly effective against desiccated *Salmonella enterica*.

Purpose: This project analyzes the antimicrobial mechanisms of acetic acid in peanut oil against desiccated *S. enterica*.

Methods: *Salmonella* Enteritidis (ATCC-BAA-1045) was inoculated on sterile stainless-steel (10^6-10^7CFU/slide) and desiccated at 75% relative humidity at 20°C. After 20 hours, slides were treated with 0mM, 50mM and 250mM acetic acid in peanut oil (AO) at 20°C or 45°C for 30 minutes. Treated cells were recovered using glass beads in buffered TSB, stained, and visualized using confocal microscopy. Levels of injured cells were determined by the difference in plate-counts on TSA versus XLD. Live/Dead stains and CTC Redox-sensor stains were used respectively, for analysis of membrane damage and changes in cellular metabolism. Quantification of microscopy data was done using NIS-elements software (Nikon Instruments). Statistical significance was quantified using two-way ANOVA ($p \leq 0.05$).

Results: Treatment with 500mM AO resulted in 3.06±0.4 and 4.91±0.2 log reduction at 20°C or 45°C, respectively, compared to non-acidified oil control (OC, 0mM acetic acid). Optical microscopy showed lysis after treatment. Treatment with lower concentrations of AO was selected to explore injury and explore the antimicrobial mechanism. After treatment with 250mM AO at 20°C, plating demonstrated that 78.5±3.4% of the surviving cells had injury, and microscopy results indicated that 78.2±8.5% had membrane damage, and 71.1±7.2% were metabolically inactive compared to the OC. Lower concentration (50 mM) AO showed a lower level of injury, cellular damage, and metabolic inactivity. Increasing AO treatment temperature from 20°C to 45°C led to significant increases ($p \leq 0.05$) in injury, membrane damage and reduction in metabolic activity in *Salmonella* for all concentrations tested.

Significance: Acidified oil-based antimicrobials can cause cellular lysis, injury, membrane damage and inhibit metabolic activity in *Salmonella* and has the potential to improve food safety in industrial settings.

P2-46 pH-Dependent Antimicrobial Potential of Plant-Derived Phenolic Acids Against *Salmonella* Typhimurium for Improving Efficacy and Reduction of Cytotoxicity

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* serovar Typhimurium (ST) remains a ubiquitous pathogen in food products that also has an increasing antibiotic resistance pattern, making the search for novel antimicrobials more critical for controlling ST. Purified phenolics, gallic acid (GA) and protocatechuic acid (PA) have known antimicrobial activity and reported protection of host cells. However, questions remain regarding their efficiency and mechanism of action in environments with varying pH, such as in DMEM used for cell lines.

Purpose: Evaluate the effects of pH on pure phenolic acids with the goal of improving antimicrobial potential and future application.

Methods: ST ATCC 14028 was inoculated in LB broth supplemented with decreasing concentrations of GA and PA starting from pre-determined bactericidal concentrations. Each solution was adjusted to a pH between 3 and 10. 96-well plates were used to allow visual evaluation of bacterial growth and was later confirmed by plating on LB agar. Percentage of cytotoxicity to an INT-407 cell line was determined using MTT assay.

Results: Compounds lost potency within specific pH ranges, even at lethal dosage, notably GA at 4 (4.5mg/mL) and PA between 5-6 (2mg/mL). However, increasing to pH 8 significantly (P<0.05) improved antimicrobial potential of GA and PA, as lethal concentrations decreased to 125µg/mL in both. When DMEM was supplemented with phenolic compounds and adjusted to pH 8, GA retained antimicrobial potency at 4.5mg/mL and 125µg/mL and showed statistically significant (P<0.05) reduction in cytotoxicity at 4.5mg/mL compared to unadjusted samples at the same concentration (35.37%) and at 125µg/mL compared to untreated control (4.86%). PA was effective up to 1mg/mL and had significant reduction in cytotoxicity (4.22%) as compared to control.

Significance: Results suggest that adjusting GA and PA to higher pH values can promote their antimicrobial effect while also serving to protect and improve their tolerance in INT-407, via reduction in cytotoxicity.

P2-47 A Pipeline Approach for the Identification of *Salmonella* Bacteriophages with Tail Spike Proteins

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Introduction: There is increasing interest in the use of bacteriophage (phage) tail spike proteins (TSPs) as antimicrobials due to their carbohydrate depolymerase activity and ability to recognize and cleave components of the lipopolysaccharide, increasing bacterial cell wall permeability, and ultimately, cell death.

Purpose: To develop a pipeline that identifies *Salmonella* phages with TSP activity.

Methods: Phages active against *Salmonella enterica* isolates from serovars (Braenderup, Enteritidis, Heidelberg, Infantis, Kentucky, Reading, and Typhimurium) that produced plaques with halos (indicative of phages with TSPs) on their host cells were isolated from raw influent wastewater. The phages were sub-cultured to ensure purity and phage DNA was isolated. To develop a novel high throughput PCR assay, the TSP gene sequence of *Salmonella* phage SB3, previously isolated and sequenced, was extracted from NCBI and homologues of this gene were identified in the same database using blastn. The nucleotide sequences of 325 gene homologues having 100% coverage and ≥70% identity with the SB3 TSP were extracted and aligned with DNAStar MegAlign. Based on multiple sequence alignments, regions of the TSPs that were highly conserved were chosen and three degenerate PCR primers with amplicon sizes ranging from 426 to 446 base pairs were derived from these regions, using the DNAStar PrimerSelect. The presence of TSPs on phages that produced amplicons of the correct size was then confirmed by transmission electron microscopy (TEM).

Results: Ten phages that produced plaques with halos were isolated. Six out of ten phages had DNA that produced amplicons of the correct size, and TEM confirmed the presence of TSPs on two of those phages.

Significance: The developed pipeline allows for a straightforward method of isolating *Salmonella* phages with TSPs which can then be used in an antimicrobial cocktail to reduce *Salmonella* populations on foods, including fresh produce and poultry.

P2-48 Multi-Year Analysis of *Salmonella* Isolates and Antimicrobial Resistance Trends in FSIS Sampling for the National Antimicrobial Resistance Monitoring System

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Introduction: The United States Department of Agriculture, Food Safety and Inspection Service (FSIS) samples certain food animal species and products for *Salmonella* and other bacteria in regulated establishments as part of the National Antimicrobial Resistance Monitoring System (NARMS). This multiagency program monitors changes in the antimicrobial susceptibility in certain bacteria isolated from clinical, retail, and food animals in the US.

Purpose: The objective of this analysis was to evaluate trends in *Salmonella* serotypes and antimicrobial resistance (AMR) in certain food animal species and products sampled as part of FSIS NARMS sampling, from 2014 through 2019.

Methods: Cecal (intestinal) content samples from food-producing animals (chickens, turkeys, cattle, and swine) at slaughter and product samples were tested. Samples were analyzed for the presence of *Salmonella* and by serotype and antimicrobial susceptibility. Results were evaluated by species to assess differences in *Salmonella* serotypes among the sample sources. Trend was assessed using Kendall tau correlation coefficient at $p < 0.05$.

Results: From 2014 through 2019, multi-drug resistance (MDR) in FSIS *Salmonella* isolates showed a net increase in both cecal (4.1%) and product samples (8.3%). Serotype Infantis isolates showed a proportional increase in both the serotype frequency and MDR in chicken cecal and product samples. An increase in resistance to at least one critically important antimicrobial was observed in all slaughter categories, except for turkeys. Serotype Infantis showed a significant increase in resistance to the critically important antimicrobials ciprofloxacin, ceftriaxone and trimethoprim-sulfamethoxazole.

Significance: The continuous detection of MDR *Salmonella* and monitoring its trends in FSIS cecal and product samples presents FSIS and the NARMS partners an opportunity to prevent and mitigate AMR threats to public health.

P2-49 Seasonal Trends in Prevalence and Antimicrobial Resistance of *Salmonella* in Animals at Slaughter

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Introduction: This study examines the occurrence of seasonal trends in *Salmonella* and antimicrobial resistance (phenotypes and genotypes) in the National Antimicrobial Resistance Monitoring System (NARMS) cecal content samples collected between 2013-2019.

Purpose: We wanted to examine seasonal variation in the occurrence of *Salmonella* and antimicrobial resistance phenotypes and genes in food animals.

Methods: We stratified publicly available NARMS cecal data by meteorological seasons and used logistic regression models to estimate sample contamination and resistance risks by season. This study incorporates 32,804 cecal isolates from beef cattle, dairy cattle, young chickens, turkeys, market swine, and sow and 6,572 *Salmonella* genomes.

Results: There was a higher probability of isolating *Salmonella* in the summer across all sources. The risk of contamination for a sow sample was the highest across all sources and seasons whereas beef cattle samples had the lowest risk. For summer, fall, spring and winter, respectively, the risks were: sow 59.8%, 55.5%, 54.4%, and 53.6%; market hogs 48%, 43.6%, 42.6%, and 42.8%; chicken 39.2%, 35.1%, 34.2%, and 33.4%; dairy cow 23.8%, 20.8%, 20.1%,

and 19.5%; turkey 16.9%, 14.6%, 14.1%, and 13.7%; beef cattle 9.6%, 8.2%, 7.9%. and 7.6%. Isolates from beef cattle collected in winter were less likely to be resistant to folate pathway inhibitors. Conversely, sow isolates collected in winter were more likely to be resistant to folate pathway inhibitors and aminoglycosides. There was no seasonal trend in resistance against cephems and quinolones among isolates from any animal source. In cattle and turkey, we identified two genes, *aadA* and *tet*(B), that were significantly less likely to appear in summer.

Significance: Distinct seasonal differences were seen in *Salmonella* isolation and antimicrobial resistance for specific antimicrobial classes and cecal animal sources. Further work is needed to explore linkage between our observations and seasonal serotype variations, potential seasonal differences in agricultural practices including those in food animal production.

P2-50 Effect of Oregano Oil Nanoemulsion to Control *Salmonella* spp. on Alfalfa Seeds and Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: Seed sprouts are particularly prone to microbial contamination due to high nutrients and moist conditions required for their production. Hence, disinfection is an imperative step in food processing to prevent the contamination of pathogens in food. Plant based essential oils (Oregano Oil) are generally recognized as safe (GRAS) for consumption in the US and shows a promising disinfection because of their antimicrobial activities.

Purpose: The purpose of this study was to determine the effectiveness of oregano oil nanoemulsion for inhibiting the growth of *Salmonella Spp.* in alfalfa seeds and sprouts.

Methods: 5% v/v Oregano oil nanoemulsion was prepared by ultrasonication using tween 80 as an emulsifier. Oil droplets size was characterized for dynamic light scattering. Six different strains of salmonella from multiple sources were used to determine the microbial inhibiting properties of oregano oil nanoemulsion.

Results: Minimum Inhibitory Concentration (MIC) of Oregano oil nanoemulsion for Salmonella is 0.078%. Minimum Bactericidal Concentration (MBC) of oregano oil nanoemulsion is also 0.078%.

Significance: These data suggests that oregano oil nanoemulsion may serve as an effective anitimicrobial treatment for seeds and sprouts against *Salmonella* spp.

P2-51 Phenotypic Antimicrobial Resistance Profile of *Salmonella* spp. Isolated from West Texas, Alabama, Georgia, and Mexico

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Introduction: Antimicrobial resistance (AMR) continues to be a public health problem, especially for critically important antimicrobials. Food-animal trade may play a role in AMR spread.

Purpose: To compare phenotypic AMR of presumptive *Salmonella* sp. isolated from beef and dairy cattle from United Stated and Mexico, to antibiotics commonly used to treat bacterial infections in health care systems.

Methods: Fecal samples were obtained from 203 dairy and beef cattle located in West United States (Texas, Alabama, Georgia) and Mexico. *Salmonella* sp. was isolated by enriching samples overnight at 37°C, in Buffered Peptone Water, and then transferred to Rappaport Vassiliadis and Tetrathionate broths as selective enrichment, incubated overnight at 42ºC, and streaked onto Brilliant Green Sulfa and Xylose Lysine Tergitol 4 agar. Antimicrobial resistance was investigated by determining minimum inhibitory concentration using a Gram-negative Sensititre™ plate (CMV5AGNF).

Results: A total of 123 presumptive *Salmonella* sp. were recovered (31 from Mexico and 92 from the U.S.). From West Texas, 8.1% of beef cattle were resistant to ampicillin, chloramphenicol, tetracycline ceftriaxone, amoxicillin/clavulanic acid, sulfisoxazole, nalidixic acid and colistin, while 89.3% of dairy cattle isolates were susceptible to all except for 10.7% only resistant to colistin. Regarding Alabama isolates (all from beef cattle), 66% expressed resistance to cefoxitin and chloramphenicol, while 33% to azithromycin and colistin. Georgia isolates (all from dairy) expressed resistance to tetracycline (26.7%), as well as cefoxitin azithromycin, amoxicillin/clavulanic acid, and colistin (20.0%). From Mexico, 30.8% of beef isolates expressed resistance to tetracycline (beef). Dairy isolates showed resistance to amoxicillin/clavulanic acid (61.1%), azithromycin (38.9%), ampicillin (38.9%), colistin (33.3%), and tetracycline (33.3%).

Significance: Mexico and USA are important food trade partners. Considering the alarming findings, there is a high risk of transferring AMR bacteria between food animals (beef and dairy cattle) from both countries. Mitigation strategies should consider international food-animal trade.

P2-52 Reduction of *Salmonella* and STEC by Citrilow Spray and Dip Treatments on Chilled Beef Trim

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Introduction: Citrilow is a meat and poultry antimicrobial treatment consisting of citric and hydrochloric acid that is applied at a pH of 1.2; however, th antimicrobial and pH effects on chilled, inoculated beef trimmings need to be evaluated to determine its feasibility for industry adoption.

Purpose: The reduction of *Salmonella* and 7 serotypes of STEC considered FSIS adulterants was measured using Citrilow as a 3-second spray and 5-second dip application, as recommended by the manufacturer, on chilled beef trimmings.

Methods: Fresh beef trimmings were cut into 1.5 $inch^3$ coupons; the coupons were divided to inoculate 4 Log or 1 Log concentrations with a *Salmonella* or a 7 serotype STEC cocktail onto the surface. Duplicate samples were prepared for either spray or dip applications. The surface pH was taken before and after treatments. Controls included uninoculated and treated and untreated samples. One set of samples was processed immediately, and one was held for 48 hrs. The samples were plated onto either XLT4 or SMAC agar with a TSA overlay and incubated for 24 hours at 37°C before counting typical colonies. The entire experiment was replicated 3 times (n=192).

Results: The results of this study indicate that both the dip and spray treatments of Citrilow significantly reduced both *Salmonella* and STEC in all applications. Both treatments showed an average of a 3.72 Log reduction of STEC, and 2.81 Log reduction of *Salmonella*. There was not a significant difference in the surface pH of samples before and after treatment; samples had an average pre-treatment pH of 6.14 and an average post-treatment pH of 5.74 (P-value = 2.68×10^{-6}).

Significance: This study can be utilized as supporting documentation for the validated use of Citrilow as an antimicrobial in the beef processing industry.

P2-53 Farm Readiness for Produce Safety Rule Inspections: Review of On-Farm Readiness Review Data from 2018–2021

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Introduction: On-Farm Readiness Reviews (OFRRs) assess produce growers' readiness for a Produce Safety Rule (PSR) inspection during a voluntary assessment of the growers' current food safety practices.

Poster

Purpose: The purpose of this study is to analyze OFRR participants' readiness to comply with the PSR requirements and identify insufficiencies of resources, knowledge and/or behavioral practices pertaining to preharvest, harvest, and postharvest activities.

Methods: Following an OFRR, assessors report the assessments of a farm's readiness into a national 20 -question multiple-choice and multiple-answer questions survey with the option to add additional comments. Questions provided data on the participants' demographics, food safety practices during preharvest, harvest and postharvest activities, readiness status and resources needed for compliance. Additional comments left by assessors were analyzed by a team of experts and were used to identify practices or topics that were not covered within the survey. Descriptive statistics with RStudio and Person chi-squared test were used to identify relationships between categories.

Results: Survey data from 1,536 OFRR visits between January 1, 2018 to June 30, 2021 was analyzed, representing 42 US states and 2 territories. Of these participants, 589 farms, sold less than $250,000 in produce sales. Over 60% of the participants did not meet the minimum PSR requirements (927/1529); of these participants, 416 farms sold less than $250,000 in annual produce sales (*n*=927). Of the participants that did not meet the minimum requirements, the biggest challenge to preparedness was time (63%, 391/620). The top three areas that were identified as needing the most improvement in food safety practices were postharvest sanitation (39%), health and hygiene (33%) and harvest sanitation (27%) (*n*=1536). The top three areas of improvement did not change by produce sales, acres or region.

Significance: Additional resources in high priority areas are needed to help growers efficiently and effectively translate learned knowledge from food safety trainings into practical behaviors.

P2-54 Systematic Review, Meta-Analysis and Thematic Synthesis of Virtual Food Safety Trainings and Education

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❖ ***Developing Scientist Entrant***

Introduction: Previous research showed that digital food safety training can be as effective as in-person training. However, information is lacking about the effectiveness in different subpopulations of food handlers.

Purpose: To assess the effectiveness of digital training on knowledge, attitudes, and behavior measures in different subpopulations of food handlers.

Methods: We conducted a systematic review, random-effects meta-analysis, and qualitative synthesis. Inclusion criteria were (1) peer-reviewed papers in English and (2) food safety topic as a digital intervention. Degree theses and conference proceedings were excluded. The databases searched until September 2020 were Ebesco, PubMed, ProQuest, Wiley Online, Scopus, Web of Science, and Cochrane. The scientific study was conducted using PRISMA guidelines incorporating a risk of bias assessment using ROBINS-I and ROB-2. Effect sizes were calculated using Hedge's G. The qualitative synthesis will use a thematic synthesis framework.

Results: After a systematic search and reverse search, 23 articles (out of 1590) were included in a meta-analysis (n=15) and qualitative synthesis (n=15). The effectiveness of digital food safety programs varied among the subpopulations but was minorly effective across all categories (G>0.23). Knowledge was moderately effective in all sub populations (G >= 0.58), except food workers (G=0.38, P= 0.028). All measures have lower levels of between-study heterogeneity (I^2 <32%). However, the sample size is too small to have strong statistical power. Multiple subpopulations identified difficulty of focusing during the digital training due to a lack of interaction. In addition, food workers were noted to encounter a challenge with varying levels of background knowledge. Identified recommendations were to: (1) design shorter training sessions, include more breaks, and (2) introduce more interactive activities and short videos.

Significance: This study identified a need for better digital training targeting attitude- and behavior- change. The findings provided guidance for future development of digital food safety training programs for different subpopulations of food handlers.

P2-55 Thermometer Use When Grilling Meat Products in a Consumer Test Kitchen

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Introduction: Observing consumers during meal preparation is a valuable tool for measuring adherence to recommended food safety practices and for crafting educational and outreach materials rather than relying on self-reported data. Limited data are available on thermometer use rates specific to grilling.

Purpose: The purpose of this study was to determine the rate of thermometer use when grilling hamburgers and bratwurst using an indoor grill in a consumer test kitchen.

Methods: Participants (n = 66) were instructed to grill bratwurst and hamburgers using an indoor grill and to prepare a ready-to-eat salad while being videotaped. Coders viewed the videos to determine the rate of thermometer use, whether doneness was checked for all patties and bratwurst cooked, and whether the thermometer was inserted in the proper location.

Results: More than half of participants used a thermometer: 55% for bratwurst and 58% for hamburgers. Among participants using a thermometer, most checked the doneness of the two hamburgers and all five bratwurst. Among participants using a thermometer, most participants failed to insert the thermometer in the proper location when checking the doneness of the hamburgers and bratwurst. Comparing thermometer use for hamburgers and turkey burgers (from a prior observation study), thermometer use was significantly higher for hamburgers (58%) compared to turkey burgers (34%).

Significance: These results highlight the need for targeted messaging to consumers about thermometer use, including the need to check temperature for doneness of all meat and poultry products regardless of cooking method, as well as instructions for proper placement.

P2-56 Do Celebrity Chef-Endorsed Food Safety Messages in Recipes Improve Consumer Food Safety Practices?: Findings from an Observation Study

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Introduction: Previous research suggests that including food safety instructions in recipes promotes the use of recommended practices; however, the effect of a celebrity chef endorsement has not been explored.

Purpose: The purpose of this study was to determine the effect of food safety messaging within recipes that were endorsed by a celebrity chef.

Methods: Participants were instructed to grill bratwurst and hamburgers using an indoor grill and to prepare a ready-to-eat salad. Participants were randomized to one of three conditions: the control group (n = 66), recipes without food safety instructions; Treatment 1 (T1) (n = 66), recipes with food safety instructions; or Treatment 2 (T2), recipes with food safety instructions and a celebrity chef endorsement (n = 68). Food safety information included instructions on washing hands at the beginning of cooking and after touching uncooked ground beef, using a thermometer to check for doneness, cleaning and sanitizing surfaces/utensils after touching uncooked ground beef, and washing fruits and vegetables by rubbing under cold water.

Results: Thermometer use was significantly higher for T1 and T2 when compared with the control group for determining doneness of the bratwurst. Handwashing attempts before meal preparation were also higher for T1 and T2. Forty-six percent of T2 participants had seen food safety instructions shared before by celebrity chefs. Most participants (81%) had not heard of the celebrity chef prior to participating. Many participants (81%) said they trusted celebrity chefs to provide information on how to cook food safely. However, only about a third agreed they would follow food safety instructions because a celebrity chef endorsed them.

Significance: These findings suggest that the addition of a celebrity chef endorsement for food safety instructions in recipes may not influence consumers' food safety behaviors. Inclusion of food safety instructions alone may be sufficient to motivate behavior change.

P2-57 Risks for Outbreak of *Listeria monocytogenes* Infections Associated with Frozen Corn Consumption in Japan

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Introduction: An outbreak of invasive *Listeria monocytogenes* infections associated with frozen corn and other frozen vegetables occurred in Austria, Denmark, Finland, Sweden and the United Kingdom from 2015 to 2018, however, there is no report on outbreaks of invasive *L. monocytogenes* infections by any foods and *L. monocytogenes* contamination levels in such frozen foods distributed in Japan is not clear in Japan.

Purpose: This study aimed to study potent risk for *L. monocytogenes* infections associated with frozen corn consumption in Japan.

Methods: A total of 41 frozen corn samples (11 domestic frozen and 30 imported frozen corns, both of which were labelled to consume after heating) were bought at grocery supermarkets in Tokyo. The samples were used to microbiological examination (TVC, *Enterobacteriaceae*, *Salmonella* spp. and *L. monocytogenes*). Growth kinetics of *L. monocytogenes* were examined by spike experiment using corn-originating *L. monocytogenes* strains in corn samples under low-temperature (<5°C). SPSS for Windows (version 27, IBM corp.) was used for statistical analysis.

Results: *Enterobacteriaceae* were detected from 7 domestic samples (63.6%, 0.7-1.2 log CFU/g) and 15 imported samples (50.0%, 0.7-2.8 log CFU/g). No *Salmonella* spp. were detected in any samples. *L. monocytogenes* were detected from 6 imported samples (20.0%). Low-temperature storage tests revealed moderate growth of *L. monocytogenes* in corns (inoculating: 2.7 log CFU/g. after 3 days: 3.3±0.1 log CFU/g, after 5 days: 4.4±0.1 log CFU/g, after 7 days: 5.5±0.1 log CFU/g).

Significance: We concluded that the risk for occurring *L. monocytogenes* infections associated with frozen corn consumption should be low, because of its low numbers of contamination, moderate growth kinetics and which is consumed after heating. However, poor hygienic handling may induce such risks and thus hygiene education to the variety of food handlers is important theme for food safety.

P2-58 An Evaluation of Produce Safety Rule Resources for Website Accessibility, Readability, and Content Quality

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Introduction: More than one-third of farmworkers do not have a high school diploma while only 23% reported that English was their most comfortable language. Farmworkers play an essential role in agriculture in the United States assisting in greenhouse and nursery and fruit and tree nut operations. It is recommended that resources be developed for this audience at an 8th-grade level.

Purpose: The purpose of this study was to evaluate existing produce safety materials from three Cooperative Extension (CE) information sources— Virginia Tech, NC State University, and Cornell University—for website accessibility/navigability, content quality, and health literacy (readability) level.

Methods: Five search terms were generated from website analytics of two CE sources using the number of downloads and clicks, and the most frequently downloaded. The top 30 results from each of the search terms from three CE sources were downloaded, cleaned, and analyzed. The evaluation tools of Web Content Accessibility Checker (WAVE), Adobe's PDF accessibility checker, and the Flesch-Kincaid (FK) method were used. Significant differences were determined using one-way MANOVA for WAVE errors, and one-way ANOVA for FK statistics.

Results: 17% (67 of 391) of the available web pages met the criteria to be analyzed. All results were significantly significant ($p<0.001$). Websites were not accessible with an average of 10.7 accessibility errors per page, an FK Grade Level of 13.4, far above 8th grade; and 72% of PDFs analyzed (13 of 18) violated accessibility rules. All web pages provided references through citations or additional website links, but 40% (27 of 67) failed to provide a last updated date.

Significance: CE materials evaluated were not generally accessible to the intended audience. Materials should be accessible to an 8th grade level and should be reviewed for accessibility prior to release. Content developers must be intentional when developing materials to ensure that they meet health literacy and communication needs of consumers because the effectiveness of training can also be influenced by accessibility.

P2-59 Content Analysis of Online Tree Nut Recipes: Soaked Nuts and Nut-Based Dairy Alternatives

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❖ ***Developing Scientist Entrant***

Introduction: Multiple foodborne outbreaks were associated with tree nuts and nut products with a soaking process. Consumers reference YouTube videos to prepare soaked nuts and nut-based dairy alternatives at home, however, few online recipes offer appropriate food safety handling instructions.

Purpose: To explore consumer handling practices for soaked nuts and nut-based dairy alternatives via content analysis of popular consumer shared recipes; (2) to evaluate the available food safety information provided by those recipes.

Methods: A keyword search was conducted on YouTube with inclusion criteria: (1) use of almonds, cashews, walnuts, or pistachios to make soaked nuts or nut-based dairy alternatives; (2) in English; (3) more than 500 views; (4) include a fermentation step (cheese and yogurt only). Videos (N = 204) were coded for general characteristics including washing and storage practices, soaking and fermentation methods, and food safety messages.

Results: Raw tree nuts (not roasted, treated, or pasteurized) were used in more than half of the recipes. Washing hands, cleaning kitchen surfaces and tools, or rinsing tree nuts were mentioned in about 3%, 19%, and 48% of the videos, respectively. Of the recipes including a soaking step, most recommended soaking for more than 8 h (75%), failed to refrigerate or specify a soaking temperature (85%), and lacked the addition of salt or an acidifying agent (93%). For fermented products, most often probiotics were used as the starter culture (63%) and left on the countertops at room temperature (35%). When storage recommendations were provided (70%), 3 to 4 days in the refrigerator was the most common.

Significance: The findings support developing science-based resources to improve consumers' tree nut handling practices.

P2-60 Food Safety Implications of Nut-Based Dairy Alternatives and Soaked Nuts

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Introduction: Tree nuts, a low-moisture food, are typically perceived as a low-risk for foodborne illness. In recent years there has been an increase in the consumption of tree nuts (dry, soaked or as plant based dairy alternatives) as well as recalls and outbreaks associated with these products.

Purpose: To understand consumers' handling and knowledge of food safety risks related to soaked tree nuts and tree nut-based dairy alternatives through an online survey.

Methods: A survey was developed and Qualtrics Inc. managed an online panel to recruit participants based off the inclusion criteria: primary household shopper and tree nut consumers who soak tree nuts or make tree nut-based dairy alternatives in the home. In October 2021, the survey questions were distributed to a convenience sample (N = 12) to test for validity and reliability. In January 2022, participants (N = 981) that met the criteria completed the survey. The survey questions focused on consumer tree nut handling, risk perception, and preferred delivery of food safety information.

Results: Almonds were the most common tree nut used for soaking nuts for direct consumption (54%) and when making nut-based dairy alternatives (67%). For direct consumption, 34% of participants soaked at room temperature, 33% for more than 8 h, and 50% lacked a control step to inhibit pathogen growth. For nut-based dairy alternatives, 42% soaked at room temperature, 33% for more than 8 h, and 38% lacked a control step. The most popular starter culture used for cheese was probiotics (65%) and for yogurt was a yogurt starter culture (70%). Nearly a third (36%) of participants preferred to use raw tree nuts and about a third (37%) believed that the health benefits of raw tree nuts outweighed the microbial risks.

Significance: The findings can be applied to the food community and food safety extension education programs to develop tree nut food safety resources.

P2-61 Food-Handling Practices of Apple Drying in Home Kitchens: A Survey

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Introduction: Fruit drying has traditionally received little food safety attention despite *Salmonella* outbreaks and recalls associated with low-moisture foods. Home apple drying raises potential concerns given consumers' lack of knowledge of food safety handling and drying in domestic kitchens.

Purpose: To identify specific knowledge gaps and food handling practices of home apple drying in the United States.

Methods: A cohort of home apple dryers (N=979) participated in an online survey through Qualtrics XM. The 69-question survey was refined based on food safety experts' written feedback and guided verbal tests with laboratory members. A Safe Practice Index (SPI) was developed based on 21 apple drying practices to quantify individual level of safe food handling. Data were analyzed using ANOVA and Eta-squared.

Results: Participants' level of safe food handling in apple drying was low. Participants had a mean SPI score of 37 out of 100 (SD=11.0). Participants below 30 years old (mean=34.6, SD=11.1), identifying as males (mean=35.9, SD=11.4) or earning less $10,000 (mean=31.7, SD=11.2) had lower mean SPI scores ($p \leq 0.05$) than other age groups, genders and income levels, respectively. Results revealed inadequate frequency of handwashing during apple preparation (52%), potential points of cross-contamination from kitchen tools (31%), a lack of pre-treatment that can reduce microbial load (28%), failure to incorporate a thermal kill step during drying, and a lack of objective measurements to ensure that target parameters are attained. Apple pre-treatment before drying was mainly used to improve sensory quality rather than to control microbial risk. When presented with some pre-treatment benefits, participants who did not pre-treat their apples considered doing so to kill bacteria.

Significance: Findings of this study can provide handling practice data to support the development of more accurate food safety risk assessment models, and to guide the development of food safety education for home fruit dryers.

P2-62 Risk Management Practices of North Carolina Animal Agritourism Operations

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Introduction: Animal-Agritourism interactions allow non-farmers to learn about animals and agriculture, however carry the risk of zoonotic infection. Activity-audience-area constraints and cost may present barriers to implementation of best-practice recommendations.

Purpose: The aim of this survey was to characterize NC animal-agritourism operations and benchmark current visitor safety practices covering multiple risks in the context of the pandemic.

Methods: An electronic survey questionnaire was developed and distributed in 2021 to assess the characteristics of North Carolina animal-agritourism operators (n=35), their operations, their confidence to effectively manage such risks, prevailing barriers to implementing safety improvements, and intention to maintain safety improvements installed in response to the Coronavirus pandemic. Questions included likert, multiple-choice and short-text answer formats. Survey was classified as exempt by NCSU IRB (protocol #20712).

Results: The most frequently reported activities were farm tours (85.7% of responding operations), direct sales at farm stands and stores (62.9%), petting zoos and animal feedings (65.7%), special events, and festivals (51.4%). The most frequent animals reported were horses, donkeys or burro (66%), goats (63%), chickens (63%), sheep (46%), and pigs (46%). A majority of operators self-report high ("Extremely or Moderately confident") confidence in their ability to assess health and safety risks to visitors, COVID-19 risk, and biosecurity risks at their operation. Operators were slightly less confident- 46% reported any level of confidence ("Extreme, Moderate, or Slight")- in their ability to assess risks to visitors at an unfamiliar operation. Animal feeding was allowed on 55% of operations. 90% of operations had hand sanitizer available, 68% had permanent restroom facilities and 55% indicated a permanent sink available. Of practices that were added in 2020, operators indicated they would continue 78% of these moving forward.

Significance: Understanding the barriers to implementation and persistent use of risk-reduction strategies, especially those that manage multiple agents, informs future programming and resource development.

P2-63 Incorporating Celebrity Chef Endorsed Food Safety Messages into Recipes for Meal Preparation in Consumer Kitchens and Their Influence on Cross-Contamination to Kitchen Surfaces during Meal Preparation

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Introduction: Consumer meal preparation in the home contributes significantly to foodborne illness in the United States. It is not known if recipes containing safe food handling instructions (SFHI) with or without a celebrity chef endorsement (CE) influences cross contamination from raw meat products to kitchen surfaces during meal preparation.

Purpose: The purpose of this study was to determine the impact of SFHI within recipes, with and without a CE, on cross contamination from raw meat products to kitchen surfaces during meal preparation.

Methods: A 2lb chub of ground beef was inoculated with 10^{9}-10^{10} CFU/g of *Escherichia coli* DH5-alpha tagged with a green fluorescent protein. The chub and a salad were prepared by participants (n=200) in test kitchens. Participants were split into two treatment groups: T1 (recipe included SFHI) and T2 (recipe included the same SFHI with CE) and a control group (recipe did not include SFHI). Kitchen surfaces and salad were sampled after meal preparation, and the tracer organism was cultured on selective media for enumeration.

Results: When cutting boards used to prepare ground beef were hand washed (n=53), the control group was found to have a significantly (p<0.05) higher prevalence of contamination (45%, n=11), followed by T1 (37%, n=19) and T2 (22%, n=23). Showing that messaging was effective, and the CE increased efficacy. However, for the sink basin, a higher prevalence of contamination was found for the control group and T2 (32%, n=65 and 35%, n=68, respectively) compared with T1 (17%, n=65; p<0.05). No differences were found for the remaining samples sampled (salad, counter, spice containers and cupboard handle).

Significance: This study provides evidence that providing recipes that include SFHI results in variable cross contamination in consumer kitchens post meal preparation. Further studies are warranted to determine which messages may be effective with an endorsement.

P2-64 Development and Implementation of a Hands-On Food Safety and Regulatory Curriculum for Members of Shared-Use Commercial Kitchens in Florida

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Introduction: Food entrepreneurs often utilize shared-use commercial kitchens (e.g., incubators, accelerators) to launch new businesses. Previous survey results, workshop evaluations, and stakeholder feedback have indicated that food safety and regulations are significant hurdles for many food businesses working out of these facilities. Feedback also indicated a need for more hands-on scenarios, examples, and activities.

Purpose: This project aims to develop hands-on curriculum modules, deliver the training to target audiences, and assess short- and medium-term knowledge gains and impacts.

Methods: Members of shared-use commercial kitchens in Florida (n = 52) completed a needs assessment survey. These results, along with previous data and feedback, were used to develop a hands-on curriculum that included a basic overview of state and federal food regulations and outlined food safety plan examples that met FSMA's Preventive Controls for Human Food Rule standards. Hands-on activities were developed that focused on sanitation controls and verification activities. In 2021, three pilot workshops were offered using the new curriculum to a total of 31 attendees. Evaluations were collected after each training to measure impacts.

Results: The curriculum developed provides a valuable tool to guide future workshops while leaving attendees with the framework to develop a food safety plan for their products. Participants of the pilot workshops reported their knowledge in six topic areas related to food safety principles and food regulations before and after the training using a five-point Likert scale. Results (n = 31) were analyzed using a paired t-test and indicated a significant increase ($P < 0.001$) in perceived knowledge across all topic areas with an overall average of 2.69 pre-training and 4.37 post-training.

Significance: Increased understanding of these topics better prepares food entrepreneurs for regulations they may encounter as their businesses grow and limits the potential for adverse food safety events affecting them as they work in a shared environment.

P2-65 Perceptions of 'Invulnerability', 'Optimistic Bias', 'Illusion of Control', and 'Superiority Bias' Regarding Food Safety Risks Among Lebanese Consumers

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Introduction: To facilitate the development of effective consumer food safety education, there is a need to identify, consider, and address consumer perception regarding food safety risks that may undermine food safety education attempts. Despite an increasing interest in food safety research studies involving Lebanese consumers, to date, none have conducted quantitative analyses to explore the perceptions of risk, control, and responsibility of consumers regarding food safety in the region.

Purpose: Consequently, the purpose of this study was to explore the food safety perceptions of Lebanese consumers.

Methods: Lebanese consumers (n=95) participated in a self-complete questionnaire to determine perceptions of risk, control, responsibility, and hygiene consciousness relating to food safety.

Results: Food prepared at home was perceived to be least likely of causing foodborne illness, whereas food prepared in retail outlets was perceived to be the most likely of resulting in foodborne illness. Correlations were determined between personal perceptions of risk, control, responsibility, and consciousness (p<.001), whereby low levels of risk were correlated with high levels of control, responsibility, and hygiene consciousness. Statistically significant differences were determined between perceived risk, control, responsibility, and hygiene consciousness for 'self' compared with 'others' (p<.005) suggesting that consumers in Lebanon exhibit perceptions of 'invulnerability', 'optimistic bias', 'illusion of control', and 'superiority bias'. The most notable finding in this study is that negative food safety experiences, such as acquiring a foodborne illness can have a negative impact upon perceptions of risk, control, and responsibility to prevent reoccurrence of foodborne illness.

Significance: The perceptions and biases identified among Lebanese consumers in this study are of great importance to help inform the development of future consumer food safety education interventions as such perceptions and experiences may undermine food safety education attempts.

P2-66 Utilising the 'Safe Recipe Style Guide' to Assess Food Safety Communication in Chicken Salad YouTube Video-Recipes

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Introduction: Previous research has explored food safety communication in broadcasted television cookery programmes and published recipe books; however, the way consumers obtain recipes is changing. Online video sharing and social media platforms allow users to view videos without the constraints of static broadcasting schedules. YouTube is widely used by consumers to access and engage with video-recipes and has the opportunity to incorporate food safety communication.

Purpose: Given the popularity of online video-recipes, this study reviewed the inclusion of food safety information in amateur cook and celebrity chef YouTube video-recipes.

Methods: Content-analysis of YouTube 'chicken salad' video-recipes (n=38) using an electronic observational checklist, was undertaken. The checklist was based upon the Partnership for Food Safety Education 'Safe recipe style guide', which was annotated with visual and verbal communication of food safety practices and malpractices relating to *'temperature', 'hand washing', 'cross-contamination',* and *'produce'.*

Results: Just 8% of video-recipes demonstrated temperature-probe usage, with amateur cooks more frequently communicating the practice to determine cooking adequacy, than celebrity chefs. None of the observed video-recipes showed visual handwashing before commencing food preparation. Furthermore, only 8% of video-recipes demonstrated handwashing after handling raw chicken. In terms of cross-contamination, amateur cooks communicated the malpractice of washing raw chicken; 5% of video-recipes included verbalisation, demonstration, and/or on-screen prompts regarding the practice. No video-recipes explained *'why'* poultry should not be washed. Washing produce was partly communicated in 11% of video-recipes, however, only one video-recipe appropriately demonstrated how salad produce should be washed.

Significance: This is the first study that has utilised the 'safe recipe style guide' as a tool to assess inclusion of food safety messages. Completion of the study has determined a lack of visual and verbal food safety communications within video-recipes. Inclusion of such information could potentially help to inform consumers of risks and safeguarding practices when preparing food in the domestic kitchen.

P2-67 Understanding Food Safety Training Barriers Among Hmong Farmers in the U.S.

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Introduction: Although cultural differences between Hmong and Americans presents unique food safety challenges, the current Food Safety training programs do not address the needs for Hmong farmers in the U.S.

Purpose: The study was to identify the specific food safety training and education needs and food safety challenges faced by local Hmong farmers with particular consideration of their cultural values.

Methods: First, five Hmong farmers from southwest Missouri were recruited for a 60 minutes interview to assess the current food safety training needs. All interviews were facilitated by a translator who speaks both Hmong and English. Second, five Produce Safety Alliance (PSA) certified trainers who

had been working with Hmong farmers previously were recruited for a 60 minutes interview to understand their training experiences while working with this population. All interviews were audiotaped, transcribed verbatim, and used for the preliminary indexing of concepts and themes. Last, food safety handling behavior observations were conducted at five Hmong farms.

Results: All Hmong farmers (n=5) identified language as a major barrier when participating Food Safety Modernization Act (FSMA) training, following by material format, time, and perceived need for getting FSMA certificates. Hands-on activities, on-farm demonstrations, mini-section training videos, and printed pictorials were the most preferred food safety training methods of Hmong farmers, and Hmong was the preferred language in which to conduct the training. Besides, PSA trainers specified the need for developing hands-on experiences/demonstrations and one-on-one consultation for Hmong farmers. Based on the farm observations, water supply, domestic animals, and trash were identified having food safety concerns on Hmong farms.

Significance: This study contributes to the understanding of the specific food safety training and education needs and challenges within local Hmong farm communities.

P2-68 The Year-Long Effect of COVID-19 on Food Safety: Consumer Practices and Perceptions Using Longitudinal Consumer Surveys and Focus Groups

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❖ ***Developing Scientist Entrant***

Introduction: Behavior models revealed that risk perception levels can lead to behavior changes during health-related situations like the COVID-19 pandemic.

Purpose: To assess consumer risk perception and practices regarding food safety for a 1-year period during the COVID-19 pandemic.

Methods: Nine waves of surveys (April 2020 to May 2021; N= 6,496, 700+ each month) were distributed to an online U.S. consumer panel. There were two sets of online focus groups: 2020 sessions (May to July 2020; n=43; via WebEx) and 2021 sessions (June 2021; N=32; via Zoom). The 2020 session volunteers were recruited from the April 2020 survey respondents while the 2021 volunteers were recruited from the May 2021 respondents. Topics for both studies included both COVID-19 and food safety practices, and anticipated practices after the COVID-19 pandemic.

Results: Consumers were significantly more concerned about contracting COVID-19 from other people than from food and by May 2021, the contracting-from-people-concern dropped to below "somewhat concerned" (43.3 out of 100). Focus group participants mentioned that because the virus was "airborne", their contracting-from-food-concerns dropped, and many consumers were going "back to normal" with their food safety habits by June 2021. Survey respondents had decreasing levels of belief that handwashing protects them from foodborne illness with May 2021(68.2±29.6) being significantly lower than March 2021 (73.7±26.2). Consumers living with older adults (age 65+) had significantly higher levels of handwashing with soap when compared with those not living with older adults. Younger adults had significantly higher levels of produce-washing with soap in all months compared to older adults (ages 55-65+).

Significance: Consumer food handling practices and risk perceptions changed throughout the pandemic and many best practices did not sustain throughout the pandemic. Consumers from certain demographic groups, like those in different age groups and high-risk individuals, need more science-based food handling information during a pandemic.

P2-69 Did COVID-19 Change How We Do Things? Critical Violation of the Restaurants in Louisiana before and during The Pandemic

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Introduction: The COVID-19 has impacted the restaurant industry negatively, from the initial shutdown, limited capacity, to full capacity now. Factors such as consumers' perception of risk, supply chain disruption, employee shortage etc. alone or together affected the business performance.

Purpose: This study aimed to compare the restaurants performance before and during the pandemic, with the intention to gain information about how restaurant industry in Louisiana responded to the global pandemic.

Methods: Data were collected from a random selection of 512 restaurant inspection records issued by Louisiana Department of Health. Each restaurant was categorized by type (fast, fast casual, sit-down) and location (urban, suburban, or rural). Restaurants went out of business were excluded. Critical violations have been coded, analyzed, and compared before (2016-2017) and during pandemic (2020-2021).

Results: Results showed that in fast restaurants, the top critical violation has shifted from employee health before the pandemic to toxicity exposure during pandemic. Fast-causal restaurants showed a shift from employee health and toxicity exposure pre-pandemic to toxicity exposure alone during pandemic. Sit-down restaurants, interestingly, showed the highest violation in cross-contamination before and during pandemic. During pandemic, there was a significant effect of restaurant type on cross-contamination. Pillai's trace indicated no statistically significant differences among violation categories by location pre- or during pandemic.

Significance: Different from other natural disasters such as hurricanes, COVID-19 pandemic impacted the restaurant industry in its unique way. As industry is still trying to recover from the disruption, this study provided valuable information on restaurants' performance before and during pandemic, which may assist in troubleshooting at recovery stage.

P2-70 Supporting Master Food Preserver Volunteers during the COVID-19 through Hybrid Food Safety Training

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Introduction: As part of the Master Food Preserver (MFP) training program, MFPs attend a three-hour food safety class. Due to the COVID-19 pandemic, the class was adapted for remote delivery.

Purpose: To evaluate the learning outcomes and satisfaction of a hybrid (asynchronous and synchronous) food safety training program for MFPs.

Methods: Eleven videos were created covering key concepts related to safe food preservation. The videos were posted on YouTube and viewed by MFPs prior to a one-hour live question and answer session. A pre-/post test was used to assess learning outcomes and the level of satisfaction with the hybrid training class.

Results: Overall, MFP participants (n=46) indicated a high level of satisfaction with the online content and the live session. Knowledge increases were observed in three different content areas: (1) microorganisms and foodborne illness (8% increase), (2) water activity (18% increase), and (3) *Clostridium botulinum* (5% increase). Since posting content on YouTube in March 2020, content has > 21,100 views.

Significance: A hybrid model was successful in providing food safety training to county-based Extension personnel. Moreover, the views on YouTube highlight interest in food safety topics by the public and the value of social media platforms in Extension programming.

P2-71 Assessment of Elderberry/Elderflower Post-Harvest and Processing Practices to Inform Extension Food Safety Education Products

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Introduction: Elderberry (*Sambucus nigra*) is an emerging non-traditional commercial crop in California and other parts of the US. Recently, elderberry products have gained attention and there is an increase demand for elderberry products nationally.

Purpose: Understand the current food safety practices related to elder fruit and flower value-added processing.

Methods: A 43 question survey was developed targeting both elderberry growers and processors. The survey was reviewed by five food safety, elderberry production, and evaluation specialists prior to dissemination. It was piloted with three elderberry growers/small-scale processors. The survey was administered online via Qualtrics and shared via email by three organizations with a self-selected elderberry-interested audience (432 recipients total) and posted to six elderberry-related groups on Facebook, with combined membership totaling over 1,000 individuals. This research protocol was approved by the Institutional Review Board at the University of California, Davis.

Results: A total of 61 elderberry processors and growers completed the survey of which 45 self-identified as processors of either elder fruit and flowers. Of those processing, 46.7%, were making a formulated product (i.e. jams, syrups, teas); 33% dried berries or flowers; and 15% frozen berries. When participants were asked to indicate applicable regulatory schemes (e.g., Cottage Foods, FSMA, etc.) the selections with the greatest number of responses were "Don't know/not sure" (27%, n = 33) and "None of the above" (27%, n = 33), indicating that there is a need for education with this target audience.

Significance: The survey information will provide the foundation for developing targeted food safety resources addressing adoption of food safety practices related to different processing activities (e.g., drying, freezing, and other value-added processing methods) of elder fruit and flowers and providing training for growers and processors on compliance with applicable regulatory requirements.

P2-72 Enhancing Fermentation Nutrition and Food Safety Extension Education Utilizing Online Platforms

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Introduction: Home fermentation continues to increase in popularity. Currently, there is limited science-based information available for consumers from Extension. Therefore, new strategies are needed to help increase consumer knowledge on the nutrition and safety of fermented foods.

Purpose: Develop a consumer education program on the science of fermentation.

Methods: A three-part webinar series was developed and delivered via Zoom. Webinars covered the definition, production, health benefits, and safety of fermented foods. Learning outcomes for the webinar series were determined by retrospective pre-/post-program evaluations. The evaluation consisted of questions where participants had to rate their understanding of key concepts before and after the webinar on a Likert scale from 1 to 5, 1) being "No understanding", and 5) "Very Good".

Results: A total 478 individuals registered for the webinar series. The number of participants in live webinars 1, 2, and 3 were 125, 131, and 99 respectively. Of those, 71 individuals completed the evaluation survey for webinar 1 (56.8% of participants), 51 (39.8% of participants) for webinar 2 and 39 (39.3% of participants) for webinar 3. Participants (>80%) reported the webinar series met most or all of their expectations and were somewhat or extremely satisfied with the content. Participants reported increases in knowledge in all areas evaluated after attending the webinars. Webinar recordings were posted on YouTube and have been viewed >1,000 times since February 2021.

Significance: The webinar was able to engage a wide range of stakeholders including consumers, industry, regulatory, and academics with interest in fermented foods during the COVID-19 pandemic.

P2-73 Work-Based Learning is Effective Tool for Training People with Significant Cognitive Disabilities or Autism Spectrum Disorder

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Introduction: People with significant cognitive disabilities and autism spectrum disorder are a willing but underutilized resource for the food industry's workforce because the necessary food safety training is infrequently offered in a style suitable for their learning needs.

Purpose: The purpose of this project was to assess the effectiveness of food safety training using an immersive, work-based learning style for persons with significant cognitive disabilities and autism spectrum disorder.

Methods: Sixty students with significant cognitive disabilities or autism spectrum disorder were enrolled in the program. Students attended food safety classes three hours per week for sixteen weeks for two summers. Classes utilized a learner-centered, interactive teaching environment and classwork was reinforced weekdays in the program's café 2019-2021. Through hands-on experience the students immediately put their knowledge into practice allowing for a significantly higher retention rate. The instructors and students developed 10 high-interest, student-centered videos with supporting lesson plans which have been shared with educators throughout Alabama via an online portal.

Results: Based on instructor observations and students' on-the-job performance, all students demonstrated food safety understanding and were able to perform food handling tasks correctly while working in the café. As selected by the instructor, six students passed the food handlers certification test and four passed a manager-level exam. Twenty-nine participants found community-based employment outside the program. The remaining students continue to learn and work in the café receiving minimum wage or higher.

Significance: Immersive work-based learning in the food service industry is an effective way to prepare young adults with job skills and knowledge needed for employment especially for those with significant cognitive disabilities or autism spectrum disorder. It is the opinion of the instructors that certification tests need to reasonably accommodate students of this population.

P2-74 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-test scores from PSA grower trainings (n=1,444) and OFRR surveys (n=52) indicated knowledge changes and highlighted areas where more education was needed to meet minimum requirements.

Results: Post-test score means (22.15/25), were significantly higher than pre-test score means (19.02/25), indicating an increase in knowledge after participation in the training (t=-2.14, P< 0.05). The OFRR surveys indicated sanitation, post-harvest water, and worker training required the most improvements and 46.15% met the requirements, 42.31% needed minor improvements, and 11.54% needed significant improvements to meet the FSMA PSR requirements.

Significance: The results of the PSA Training and the OFRR program 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.

P2-75 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 22 institutions aimed at enhancing produce safety in 13 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 courses to assess short-term knowledge gains from October 2020-September 2021 across the south. 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: Of seven practices included in the FSPCA PCQI behavior change surveys (n=16) the most frequent behavior change was fine-tuning existing food safety plans (37.5%). PSA post-test scores (n=452) were significantly higher than pre-test scores ($T = -37.52$, $p < 0.05$), indicating a significant increase in knowledge. Of 13 practices included in the PSA surveys (n=49) the most frequent behavior changes were creation or modification of food safety record-keeping systems (59.18%), implementing new or different methods for cleaning or sanitizing food contact surfaces (59.18%), beginning to write or modify farm food safety plans (55.10%), and implementing new or different practices for monitoring on-farm facilities (55.10%).

Significance: Additionally, 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.

P2-76 Assessing Knowledge Gained from Produce Safety Alliance Grower Trainings Administered by the Southern Center for FSMA Training

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Introduction: The successful implementation of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) depends on the effective delivery of training courses to growers.

Purpose: The purpose of this study was to evaluate the effectiveness of Produce Safety Alliance (PSA) Grower Training courses administered between 2016-2019 using data from pre- and post-training knowledge assessments.

Methods: Knowledge assessment data from paper tests were entered into a Qualtrics database, representing trainings from nine states: Alabama, Arkansas, Florida, Georgia, Kentucky, North Carolina, South Carolina, Texas, and Virginia. Tests that were missing year or state information were excluded from analysis; 2,522 pre-tests and 2,490 post-tests were analyzed. Scores for the 25-question assessments were calculated by assigning one point for each question and module scores were rescaled to a 10-point scale to allow for comparisons. Significant differences ($P < 0.05$) were compared among average pre- and post-test scores for each module and across years and states by Welch's two-sample t-test and Tukey's honest significance test, respectively. Pearson's chi-squared test was used to assess the differences in correct and incorrect responses for each question between pre- and post-tests.

Results: For all states and years combined, average post-test scores (20.30) were significantly higher ($P < 0.001$) than pre-test scores (15.91). For most states (6/9), scores increased by greater than 4.2 points between pre- and post-tests. Training participants showed the most improvement from pre- to post-test on the Postharvest Handling and Sanitation module (2.83). The module, How to Develop a Farm Food Safety Plan, had the lowest average pre- and post-test scores of 4.26 and 6.80, respectively. Six questions of concern were identified which had the lowest percentage of correct responses in either pre-tests, post-tests, or both.

Significance: Understanding the effectiveness of PSA Grower Training courses will lead to program improvements aimed at filling these crucial knowledge gaps.

P2-77 How Food Processors are Responding to Regulatory and Environmental Changes

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Introduction: Many changes have occurred in the scientific and regulatory environment for food processors. Processors have had to change many of their daily procedures. This situation has created a new set of challenges and responses. Much has been written about what processors should be doing, but it is important to find out what they have actually been doing.

Purpose: The purpose of this survey study was to better understand these changes, the responses implemented by food processors and areas in which they plan to focus their resources.

Methods: *Food Safety Magazine* has been conducting a series of surveys over a 3-year period ending in June 2021 involving more than 1,000 food processing locations throughout Europe, the U.S. and Canada. Survey questions were asked about current food safety practices, how these practices have changed since the implementation of the Food safety Modernization Act (FSMA), and where processors plan to make further investments over the next 2 to 5 years.

Results: Processors have been making numerous changes to their operations, including the development of Hazard Analysis and Risk-Based Preventive Controls programs, but they report prioritizing upgrades to their employee training programs, putting additional resources into microbiological and pathogen control programs, increasing outsourcing to commercial laboratories for their testing and enhancing supplier compliance and supply chain programs, especially with their international suppliers. The data also show that processors are investing more in the validation of processes than a reliance on analytical testing. Recent results have also included processor's reactions, responses, and recovery to the disruptions brought on by the COVID pandemic.

Significance: These data suggest that regulatory and technical challenges have significantly changed food processing practices since the implementation of FSMA, with recovery from the pandemic accelerating many of those changes and creating new ones. A better understanding of these changes is important for other stakeholders, including processors, regulators, and the service companies who support the industry.

P2-78 Home-Canning: Preliminary Results of Electric Pressure Cookers for Canning Low-Acid Foods

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Introduction: The pandemic caused a resurge of interest in home canning. Unsafe home canning at its highest risk could cause death and complications due to *Clostridium botulinum* when unsafe canning recipes or methods are used that do not provide an adequate kill step. New appliances, such as electric pressure cookers/canners are marketed for home food preservation, however, the validity of these appliances to meet pressure and temperature are not well known due to company proprietary reasons.

Purpose: Three electric pressure cookers were tested to determine their efficacy to safely reach pressure and temperature for green beans, tomatoes, and chili con carne using the National Center for Home Food Preservation recipes for safe home canning guidelines for an elevation range of 0' to 1,000'.

Methods: Presto, Carey, and Power Pressure Cooker XL were used in this study. Pint-size mason jars were used, and green beans, tomatoes, and chili con carne were tested in triplicate for each cooker. Four jars of food were fitted with ThermoWorks HiTemp 140 wireless-temperature probes and two Omega submersible pressure data loggers were placed in the inner pot. Manufacturer instructions were reviewed against stovetop pressure canners instructions.

Results: All cookers were appropriate to use up to 2,000', one brand omitted an important depressurizing step and time ranges for safe processing. The maximum-unadjusted ranges for temperatures and pressures across all tested models were 117.22°C-119.09°C and 26.85 to 27.77 PSI respectfully, while D-value calculations are underway.

Significance: This study shows that more recipe-electric pressure cooker/canner validation studies are needed at elevations 0'-1,000' and above, as well as for a variety of low and high acid foods. Encouraging awareness that the science may or may not support new appliances for home canning from Extension Agents/Specialists is important to prevent foodborne illness.

P2-79 Analyzing Virtual Platform Effectiveness to Interact with Produce Safety Stakeholders

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Introduction: As food safety education, outreach, and extension activities are increasingly transitioned to virtual forums, an emphasis has been placed on converting existing programs (in-person presentations) into web-based equivalent programs (online webinars); however, stakeholders are often unwilling to commit to extended periods on virtual platforms and prefer shorter delivery periods.

Purpose: To analyze virtual methods of content delivery for engagement, interest, and content value with stakeholders in the fresh produce industry.

Methods: A 90-min webinar series was carried out monthly to tackle challenging produce safety issues within the industry using a speaker presentation or panel, question and answer session, and post-webinar survey (n = 3). A social media outreach program was also designed to aid growers in identifying food safety experts, address common questions, and increase 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 80 d (n = 51). Data was collected using Zoom and Twitter Analytic software to assess engagement (attendance rate; engagement rate), interest (registration; likes), and content valued as most important on the platform (survey response; retweets) using Kruskal-Wallis and Dunn tests in RStudio Version 4.1.2.

Results: Average webinar registration and attendance were ca. 522 and 374, respectively, with attendance rate >70%. Both engagement and value increased over time with webinar content, but interest did not. Primary tweet content was divided into advertisements (13.7%), education (37.3%), personnel introductions (17.7%), publications (15.7%), research updates (11.8%), and other (3.9%). Engagement differed by tweet content (P=0.006), but interest (P=0.2602) and value (P=0.1317) did not. Publication (P=0.081) and education (P=0.076) tweet content were significantly more engaged with than advertisement (P=0.042), but not personnel, research updates, or other tweets.

Significance: Virtual methods of content delivery can engage produce stakeholders in science-based produce safety publications and education content.

P2-80 Inactivation of *Listeria innocua* in Raw Cashew Milk Treated with High Pressure Processing (HPP)

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Introduction: High pressure processing (HPP) is a nonthermal processing method that could be used to pasteurize plant-based milks to inactivate pathogens and reduce spoilage microorganisms, thereby extending shelf-life.

Purpose: The purpose of the study was to determine if HPP can provide a microbial reduction and shelf-life for cashew milk comparable to that of pasteurization for cow's milk, approximately 14 days.

Methods: *L. innocua* ATCC 33090 and ATCC 51742 were cultured and cold adapted at 15˚C for 72 h before inoculating approximately 10^7 CFU/mL into cashew milk. Samples were treated in a Hiperbaric 55L machine at 590 MPa for 6 min using chilled (4˚C) or room temperature (RT) water as the pressurizing fluid. Samples were enumerated on recovery media to recover healthy and sub-lethally injured cells. The limit of detection was 1 log CFU/mL, which is the value reported when no growth was observed. Microbial reductions were calculated using the microbial counts of the pressure-treated sample at day 1 and the untreated control at day of HPP treatment. Pathogen recovery was monitored for up to 35 days post-HPP treatment.

Results: Greater than 5 log reductions in *L. innocua* were achieved in HPP-treated cashew milk using chilled (5.45 ± 0.19 log) and RT (5.41 ± 0.04 log) water. The recovery of *L. innocua* in the milk was below 2 log CFU/mL until day 21 when chilled water was used and was still below 2 log CFU/mL by day 35 when RT water was used.

Significance: The pressure treated cashew milk at 590 MPa 4˚C and 590 MPa RT do have a shelf life comparable to that of pasteurized cow's milk, showing that HPP could be an alternative processing method for cashew milk.

P2-81 Impact of Surface Topography and Shear Stress on Single and Dual Species Biofilm Formation by *Listeria monocytogenes* in Presence of Promotor Bacteria

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Introduction: Pathogens can form biofilm on equipment surface and subsequently contaminate foods during processing. Promotor bacteria *Ralstonia insidiosa* may facilitate stronger biofilm formation of pathogens on equipment surface.
Purpose: Single and dual species biofilm formation of *L. monocytogenes* on food contact surfaces under different hydrodynamic shear stresses was investigated.
Methods: Biofilms were grown on stainless steel (SS), PTFE, polycarbonate (PC) and EPDM coupons in a CDC bioreactor containing 10% TSB containing *L. monocytogenes* alone or with *R. insidiosa* for 48 h. *L. monocytogenes* and *R. insidiosa* populations in biofilms from surface coupons (n=144) were determined by spiral plating on MOX and TSA, respectively. The log CFU/cm^2 from three individual replicates were analyzed to determine significant differences due to surface material, shear stress, and presence of *R. insidiosa*. Surface topography of equipment surfaces were measured using profilometer and scanning electron microscopy.
Results: *L. monocytogenes* recovered from dual-species biofilms from SS, PTFE and PC surfaces were significantly higher at the lowest shear stress of 0.013 N/m^2 compared to 0.043 and 0.088 N/m^2. At the highest shear stress of 0.088 N/m^2, *L. monocytogenes* populations were significantly higher on PTFE (8.17 log CFU/cm^2) and EPDM (7.93 log CFU/cm^2) compared to SS 316L (7.38 log CFU/cm^2) and PC (7.57 log CFU/cm^2). *R. insidiosa* bacterial populations were similar on all the materials at 0.013 N/m^2 shear stress. *L. monocytogenes* recovered in dual-species environment at 0.013 N/m^2 (8.21 log CFU/cm^2) were significantly higher than in single-species biofilms (4.98 log CFU/cm^2); similar trend was observed at other shear stresses. PTFE and PC surface had higher surface roughness (3.17 and 2.36 µm, respectively) compared to EPDM (1.11 µm) and SS 316L (0.71 µm).
Significance: Surface topography and shear stress impact *L. monocytogenes* biofilm formation in a dual species environment. *R. insidiosa* promotes stronger *L. monocytogenes* biofilm on surface material.

P2-82 Screening of *Listeria monocytogenes* Strains for Blanching Validation

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Introduction: The US frozen food economy is worth $56 billion with frozen vegetables and potato products accounting for approximately 14.3% market share. These products are blanched using hot water or steam, to inactivate enzymes, expel gases, soften tissues, remove off-flavor or enhance color prior to freezing but blanching was not intended as a microbial inactivation step for foodborne pathogens nor was it a validated process. Recent recalls, e.g. frozen green beans and mixed vegetables, have heightened the significance of foodborne pathogens in the industry affecting product sold in over 100 countries including USA.
Purpose: The study was to screen various outbreak strains of *L. monocytogenes* for use in blanching validation study.
Methods: Individual *L. monocytogenes* strains (n=11) were grown using Tryptic Soy Agar with Yeast Extract (TSAYE) with Modified Oxford Medium (MOX) overlay and approximately 7 log CFU/mL were inoculated into 0.1% peptone water or various fresh produce (peas and corn). Blanching was conducted in a water bath at various temperatures and times to obtain inactivation curves and derivation of D- and z- values. Analyses were conducted using Microsoft Excel and for the construction of primary and secondary models.
Results: *L. monocytogenes* strains L303 and L338, derived from cabbage and an environmental isolate respectively, demonstrated higher heat resistance at 65ºC with up to 7 log inactivation over 180s while other *L. monocytogenes* strains had similar inactivation within 60s. A five strain *L. monocytogenes* cocktail (L303, L338, L356, L357 and L358) was further inoculated into peas and heat treated and with $D_{65ºC}$, $D_{70ºC}$ and $D_{75ºC}$ at 27.5s, 6.8s and 1.69s respectively and z-value of 8.26ºC. Additional fresh produce items are being evaluated and the data compared to those in literature.
Significance: The data generated can be utilized by industry for validation studies, assist in the development of industry guidance and provide supplement data to current Validation of Blanchers guidance.

P2-83 Validation of *Salmonella* Typhimurium MHM112 as a Surrogate for Inactivation of Pathogenic *Salmonella* Using Plasma-Activated Water

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❖ ***Developing Scientist Entrant***

Introduction: Plasma-activated water (PAW) is generated by exposing water to cold atmospheric pressure plasma. PAW is an emerging sanitizer for food/food contact surfaces which has reactive nitrogen and oxygen species.
Purpose: The purpose of this study was to provide plasma scientists and the industry with a validated surrogate for pathogenic *Salmonella* inactivation with PAW.
Methods: During PAW generation, the volume of deionized water (DI) being treated, plasma exposure time, and the distance between plasma jet nozzle and water surface play important role in determining physicochemical properties and inactivation efficacy. Using a response surface methodology from our pilot study, two optimized conditions (#1: 250 mL, 15 min, 6.5 cm, and #2: 350 mL, 15 min, 4.5 cm) were chosen to generate PAW. Three PAW batches at each of these conditions were prepared consecutively and combined to make larger volumes to understand the effect of mixing and storage. The mixed batches were investigated immediately (t_1 = 0 min) as well as after storage (t_2 = 25 min) at 40 – 46 ˚C. The microbial cultures were grown on agar and suspended in tryptic soy broth. At both time points, 3 min PAW treatment of planktonic cells of *Salmonella* Typhimurium MHM112 (avirulent strain) and a *Salmonella* cocktail prepared using 6 pathogenic strains were evaluated in triplicate (student's t-test at $p<0.05$).
Results: There was no significant difference in inactivation by PAW at each time point between the avirulent strain and the pathogenic strains for both PAW processing conditions. In addition, the storage (25 min at 40 – 46 ˚C) did not change the microbial inactivation efficacy of PAW indicating that this strain can be used as a surrogate under all tested conditions.
Significance: The avirulent *S.* Typhimurium MHM112 can be potentially used as a *Salmonella* surrogate for PAW-mediated inactivation of *Salmonella* suspensions in broth.

P2-84 Impact of Natural and Probiotic Fermentation on Anti-Nutrient Factors (ANFs) and Antioxidant Contents in Legumes and Cereals

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❖ ***Developing Scientist Entrant***

Introduction: Sorghum and cowpeas are underutilized crops due to the presence of antinutritional factors (ANFs) which interference with the body's ability to absorb certain nutrients. Fermentation processes, represent important technology strategies for reducing ANFs and enhancing nutrient qualities in foods.
Purpose: Determine the impact of natural and probiotic fermentation on ANFs levels, phytochemical composition of sorghum and cowpeas

Methods: Three accessions of sorghum (onyx, red, and lemon yellow) and two varieties of cowpeas (iron clay and red ripper) were subjected to natural and probiotic (*Lactobacillus paracasei* & *Lactobacillus acidophilus*) fermentations. Non-processed samples served as controls. Subsequently, samples were analyzed for ANFs (oxalate, saponins and tannin), and antioxidants (AOC); total phenolics content (TPC), total flavonoids content (TFC), FRAP, DPPH. Polyphenol compounds were investigated using reversed phase high performance liquid chromatography- ultraviolet (RP-HPLC-UV) system. The data was analyzed using a one-way ANOVA ($p \leq 0.05$).

Results: The study showed that tannins were the significant ANFs observed in the raw sorghum and cowpeas varieties. The highest saponin (mg DE/g) content among the sorghum varieties was in Onyx (624.20±0.10) while lowest content was in lemon yellow (34.21±0.04). Tannins (mg CE/100g) were highest in Onyx (72.93±0.73). The oxalates contents were significantly similar in all the sorghum varieties. Among the cowpea varieties, red ripper exhibited higher ANFs. The higher TPC and TFC in the sorghum and cowpea varieties justified their greater AOC measured by DPPH free radical and FRAP assays. Our results further indicated that lactic acid fermentation led to decreased ANFs and enhanced polyphenols and associated AOC. Chromatographic investigation showed the presence of major polyphenolic compounds in the samples.

Significance: This study reveals that natural and probiotic fermentation reduce ANFs and improve the nutritional qualities and antioxidant properties. The results could be used as basis for value addition in complementary food applications and provide functional and sustainable food products with nutraceuticals benefits for the food industry

P2-85 Advancements in UV Treatment of Highly Opaque Fluids: Evaluation of Microbial UV Sensitivity (D10 Value) in Skim Milk

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Introduction: UV-C irradiation has been demonstrated to be an effective means of inactivating a range of microorganisms. However, the technique for determination of UV sensitivity of microorganisms in highly opaque scattering fluids like skim milk (SM) have not yet been standardized. To obtain accurate and reproducible microbial UV sensitivity results in highly opaque fluids, it is imperative to provide adequate mixing and consider fluid optical properties (absorbance, scattering, reflection) for accurate estimation of average delivered UV dose.

Purpose: We aimed to develop a standard protocol for the determination of microbial UV sensitivity in SM at 253.7 nm wavelength.

Methods: Optical properties of the fluids were measured using double beam spectrophotometer. UV sensitivity of *E. coli* ATCC 25922 in phosphate buffer saline, SM and humic acid (absorbance and pH similar to SM) was determined using a near collimated beam system. To provide sufficient mixing, optical path length was optimized to 6 mm and treated at average UV dose ranges from 0-6 mJ/cm^2 (n =3). SM absorbance, reflectance, scattering and light divergence factors were considered for calculation of average UV fluence rate and thereby UV dose. Scattering factor was estimated by using radiative transport equation. Tukey HSD was used to assess the statistics.

Results: The absorption and scattering coefficients of SM was noticed as 4.107 ± 0.02 and 4.845 ± 0.16 (base-e, mm^{-1}), respectively. Regression analysis was conducted and D_{10} value of *E. coli* in phosphate buffer saline, humic acid and SM were calculated as 3.32 ± 0.02, 3.20 ± 0.10 and 3.31 ± 0.07 mJ/cm^2. Statistical data showed that there was no significant difference ($p > 0.05$) in the UV sensitivity of *E. coli* in different fluids which reveals the accurate average UV dose calculation in SM.

Significance: The developed experimental protocol could be useful to determine standard microbial UV-C sensitivity in highly opaque viscous scattering fluids.

P2-86 Inactivation Strategy for Reduction of Microorganisms during Rice Cakes Manufacturing Process

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Introduction: This study was conducted to establish effective reduction strategy during the manufacturing process of rice cakes.

Purpose: As a strategy for reducing microorganisms in the rice cake manufacturing process, the washing process of rice and the cooling process of rice cake after steaming were selected as they are considered the important contamination points.

Methods: Simple washing (3 times) and ultrasonic washing (40 kHz, 10 min) were used in the rice washing process, and sodium hypochlorite (100ppm, 5 min) and chlorine dioxide (20 ppm, 5 min) were used as chemical disinfectants. Combination treatment of ultrasonic washing and the two chemical disinfectants was also performed. The cooling process of rice cakes was carried out by UV (30 min, 60 min) and general drying (5, 12 hours).

Results: In the single treatment, it was found that the chemical disinfectant was more effective than the ultrasonic cleaning in a single treatment of each method, and sodium hypochlorite showed a greater reduction effect than chlorine dioxide. In the case of combined treatment, the combination treatment of chemical disinfectant and ultrasonic cleaning was more effective than the combination treatment with water, and there was no significant difference according to the type of disinfectant. In the heating process, which is an important control point of HACCP, total aerobic bacteria, coliforms, *Bacillus cereus* and *Escherichia coli* were all inactivated. UV sterilization was applied as non-heat sterilization methods and compared with the general drying method during the cooling process of rice cakes.

Significance: The total number of bacteria and coliforms, *B. cereus*, and *E. coli* were not found after the treatment, but total number of bacteria have increased after 12 hours in naturally dried rice cakes, indicating the UV sterilization process could be applied as an important control point for rice cakes.

P2-87 Monitoring of Rice Cakes Manufactured by Small-Scale Business in Korea

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Introduction: Rice cake is becoming an excellent substitute as a food culture with increasing consumption of eating out and convenience food has been established, and the consumption pattern of rice has changed from traditional rice to convenience food processed products such as seolgi and tteokbokki rice cakes.

Purpose: This study evaluated microbial contamination levels of Korean traditional rice cakes (*Garaetteok*, *Injeolmi*, *Gyeongdan*), and manufacturing environment of small-scale businesses in Korea.

Methods: The monitoring of this study was conducted on raw materials, samples for each manufacturing process, manufacturing equipment and facilities from April 20 to 23, 2021 targeting a small-scale rice cake manufacturer (company C) that has not introduced HACCP located in Jinju, Gyeongsangnam-do, South Korea.

Results: The contamination levels of total aerobic bacteria, coliforms, and *B. cereus* in raw materials were 3.76-4.48, 2.21-4.14, 1.02-1.15 log CFU/g respectively, and *E. coli* was not detected. As a result of the analysis of the contamination level by manufacturing process of rice cakes, the contamination level of total aerobic bacteria, coliforms and *B. cereus* of the raw material decreased after the washing process, but it increased again during the soaking and grinding process. However, after the steaming steps, the contamination level increased again during the molding and cooling process, suggesting caution in managing cooling water and molded rice cakes in the process.

Significance: These results suggest that the safe management of cooling water and caution in the drying process after steaming of rice cakes to control cross-contamination is necessary. Also, it is considered that food safety strategy for processing equipment and environments to reduce microbiological risks through systematic cleaning and disinfection.

P2-88 Effect of Different Environmental Stresses on Foodborne Pathogens Response to Select Chemical Treatments

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❖ ***Developing Scientist Entrant***

Introduction: Different pre- and post-harvest stresses shown to affect the physiological properties of foodborne pathogens and their subsequent response to various intervention treatments is not well understood.

Purpose: To determine the effect of different environmental stresses on foodborne pathogens response to select chemical treatments.

Methods: Foodborne pathogens namely *S. Typhimurium, E. coli* O157:H7 and *L. monocytogenes* were subjected to six different pre-growth stress conditions: (i) acid (pH of growth media at 5-5.5), (ii) high salt (additional 4% NaCl in growth media), (iii) desiccation (water activity maintained below 0.95), and (iv) temperature (14, 23 and 37°C). These cells were subjected to treatment with different active concentrations of sodium hypochlorite (100 to 200 ppm), peracetic acid (40 to 80 ppm) and 0.5% lactic acid for up to 1 min. The survivors were enumerated by plating on both selective and non-selective media. Furthermore, sublethal injury of cells was determined by the difference in enumerated populations between selective and non-selective media. All the experiments were conducted in triplicates, and the data was analyzed by ANOVA using SPSS™.

Results: Pre-growth stress conditions showed significant effect ($P \leq 0.05$) on the response of tested organisms to various chemical treatments when compared to cells grown in growth media at 37°C. For example, when desiccated, salt and acid stressed cells of *Salmonella*, *E. coli* O157: H7 and *L. monocytogenes* subjected to lactic acid at 0.5% (v/v) showed a reductions in the range of 0.44 and 0.75 log CFU/mL. Under the tested conditions *Salmonella* showed highest sensitivity to chemical treatments followed by *E. coli* O157:H7 and *L. monocytogenes*. Increasing the concentration of sanitizer(s) increased the reductions. In addition, *L. monocytogenes* showed highest percentage (20 to 66%) of sub-lethally injured cells when compared to other pathogens.

Significance: The findings highlight the need to consider pre-growth conditions as important factor for validation of physical and chemical intervention treatments.

P2-89 Statistical Process Control Using Microbial Indicators in a Commercial Beef Processing Facility

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Introduction: Ongoing enumeration of microbial indicators can provide robust data sets to assess hygienic performance and establish statistical process control parameters in commercial beef processing facilities to support food safety management decisions for regulatory compliance.

Purpose: To estimate statistical process control parameters that can be used for food safety management in a beef processing plant as well as the establishment of baselines to compare different intervention schemes, sanitary dressing improvements and process modifications.

Methods: For sample collection, pre-hydrated 25mL buffered peptone water (BPW) swabs were taken using 3M™ cattle 100cm^2 template at seven different points in the processing line including pre-evisceration, post-evisceration, final rail, after intervention, after chilling, cold carcass and after fabrication, on four different carcass locations. Samples were collected during eight different days and three samples per sampling point were taken. Swabs were homogenized and serially diluted and tested for aerobic counts(AC), Enterobacteriaceae(EB), and *Escherichia coli*(EC). Samples were enumerated using the TEMPO® system. Process control parameters were estimated with Shewhart's-methodology for each sampling location including the expected average, an upper control limit, and a lower control limit for the operation.

Results: Counts were reduced for all indicators from pre-evisceration until after carcass intervention but increased during fabrication. For EB and EC, the natural variation in the "After Intervention" step was small (0.35, 0.11 LogCFU/100cm^2, respectively) when compared with the rest of the sampling locations. For AC, a similar natural variation was observed throughout the whole processing line (0.77-0.94LogCFU/cm^2). The average-run-length(ARL) is 370.4 meaning that it can be expected to find one sample every 370 samples outside the control limits just by random chance.

Significance: Parameters estimated in this study will help to develop a baseline of quality indicators for microbial process control in beef processing plants and will serve to support decision making for process improvement, assessing the effectiveness of sanitary dressing procedures, and evaluation of microbial interventions schemes and efficacy of corrective action implementations.

P2-90 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.

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; Bioneer, Daejeon, Korea) 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 2 days 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. The relative gene expressions were calculated, using the $2^{-\Delta\Delta Ct}$ method.

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: This finding suggests that the knock-down of *irf3,* using siRNA, increases the susceptibility of HepG2 cells to HAV infection.

P2-91 Efficacies of Ascaroside Treatment in the Control of *Salmonella enterica* on Alfalfa and Fenugreek Seeds and Sprouts

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Introduction: A novel, natural, and effective antimicrobial intervention is in demand for improving the microbial safety of sprout seeds and vegetable sprouts.

Purpose: This study assessed the efficacy of ascaroside treatment in the control of *Salmonella enterica* on alfalfa and fenugreek seeds and spouts during the sprouting process.

Methods: Alfalfa and fenugreek seeds were decontaminated with 20,000 ppm of NaClO, neutralized with Dey-Engley broth, and dried for 1 h at room temperature. The seeds were then treated with 1 mM or 0.001 mM of ascaroside and dried for another hour before being inoculated with lyophilized *S.* Cubana or *S.* Stanley cells in sandy soil (10^4 CFU/g). Treated seeds and untreated controls were planted on 1% water agar plates for germination at 25°C

Poster

in the dark. Seeds or sprout samples were collected on days 1, 3, 5, and 7 of the sprouting process, and the population of *Salmonella* was subsequently determined. Results were fit into the general linear design and analyzed using the ANOVA linear model.

Results: Results of the Type III error tests show that all four experimental variables, including sprout seed, *Salmonella* strain, ascaroside treatment, and sprouting time, were significant ($P<0.05$) factors influencing the growth of the pathogen. The populations of *Salmonella* were significantly higher on fenugreek than on alfalfa sprout samples. *S*. Stanley had a significantly higher population than *S*. Cubana on sprout samples. The population of *Salmonella* increased from day 0 to day 3 and reached its peak population on Day 5. Treatments with both concentrations of ascaroside significantly decreased the populations of *Salmonella* compared to the controls. The overall *Salmonella* population reduction was 4.39 log CFU/g by treatment with 1 mM ascaroside, and 0.92 log CFU/g by treatment with 0.001 mM of ascaroside.

Significance: Ascaroside treatment could be potentially used to improve the microbial safety of sprout seeds and vegetable sprouts.

P2-92 Impact of Incorporating *Salmonella* Serotype into FSIS' Performance Standards

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Introduction: The Food Safety and Inspection Service (FSIS) administers raw poultry performance standards. Establishments' products are categorized based on the number of positive samples relative to a product-specific standard (higher category denotes more positive samples). FSIS counts any *Salmonella* positive sample toward the performance standard, but there is increasing evidence that *Salmonella* serotypes have different likelihoods of causing human disease.

Purpose: Evaluate the potential impact of alternative performance standard schemes that incorporate serotype.

Methods: Alternative product categories (1, 2, or 3) were determined for two non-overlapping 52-week windows. For scheme 1, *Salmonella* Kentucky positives were given a weight of 0.1. For scheme 2, the ratio of the percentages of each serotype in FoodNet cases to the percentage of each serotype in FSIS' product positive samples (case-to-poultry ratio) was used as a weight. The sum of weighted positives determined the alternative category based on the current standard and the maximum positive rate.

Results: Between January 2019 and December 2020, FSIS collected over 54,000 samples, of which 4,520 (8.3%) were *Salmonella* positive(60 different serotypes). Among 23 serotypes detected at least 10 times, percentages ranged from 0% to 16% for FoodNet cases and 0.25% to 26% for FSIS samples. Cases-to-poultry ratios ranged from 0 to 32. Among 721 categorized products, under scheme 1, 86% did not change category, 14% moved to a lower category and 0 moved to a higher category. Under scheme 2, 71% did not change category, 27% moved to a lower category, and 2% moved to a higher category, including 7 that moved into Category 3.

Significance: This study demonstrates the value of using serotype data to model the potential impact of alternative performance standard schemes. FSIS will be considering alternative raw poultry performance standard schemes that account for serotype or another subtype classification to better prevent human illness.

P2-93 *Listeria monocytogenes* at the Food-Environment Interface: The African Perspective

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Introduction: The African continent bears the highest burden of foodborne diseases in the world.

Purpose: This article reviews the African food systems and the risk of *Listeria monocytogenes.*

Methods: The African continent bears the highest burden of foodborne diseases in the world. Despite this challenge, research on foodborne pathogens in the continent has not received commensurate. *L. monocytogenes* is a ubiquitous organism that can contaminate foods at several stages along the food value chain. Contaminated processed ready to eat (RTE) foods are the main vehicles of transmission leading to listeriosis outbreaks with often high fatality rates. Currently, Africa is the continent experiencing the highest rate of urbanization which, in turn, is driving a rapid transformation of food systems and consumer food choices that include increased consumption of convenience RTE foods. With the proliferation of urban informal food value chains, street food vending, and poor sanitation, the threat of *L. monocytogenes* as a food safety challenge is certainly growing. Remarkably, the continent experienced the world's biggest outbreak of listeriosis in South Africa in 2017-2018. To call attention to the risk of *L. monocytogenes*, this article reviews the ecology and transmission cycle of the pathogen in the environment-food interface taking into consideration, the unique conditions of the African continent. The implementation of prevention and control approaches, genomic surveillance, outbreak investigation approaches, the establishment of microbiological criteria as well as risk-assessment procedures in the context of the disease in Africa are areas of attention that have been analyzed in depth.

Results: A detailed review of the prevalence, antibiotic resistance profiles and surveillance and control methods for *L. monocytogenes* was done.

Significance: The review will help food safety and public health authorities in designing food safety protocols to deal with *L. monocytogenes* in Africa.

P2-94 Biomapping of *Enterobacteriaceae* Counts and Aerobic Plate Counts Using Hygiena's Microsnap™ throughout a Poultry Processing Facility

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Introduction: The poultry processing industry continuously seeks new technologies to assess process hygienic performance to contribute with compliance with performance standards; rapid methods for enumeration of indicator organisms in raw carcasses and parts are needed for timely decision-making in food safety management systems.

Purpose: This study was designed to assess the performance of the MicroSnap™ new rapid (6-8h) method for microbial indicator enumeration, including *Enterobacteriaceae* (EB) and Aerobic count estimations, in chicken rinses collected in a commercial chicken processing facility.

Methods: Whole carcass and parts poultry rinses were collected from seven different locations in a commercial processing facility, including: Rehanger, Post-Evisceration, Post-Cropper, Inside-Outside Bird Washer (IOBW), Pre-Chiller, Post-Chiller and Wings. Microorganism levels were estimated at each sampling point using the TEMPO® system. In addition, 1mL from each sample were placed into the MicroSnap™ device for each microorganism and incubated for 6 hours (AC) and 7 hours (EB) and analyzed in the EnSURE™ Touch luminometer. All data was $\log_{10}$ transformed before statistical analysis using R software.

Results: AC and EB counts showed a significant difference between the seven zones ($P< 0.001$) as expected. Although, they both showed microbial reduction at different stages of the process, the biggest decrease occurred in the Post-Chiller location. The difference from the Pre-Chiller to the Post-Chiller sampling zone was 1.91 Log CFU/mL (AC) and 1.18 Log CFU/mL (EB). The rapid testing method when compared with the standardized methodology result in a slope of 0.79 ($P < 0.05$) and 0.82 ($P < 0.05$) Log CFU/mL for AC and EB, respectively.

Significance: The performance of the MicroSnap™ system in this study indicates that it can be potentially used as a rapid alternative for indicator enumeration in poultry processing chicken rinsates. In addition, the portability of the test method, allows processors to implement enumeration schemes to complement their process control systems in support of food safety management.

P2-95 Withdrawn

P2-96 Withdrawn

P2-97 ISO Validation for Pathogen Detection in Food and Environmental Samples Utilizing the Hygiena™ Foodproof *Listeria* Genus Plus *Listeria monocytogenes* Multiplex PCR Assay

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Introduction: Due to the extreme risk of illness from foods contaminated with *with Listeria monocytogenes*, many Ready-to-Eat products and food production environments are highly monitored utilizing *Listeria* spp. as an indicator. However, currently available PCR assays require a separate secondary test to identify *L.* monocytogenes, which causes delays for corrective actions and increases laboratory labor.

Purpose: The purpose of these studies was to validate the **food**proof *Listeria* plus *L. monocytogenes* PCR LyoKit for detection and discrimination between *Listeria monocytogenes and food relevant Listeria* species (*Listeria* sensu stricto) across food and environmental samples.

Methods: Validation of the alternative method was performed for 5 food categories (25 g) and environmental samples. The study comprises 50 inclusivity and 30 exclusivity strains per target, sensitivity, relative level of detection (RLOD) and collaborative studies.

Samples were enriched in Half-Fraser Broth 1:10 at 30°C for 24 h-46 h and in Actero™ *Listeria* Enrichment Broth 1:7 at 36°C for 20-24 h. Following incubation, DNA extraction was performed with **food**proof StarPrep Two Kit, then lysates were analyzed by real-time PCR. All samples were compared to the cultural reference method.

Results: The validation study (NordVal No. 054) indicated that the alternative method performs equally compared to reference method EN ISO 11290-1:2017 and fulfills the validation criteria according to EN ISO 16140-2:2016. The specificity studies yielded 100% inclusivity of the 50 target strains and 100% exclusivity of the 30 non-target strains. Additionally, the acceptability limits for the sensitivity and the RLOD studies for all categories and enrichment protocols were met.

Significance: The validation of the **food**proof *Listeria* multiplex assay provides many industries with the ability to quickly screen for *Listeria* and then immediately identify if corrective actions need to occur for *Listeria* monocytogenes in a single test. *This improves operational efficiencies throughout food production and testing laboratories.*

P2-98 Development and Validation of Hygiena™ Real-Time PCR Assay for the Detection and Identification of Coagulase Positive *Staphylococcus* Species and *S. aureus*

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Introduction: Roughly 25% of people have *Staphylococcus* aureus present on their skin which can lead to a large rate of food poisonings from food service workers and ready-to-eat foods. Additionally, this pathogen contributes to highly contagious mastitis in dairy cows resulting in animal health and raw milk implications. Culture methods are too laborious, time-consuming, and difficult to interpret to provide rapid results to identify and control the spread of infections.

Purpose: The aim of these studies was to develop a rapid, real-time PCR assay that detects all coagulase-positive *Staphylococcus* species (CPS) and simultaneously identifies the most relevant CPS species, *Staphylococcus aureus.*

Methods: The sensitivity of the *Staphylococcus* detection assay was compared to the ISO 6888-3 culture reference method with various matrices such as infant formula, whey powder, milk powder, cheeses, ice cream, and salad dressings. The matrices were spiked with *Staphylococcus* (*S. aureus* and CPS) at 1-10 CFU/g(ml) and were enriched with Giolitti-Cantoni broth according to ISO 6888-3. Following incubation, extraction was performed utilizing **food**proof StarPrep Two kit (single-well or 8-Strip), with lysates analyzed using real-time PCR. Specificity was determined by testing a panel with more than 150 isolates total, with 55 isolates of *Staphylococcus aureus* and 23 other coagulase-positive *Staphylococci*.

Results: All PCR results were not significantly different compared to the reference culture method. Utilization of the **food**proof assay in combination with the extraction and enrichment methods identified, at minimum 10^3 CFU/ml after enrichment were consistently detected across the wide variety of matrices. The specificity of the kit (100%) was confirmed from the inclusivity and exclusivity panel.

Significance: Providing the animal health, Ready-to-Eat, and food service industries with a rapid, easy to use PCR method for coagulase-positive *Staphylococcus* species, that meets all regulatory requirements, can drastically improve contamination management of this highly infectious pathogen.

P2-99 Validation of the Hygiena™ foodproof® *Enterobacteriaceae* Plus *Salmonella* Detection PCR Kit Compared to ISO Reference Methods for Infant Cereals, Infant Formula with or without Probiotics and Ingredients, and Production Environmental Samples

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Introduction: Throughout the production of infant formula and cereals, regulatory agencies not only require pathogenic screening for *Salmonella* and *Cronobacter* spp. but also require continued monitoring of the environment for hygienic indicators such as *Enterobacteriaceae*. PCR technologies have improved for pathogens; however, indicator testing continues to lack in advancements for sensitivity, specificity, and time-to-results.

Purpose: The aim of this validation was to provide infant formula industries with a real-time PCR assay that detects both *Salmonella* and *Enterobacteriaceae* from a single enrichment and PCR reaction.

Methods: The alternative method was compared to cultural ISO methods by ADRIA Développement for the detection of *Enterobacteriaceae* (ISO 21528-1:2017) and *Salmonella (*ISO 6579-1:2017) in infant cereals, infant formula with or without probiotics and ingredients (375 g), and production environmental samples (up to 200 g). A 1:10 enrichment with buffered peptone water (supplemented with 10 mg/L vancomycin for probiotic containing samples) was created and incubated at 37°C for 16-20 h. Following incubation, DNA extraction was performed using **food**proof® StarPrep One and Three extraction kits, with analysis using real-time PCR. Specificity panels were evaluated to ensure inclusivity and exclusivity of PCR targets.

Results: The **food**proof® *Enterobacteriaceae* plus *Salmonella* Detection PCR assay in combination with the extraction options successfully yielded results comparable to the reference methods to gain ISO validation. The specificity studies produce 100% inclusivity for the 53 *Enterobacteriaceae and* 100 *Salmonella* target strains with 100% exclusivity of 30 non-target strains.

Significance: The novel Hygiena™ **food**proof® method advances indicator monitoring by providing a rapid, PCR-based technology for *Enterobacteriaceae* with further identification of *Salmonella* in a single enrichment and test. Not only does this method provide more sensitive and specific results for indicator testing, but it also reduces technician resource requirements by avoiding multiple consumables, testing platforms, and data entry.

P2-100 Validation of the Hygiena™ foodproof® *Salmonella* Genus Plus Enteritidis and Typhimurium PCR Kit for Raw and Ready-to-Cook Meat and Poultry Products and Environmental Samples

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Introduction:
Identification of the most relevant serovars for human and animal health, *S.* Enteritidis and *S.* Typhimurium, is critical when managing the food production chain from farm-to-fork to reduce consumer risk. Global regulations concerning these serovars require test methods to be robust in sensitivity and specificity to ensure reliable and accurate results.

Purpose:
This study evaluated the **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit in combination with **food**proof® StarPrep Three Kit for extraction compared to ISO 6579-1:2017 and ISO/TR 6579-3:2014 reference methods according to the requirements of DIN EN ISO 16140-2:2016 and the NordVal International validation protocol.

Methods:
Sensitivity, relative level of detection (RLOD), and specificity studies were conducted by Campden BRI for the method comparison part of the NordVal International validation study. For sensitivity and RLOD studies, artificially contaminated samples of raw meat and poultry, ready-to-cook meat and poultry products and environmental samples were incubated for 16-20 hours at 37°C, following sample preparation according to ISO 6887 methods. After incubation, DNA extraction was performed in both single tube and 8-strip formats followed by real-time PCR analysis. Specificity testing included 25 *S.* Enteritidis, 25 *S.* Typhimurium, 75 *Salmonella* spp. strains, and 30 non-target strains.

Results:
Based on experiment results and an interlaboratory study, the assay met all requirements compared to ISO reference methodologies to receive certification (NordVal No. 055). The observed sensitivity and RLOD values met the acceptability limits for paired studies for all three targets. Specificity claims for the assay and extraction method combination successfully detected and identified 100% of target strains with 100% exclusivity of non-target strains.

Significance:
This validation provides a multiplex real-time PCR assay that is a rapid and reliable alternative method for the detection of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw and ready-to-cook meat and poultry products and environmental samples.

P2-101 Combined Effects of UV-C and Superheated Steam on Inactivation of *Enterococcus faecium* and *Geobacillus stearothermophilus* Spores on Stainless Steel: Influence of Food Residue

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❖ ***Developing Scientist Entrant***

Introduction: There is a need for novel dry sanitation technologies for treatment of food plant surfaces. Superheated steam (SHS) and UV-C both represent promising technologies; however, there is limited data on their combined effects.

Purpose: The objective was to investigate the individual and combined effects of UV-C and superheated steam on the inactivation of *Enterococcus faecium* and *Geobacillus stearothermophilus* spores on stainless steel in the presence of food residue.

Methods: *E. faecium* NRRL B-2354 and *G. stearothermophilus* ATCC7953 spores were spot-inoculated (SP), or inoculated wheat flour (FL,0.30±0.01mm) and dry milk film (DM,0.30±0.02mm) were coated on stainless steel coupons (34.9mm×23.8mm×4.8mm). Four different treatment conditions were used: 1) UV-C (6 cm from UV-C LED at 255 nm), 2) SHS (180°C in the preheated chamber), 3) UV-C followed by SHS, and 4) SHS followed by UV-C. The experiments were carried out in triplicate.

Results: The fluence rate of UV-C was 0.4140±0.0004 mW/cm^2. The Weibull model was used to evaluate the UV dose (δ_{UV}) and SHS treatment time (δ_{SHS}) of first decimal reduction (R^2>0.95). After UV-C pre-treatment for 8 mJ/cm^2, δ_{SHS} of *G. stearothermophilus* were 89.1s, 245.3s, and 241.1s for SP, FL, and DM, respectively, which were similar to δ_{SHS} values without UV-C pre-treatment (89.7s, 268.4s, and 228.9s for SP, FL, and DM, respectively). In contrast to δ_{SHS}, δ_{UV} of *G. stearothermophilus* after SHS treatment increased from the δ_{UV} without SHS treatment (from 3.6 to 6.0 mJ/cm^2, from 2.6 to 249.2 mJ/cm^2, and from 165.9 to 430.6 mJ/cm^2 for SP, FL, and DM, respectively). Similar trends were also observed for *E. faecium*.

Significance: Combined UV-C followed by SHS treatment may be an effective sanitation approach achieving up to 5-log reduction of *G. stearohermophilus* spore using 8mJ/cm^2 of UV-C irradiation followed by 300 s, 500 s, and 500 s of SHS treatment for SP, FL, and DM, respectively.

P2-102 Validating Commercial-Scale Dry-Roasting Process for Hazelnuts Using *Enterococcus faecium*: Critical Limits Depend on Roaster Design

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Introduction: Dry roasting equipment used for hazelnut processing is diverse in design and biological verification is necessary to identify appropriate critical limits to establish process controls to mitigate *Salmonella* contamination.

Purpose: To identify critical limits that achieve a 5-log reduction of *Enterococcus faecium* ATCC 8459 (*Salmonella* surrogate) in two unique commercial-scale hazelnut dry roasting systems.

Methods: Hazelnut kernels inoculated with *E. faecium* and transported to two commercial hazelnut processors for roasting. Roaster A (Buhler Aeroglide) is a continuous belt roaster with temperature, belt speed, and bed depth as processing parameters. Roaster B (Sivetz) is a batch air roaster with target temperature and total dwell time as processing parameters. Roasted nuts were analyzed for survivors using standard plate counting methods on tryptic soy agar (TSA) overlaid with m-Enterococcus Agar (mEA) and incubated at 37ºC for 72 h.

Results: Roaster A: Roasting parameters of 190ºC/375ºF for 12.9 min resulted in 6.9 log CFU/g reduction of *E. faecium* when the bed-depth was minimal (1 inch); however, *E. faecium* was not sufficiently inactivated (2.5 log reduction) when bed depth was increased to 3-inches.

Roaster B: Cumulative roasting time to achieve 149ºC/300ºF (5.6-7.0 min), 157ºC/315ºF (6.6-8.3 min), and 165ºC/330°F (7.6-9.7 min) resulted in log reductions of 5.4±0.6, 5.8±0.4, and 5.8±0.6, respectively.

Significance: Results from this study demonstrate significantly different efficacy of roasting temperatures and dwell times on the inactivation of *E. faecium* and clearly support the necessity of biological verification studies at commercial scale.

P2-103 Combining Postbiotic Metabolites (*Lactiplantibacillus plantarum* [M.21]) with Eugenol and Thymol Against Pathogenic Microbial Biofilms on Food-Processing Surfaces and MBECTM Biofilm Device

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❖ ***Developing Scientist Entrant***

Introduction: Humans are continually on the lookout for new ways to keep food safe from harmful chemical and microbiological deterioration, as well as foodborne illnesses. Foodborne pathogens can develop biofilms on food and food-processing appliances.

Poster

Purpose: The aim of this study was to investigate the efficacy of metabolic byproducts from Kimchi-derived *Lactiplantibacillus plantarum* M.21 and plant-derived essential oils (EO) as "green" anti-biofilm agents.

Methods: Postbiotic production and metabolic profiling, minimum inhibitory concentration (MIC), *in vitro* susceptibility and antibiotic test, stability assay, antimicrobial gene detection, metabolic and activity ion leakage measurement, biofilm inhibition on stainless steel, rubber, low-density polyethylene plastic, MBEC™ biofilm device, and visualization by confocal microscopy were performed to identify the anti-biofilm mechanism by combination of postbiotic with EO on food processing surfaces.

Results: Postbiotic was synthesized from lactic acid bacteria (LAB) isolate and analyzed their metabolic components to identify the antimicrobial potentiality against three pathogenic microorganisms (*Vibrio parahaemolyticus, Pseudomonas aeruginosa*, and *Escherichia coli*). Moreover, the post, biotic exhibited residual antimicrobial activity at diverse environmental conditions and identified the bacteriocin-coding genes in the LAB M.21 strain. The MIC assay revealed that the combination of sub-MIC (0.5 MIC) of postbiotic and EO could significantly inhibited the biofilm cells on food-processing surfaces (e.g., rubber, stainless steel, plastic) and MBEC™ biofilm-forming device.

Significance: The *in vitro* studies proved the potentiality of LAB-produced postbiotic and EOs as bio-protective agents in the food processing industry.

P2-104 Inhibition of Biofilm Formation, Quorum Sensing Signaling,and Virulence Genes of Foodborne Pathogens *Salmonella* Typhimurium and *Escherichia coli* Using Flavourzyme

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Introduction: *Salmonella* Typhimurium and *Escherichia coli* are two major and highly resistant foodborne pathogens. Due to several negative effects of chemical treatments, enzyme-based techniques are currently receiving great attention.

Purpose: The purpose of the study was to investigate the antagonistic effect of Flavourzyme and find out how it interrupts the biofilm formation and pathogenicity of *S*. Typhimurium and *E. coli* at a molecular level.

Methods: The minimum inhibitory concentration (MIC), sub-minimum Inhibitory concentration (sub-MIC), antibiofilm assessment by crystal violet and MBEC™ biofilm device assay, epifluorescence microscopic analysis, confocal laser scanning microscopic (CLSM) analysis, AI-2 verification with bioluminescence assay and high-performance liquid chromatography, and genomic expression analysis by quantitative real-time polymerase chain reaction were performed to determine inhibitory mechanisms of Flavourzyme against *S*. Typhimurium and *E. coli*.

Results: The present study revealed 4.0 and 5.5 log CFU/peg inhibition of biofilm formation by *S*. Typhimurium and *E. coli*, respectively, on the MBEC™ biofilm device treated with sub-MIC of Flavourzyme for 24 h. Visual examinations supported the inhibition of biofilms. For both bacteria, the enzyme exhibited quorum-quenching activity, preventing autoinducer-2 production. In addition, Flavourzyme significantly suppressed the relative expression levels of biofilm-forming, quorum sensing, and virulence regulatory genes.

Significance: The current study suggests Flavourzyme as a preventive agent against foodborne pathogens that possibly acts by inhibiting bacterial self-defense mechanisms following disruption of cellular proteins, which can be used to ensure food safety.

P2-105 Exposure to Protective Culture *Hafnia alvei* Attenuates *Salmonella* Virulence in Food and Intestinal Models

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Introduction: *Hafnia alvei* B16 (HA) is commercially available protective culture with demonstrated inhibitory effects on *Salmonella* growth. However, its ability to attenuate pathogen virulence in food and exhibit probiotic effects against pathogenic infection in the host remains uninvestigated.

Purpose: The purpose of this study was to determine the potential for HA to attenuate *Salmonella* virulence in food and to act as a probiotic by protecting against pathogen infection in human Caco-2 epithelial cells.

Methods: Multi-drug resistant *Salmonella enterica* serovars Typhimurium and Newport were used. Each serovar was cultured alone (control) or cocultured with HA in UHT milk and incubated at 37°C for 24 h in order to determine the antivirulence effect of HA. Subsequent changes in pathogen adhesion and invasion, and virulence gene expression were determined using Caco-2 monolayers and quantitative RT-qPCR. To determine the probiotic effect of HA, monolayers were pre-treated with HA before infection with *Salmonella*. Changes in adhesion and invasion were then evaluated. All experiments were repeated three times and significance was considered when $P < 0.05$.

Results: Exposure to HA in milk reduced the subsequent adhesion and invasion of *S*. Typhimurium by 95.23% and 86.95%, respectively, compared to control ($P < 0.02$). Comparatively, HA did not affect the subsequent adhesion of *S*. Newport but decreased its invasion by 86.58% compared to control ($P < 0.03$). Coculture with HA decreased the expression of hilA, ssrB, and sopD in *S*. Typhimurium, whereas expression of flhD and sopD was decreased in *S*. Newport. In probiotic assays, HA achieved adhesion capacity of 2.18% relative to the inoculums. Pre-treatment of monolayers with HA reduced invasion of both serovar by ≥86% ($P < 0.03$).

Significance: These findings demonstrate the potential ability of HA to enhance the safety of food products by attenuating *Salmonella* virulence, and to exert probiotic effects in the host through protection against *Salmonella* infection.

P2-106 Assessment of Food Safety Culture in Food Production and Finished Goods Warehouse Facilities in a Low-Risk Food and Drink Manufacturer

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Introduction: The food and drink manufacturing and processing industry (FDMP) has not only a legal, but a moral responsibility to control food-safety to protect the consumer. Loss of food-safety control can also jeopardise the company reputationally/financially. A positive food-safety culture (FSC) forms the foundations of a robust food-safety management system and determining targeted improvement mechanisms may strengthen a business' FSC.

Purpose: FSC assessment in a low-risk FDMP company within food-production and warehouse employee groups to identify areas for improvement.

Methods: Using a bespoke FSC measurement mechanism, attitudes associated with key FSC parameters (People/Process/Purpose/Proactivity) were assessed from employees working in food production and finished goods warehouse facilities. Attitudes were assessed using a 5-point Likert scale and data was analysed to determine targets for FSC improvement.

Results: Quantitative data indicated food-production(n=93) and warehouse(n=27) employees' attitudes towards FSC parameters. Overall, there were more positive attitudes towards general food-safety attributes were determined from food-production-employees (mean:4.2,SD:1.1) compared with warehouse-employees (mean:3.9,SD:0.1). Differences between management and operative employees were determined particularly toward FSC awareness (food-production-management mean:3.8,SD:1.0/food-production-operative mean:3.3,SD:1.3) (warehouse-management mean:3.5,SD:0.5, warehouse-operative mean:3.3,SD:0.2).

Overall, 94.3% of food-production-management agreed that they "took pride in their work" compared with 56.9% of food-production-operatives. In contrast, 100% of warehouse-employees agreed with the statement. This identifies a specific employee group for targeted improvement interventions. Conversely, warehouse-employees disagreed that "they have witnessed food-safety being compromised in favour of efficiency" (operative: mean:3.5/

management: mean:4.0,SD:0.4) signifying that intervention strategies can be focussed on both employee groups. Factory and warehouse attitudes toward communication were similar between food-production and warehouse-employees (warehouse mean:3.0/food-production mean:2.9), there was a larger variance in attitudes from factory-employees (warehouse SD:0.4/food-production SD:1.32) indicating a need for communication improvement across the business.

Significance: Key findings procured will enable the development of targeted interventions for specific employee groups within the business including increasing pride in food-production-operatives.

P2-107 Antimicrobial Activity of a Photocatalytic Titanium Dioxide-Coated Stainless Steel

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❖ ***Developing Scientist Entrant***

Introduction: Titanium dioxide (TiO_2) shows strong antimicrobial activity when incorporated as coatings on food contact surfaces (FCS), but the durability of such coatings warrants further investigation to fully utilize this technology in the food industry.

Purpose: This research aimed to create a photocatalytic, TiO_2 stainless steel coating with optimized photocatalytic activity and durability, and test the optimized coating against foodborne pathogens for FCS applications.

Methods: The synthetic factors of the photocatalytic sol-gel TiO_2 coatings on food-grade stainless steel were varied via two different protocols. Protocol 1 was to produce porous anatase TiO_2 coatings, while Protocol 2 was to produce non-porous anatase TiO_2 coatings. The antimicrobial properties were examined by exposing *Escherichia coli* O157:H7 and *Staphylococcus aureus* under the following treatments: i) Antimicrobial coating irradiated with UV light (C-UV); ii) No antimicrobial coating irradiated with UV light (NC-UV); iii) Antimicrobial coating with no UV light irradiation (C-NUV), and iv) No antimicrobial coating with no UV light irradiation (NC-NUV).

Results: The gel obtained by a peroxo-catalyzed sol-gel reaction aged for 200 h, spun at 2000 rpm, and sintered at 595 °C was found to produce the optimal antimicrobial coating. The C-UV and NC-UV treatments showed significant reductions ($P \leq 0.05$) in numbers of both pathogenic bacteria starting from 3 h, as compared to the C-NUV and NC-NUV treatments. Around 7.5-log and 6.5-log reductions in *E. coli* O157:H7 and *S. aureus*, respectively, were obtained on coated stainless steel surfaces after 24 h of UV light irradiation (C-UV), as compared to the NC-NUV and C-NUV treatments.

Significance: The photocatalytic, mechanical, and antibacterial properties of the coating make it an attractive tool as a sanitation strategy that could synergistically be applied with other current sanitation methods on FCS to promote food safety.

P2-108 Persistence of *Salmonella* Javiana and *Listeria* spp. in Hydroponic Nutrient Solution at Different Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: Leafy green consumption has been increasing due to the various associated health benefits. To meet consumer demands, produce seasonal leafy vegetables year around, and grow fresh produce in controlled environments, hydroponic systems (soilless cultures) are being increasingly used and adopted by growers. Thus, it is important to characterize the microbial risks within these unique environments.

Purpose: To determine the persistence of *Salmonella* Javiana, *Listeria monocytogenes*, and *Listeria innocua* in hydroponic nutrient solution (NS) at 25°C, 30°C, or 37°C, over a 21-day period to mimic time from seedling to mature lettuce in hydroponic system.

Methods: The modified Hoagland's NS (beneficial for lettuce growth in hydroponic system) at pH 5.5 was prepared. Bacteria were inoculated separately at 6 log CFU/mL in 100ml of NS in 250ml glass bottles. The inoculated and control NS bottles were placed in a shaking incubator at 50 rpm and maintained at 25°C, 30°C, or 37°C. Samples were collected on day 0, 1, 3, 5, 7, 14 and 21, followed by serial dilution and plating on MOX (*Listeria* spp.) and XLT-4 (*S.* Javiana) selective media. Experiments will be completed in triplicate for each temperature.

Results: At 25°C, 30°C and 37°C, *S.* Javiana persisted throughout the 21-day study period with a reduction of 1.2, 4, and 4.2 CFU/ml. However, *L. innocua* and *L. monocytogenes* was detectable up to 8 to 21 days and 2 to 15 days respectively, demonstrating a 6 log reduction at all temperatures.

Significance: Understanding bacterial persistence in NS will aid future work characterizing risk of pathogen internalization in lettuce during hydroponic production. Based on these data, *Salmonella* may be a greater risk when compared to *L. monocytogenes*; thus, pathogen persistence in the presence of lettuce roots will be evaluated to further substantiate this.

P2-109 Use of an *Escherichia coli* Pilin Gene (*tra*A) to Identify Human Fecal Contamination

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❖ ***Developing Scientist Entrant***

Introduction: An elevated number of *Escherichia coli* in food or water is indicative of fecal pollution, one of the major causes of foodborne disease outbreaks, but it does not provide any information on the pollution sources which is critical for developing effective steps to improve food safety.

Purpose: The objective of this study was to identify a genetic marker in *E. coli* that could be used to identify human, cattle, chicken or pig feces for controlling fecal pollution in our food supply chain, including irrigation water.

Methods: Nucleotide polymorphisms of 10 pili-related genes among 80 publicly available genomes of *E. coli* (20 each from human, chicken, cattle and pig) were analyzed by using the multiple sequence alignment program, Multalin. Any gene showing a significant nucleotide polymorphism was used to develop an end-point PCR assay. The resulting PCR was evaluated for its sensitivity and specificity in detecting *E. coli* of human, cattle, chicken or pig origin, using genomic DNA extracted from 205 *E. coli* fecal isolates (48 human, 55 chicken, 50 cattle, 52 pig).

Results: The pilin gene, *tra*A, showed a significant polymorphism among the 80 *E. coli* genomes and a signature sequence was identified to be specific to 11 out of 20 (55%) human-origin *E. coli* genomes. Based on the sequence, HtraA-PCR was developed for rapid detection of human-origin *E. coli*. The results demonstrated that 58.3% of human *E. coli* isolates was positive, while only 1.3% nonhuman-origin *E. coli* isolates (two cattle, no chicken, no pig) was positive in this PCR. Thus, sensitivity and specificity of HtraA-PCR was 58.3% and 98.7%, respectively, for detecting *E. coli* of human origin.

Significance: HtraA-PCR may provide a new tool for rapid and accurate detection of human *E. coli* and therefore, human fecal contamination in our food supply chain.

P2-110 Bio-Mapping of *Salmonella* Levels in Two Commercial Poultry Processing Facilities to Establish Statistical Process Control Parameters, Assess the Performance of Antimicrobial Intervention Schemes and Implement Risk-Based Food Safety Management Decisions

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Introduction: Pathogen control in poultry processing facilities, including the ones implementing the New Poultry Inspection System (NPIS) 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.

Purpose: To develop product-specific *Salmonella* level baselines for two commercial poultry processing facilities operating under the NPIS at line speeds of 175 birds per minute.

Methods: Two commercial processing facilities, plants A and B were sampled during 13-week period following the sampling scheme of the USDA-FSIS performance standards. Seven locations throughout each facility were sampled: Live Receiving (LR), Rehang (R), Pre chill (PRE), Post chill (POST) and parts: Wings (W), Thighs (T) and Breast (B). At each location, two samples were taken per repetition, 14 per shift. A total of 52 samples were taken for each process location. *Salmonella* counts and prevalence were determined using the BAX® SalQuant™ system and the detection *Salmonella* essays, respectively. All counts were transformed to Log CFU/mL of rinse.

Results: For plant A 116 of 322 samples were suitable for enumeration using the BAX® System SalQuant™, the majority found at LR, a mean difference of 2.48 Log CFU/Carcass was found between the LR and POST sampling points. For plant B 131 of 364 samples were suitable for *Salmonella* enumeration, LR were the point where the most countable samples were found, a mean difference of 2.33 Log CFU/Carcass was found between LR and POST sampling points. *Salmonella* counts were significantly different (P< 0.05) in all sampling points for both facilities.

Significance: In plant bio-mapping studies with *Salmonella* levels 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 of overall process improvement.

P2-111 Patented Organic Peracetic Acid and Hydrogen Peroxide-Based Sanitizing Solution Achieves >5 Log CFU/g Reduction in *Salmonella* Surrogate *Enterococcus faecium* NRRL B-2354 on Brazil Nuts at an Industrial Scale

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Introduction: *Salmonella*-contaminated Brazil nut recalls warrant implementation of a pathogen control method. One promising method involves application of a patented peracetic acid and hydrogen peroxide sanitizing solution followed by drying.

Purpose: The study objectives were to: 1) identify a suitable *Salmonella* surrogate for Brazil nuts, 2) determine parameters for solution application and drying for a > 5 log CFU/g reduction while maintaining sensory attributes, and 3) validate these parameters in commercial equipment.

Methods: Irradiated Brazil nuts were inoculated (20 mL/kg overnight culture) with *E. faecium* or *Salmonella* (serovars Newport, Senftenberg, Oranienburg, Saintpaul, Typhimurium DT104 and Cubana) followed by overnight acclimatization. Samples were treated (20, 40, 60, and 80 mL/kg). Recovery was compared before and after treatment (tryptic soya agar; n=3).

For efficacy testing, *E. faecium* inoculation rate was increased (60 mL/kg) to ensure 5 log CFU/g reduction detection. Inoculated samples were treated and dried (40 mL/kg, 93.33 °C). Recovery was compared before and after processing (Slanetz and Bartley agar; n=5). Uninoculated and treated samples were evaluated for sensory (5-point hedonic scale for blind smell, appearance, taste, and texture assessment, n = 5 participants).

For validation, 27 kg of inoculated Brazil nuts (60 mL/kg overnight culture, food colouring added as a visual indicator) were mixed with fresh Brazil nuts (total volume 798 kg), treated in a commercial applicator and dryer (40 mL/kg, 93.33 °C). Samples were collected every minute from the dryer exit, and inoculated nuts were sorted out. Recovery (n=11) was compared to untreated, inoculated samples (n=10, Slanetz and Bartley agar).

Results: *E. faecium* was an appropriate *Salmonella* surrogate. A 6.04 and 5.91 log CFU/g reduction of *E. faecium* was achieved at lab and industrial scale, respectively.

Significance: The commercial applicator and dryer is a suitable pathogen control method for Brazil nuts, achieving a > 5 log reduction in a *Salmonella* surrogate while maintaining sensory attributes.

P2-112 Validation and Reproducibility of a Quantitative Survey of Correlation between Operating Conditions and Food Safety in Child Development Centers

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Introduction: Food safety conditions in childcare centers have significant influence on the health of preschoolers, therefore operating conditions of childcare centers need to be in accordance with food safety rules to prevent foodborne illness.

Purpose: We analyzed and validated the application of a survey to measure the correlation between operating conditions and food safety in child development centers.

Methods: This descriptive and cross-sectional study with a quantitative approach was conducted between July and September 2018 in 20 Child Development Centers located in Monteria, Cordoba – Colombia. A 28-question survey was designed using a correlational matrix with the operating conditions established by the Instituto Colombiano de Bienestar Familiar (ICBF) for the institutional modality and the five most often food safety risk factors responsible for foodborne illness, according to the Centers for Disease Control and Prevention (CDC). Three levels of compliance in food safety conditions were defined: low, medium, and high. The statistical validity of the survey was measured by the Cronbach's Alpha coefficient and data were analyzed through descriptive statistics and the SPSS program.

Results: Internal consistency of the survey, according to the Cronbach's Alpha, was 0.909; demonstrating reliability in the designed instrument. After analyzing the 28 safety conditions, the study revealed that 20 of them showed high level of compliance, while the other 8 showed medium or low compliance. These 8 food safety conditions later were subjected to association, dependence and independence tests with other variables of interest, showing significant independence in the chi-square independence test, and non-significant associations according to the Cramer's V statistic.

Significance: The applied survey provided useful information about the food safety conditions in Child Development Centers, concluding that conditions with medium and low performance are related to the 5 safety conditions that cause the majority of Foodborne Diseases according to the CDC.

P2-113 Independent Laboratory Study for the GENE-UP® Pathogenic *Escherichia coli* Method

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Introduction: The GENE-UP® PEC employs real-time PCR technology for the accurate detection of Shiga toxin – producing *Escherichia coli* (STEC) from select food matrixes. The assay utilizes dual Fluorescence Resonance Energy Transfer (FRET) hybridization probes. These probes consist of two different, short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the reaction cycle. FRET occurs only after the two probes come in close proximity from hybridizing to the template DNA. After the PCR cycling program finishes, the PCR product(s) are melted to determine the presence of the target DNA. The software interprets data for each sample and generates a positive, negative, or inhibited result.

Purpose: The purpose of this AOAC® Independent Laboratory Study was to compare the candidate method to FDA/BAM Chapter 4A for bagged spinach.

Methods: The rapid method was compared to FDA/BAM Chapter 4A: Diarrheagenic *Escherichia coli* following an unpaired study design for the matrix study.

Results: Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. No statistically significant difference was observed between two methods.

Significance: This novel assay allows for fast, reliable detection of STEC. The kits are performed with the utilization of the GENE-UP Thermocycler. The GENE-UP® Thermocycler detects fluorescence at several wavelengths (channels) to allow multitarget detection in the same reaction.

P2-114 Combining Hollowfiber Concentration with the Automated Liquid Crystal Detection Technology for Detection of Shiga Toxin Producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC), cause illnesses ranging from mild diarrhea to ischemic colitis and hemolytic uremic syndrome (HUS), are a major concern for the food industry.

Purpose: This study utilized a commercial concentration tool and an automated detection assay to shorten enrichment time for the detection of STEC (O26, O45, O103, O111, O121, O145, O157:H7) in pure culture and *E. coli* O157:H7 in baby spinach, and also investigated pooling as a method to optimize throughput and control cost.

Methods: Three test protocols were conducted to evaluate the effect of hallow fiber concentration on autoXpress detection of STEC. First, the detection limit was determined for each STEC grown in pure culture and concentrated. Second, detection of *E. coli* O157:H7 enriched in raw baby spinach for 6, 6.5, and 7 hours was determined. The post-enrichment samples were tested with and without concentration to determine detection accuracy. Third, Samples incubated for 7 – 9 hours were diluted and tested with and without concentration.

Results: For the individual serovars, the automated assay detected $\log_{10}$ 4.56 to 4.98 CFU/ml when grown in pure culture and concentrated. *E.coli* O157:H7 enriched in baby spinach and incubated for 6, 7 or 7.5 hours were accurately detected when concentrated but poorly detected in unconcentrated samples. A final set of samples was diluted in 2 volumes of media, incubated for up to 9 hours and evaluated with or without concentration. Concentrated samples were accurately tested after 7, 8, or 9 hours whereas non-concentrated samples were not detected.

Significance: The test results show that sample concentration can shorten enrichment time for autoXpress samples when pooling multiple samples prior to concentration could potentially diminish the concentration costs.

P2-115 Multi-Laboratory Validation Study of a Real-Time Quantitative PCR Method for Detection of *Salmonella* in Baby Spinach

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Introduction: The FDA BAM *Salmonella* culture method has been widely used for detecting the pathogen in food matrices during outbreak investigations. However, the method takes at least 3 days for a negative or presumptive-positive result.

Purpose: To reduce testing time and cost, the FDA developed a real-time quantitative PCR (qPCR) method to effectively detect *Salmonella* within 24 hours, using the ABI 7500 PCR system. The method has been evaluated to be used as a rapid screening method in Single Laboratory Validation (SLV) studies. This study was to evaluate the method reproducibility on baby spinach using a Multi-Laboratory Validation (MLV).

Methods: Sixteen laboratories from FDA ORA and state public health departments participated in the study. It consisted of two rounds of analysis of twenty-five samples. The 25 samples included 8 high level, 8 low level, and 8 uninoculated samples, plus one sample for total aerobic plate count. All samples were analyzed with both the qPCR alternative and the BAM reference culture methods. Statistical analysis was performed per ISO16140-2:2016 (Microbiology of the Food Chain-Method Validation-Part 2).

Results: The first MLV round yielded a positive rate (84%) from the low-level samples that was higher than the FDA Microbiological Method Validation Guideline required level of 50% ± 25%. The second MLV round yielded 67% positive from the low-level samples, which met the requirement. Statistical analysis showed similar detection rates of the two methods. The reproducibility among laboratories of the qPCR method was high as demonstrated by the low values of standard deviation of laboratory effects (0.136) and intra-laboratory correlation coefficient (nearly 0).

Significance: The MLV study demonstrated that the qPCR method was sufficiently sensitive, specific, and reproducible for detecting *Salmonella* in baby spinach as a rapid screen method. After being validated with another food, this method is anticipated to be incorporated into the FDA BAM, which serves as an important tool in outbreak investigation and regulatory testing.

P2-116 Rapid Detection of *Salmonella enterica* in Fresh Produce by a Novel Microarray-Based PathogenDx System

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Introduction: Consumption of fresh produce contaminated with *Salmonella enterica* may result in significant risk of foodborne illnesses. The diverse matrices pose great challenges for rapid *Salmonella* detection.

Purpose: The efficacy of PathogenDx microarray-based detection system for the rapid detection of *Salmonella* in fresh produce was investigated.

Methods: A pre-PCR sample preparation protocol including enrichment in universal pre-enrichment broth for 3 h with a sample concentration step by using a concentrator or 6 h enrichment without a concentration step was used for detecting *Salmonella enterica Newport* (~ 6 CFU/25 g) from kale, spinach, Romaine lettuce, and Iceberg lettuce (N=205). Samples were simultaneously analyzed by the FDA-BAM *Salmonella* detection procedure.

Results: The system correctly identified all 108 *Salmonella* strains and 35 non-*Salmonella* strains. Among 205 produce samples tested, 98%, 93%, 76%, and 60% of Romaine lettuce, Iceberg lettuce, kale, and spinach samples were detected after 3 h of enrichment with sample concentration. After 6 h of enrichment, 100%, 98%, 90%, and 82% of Romaine lettuce, Iceberg lettuce, kale, and spinach samples were successfully detected. The overall analysis time of this methodology was between 8-11 h, in contrast to 4 to 5 days of analysis time for the traditional culture method.

Significance: The Detect^{X} Food DNA microarrays allow for the rapid detection of multiple pathogens from a single sample with minimal enrichment, providing results in a single shift. Here we have demonstrated the utility of this assay for the rapid detection of *Salmonella* in fresh produce.

P2-117 Cold Stress Growth and Population Dynamics of *Escherichia coli* in Leafy Greens Using Real-Time PCR and Whole Genome Sequencing

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Introduction: Pathogen testing on leafy greens and in growing/processing environments is a critical tool to help industry identify risks and prioritize mitigation strategies. A wide variety of environmental conditions intrinsic to the industry creates a need to understand how physiological stress affects rapid pathogen detection methods.

Purpose: To determine the effect of cold stress on composition of enrichment cultures and detection of a putative slow growing STEC (O26) by real-time PCR (rtPCR).

Methods: Romaine samples were inoculated in triplicate with *E. coli* O26:H11 (CDC 03-3014) at high, medium and low levels (92, 10, and 1 CFU/375g respectively). Half the medium- and low-level samples were cold-stressed at 4°C for 48h. All samples were enriched in modified tryptone soy broth (mTSB)

Poster

at 41.5°C. After 4, 6, 8, 10, and 16 hours, enrichment aliquots were tested for STEC using rtPCR platforms (Eurofins Genescan Technologies BACGene™ and Hygenia BAX® System). Additionally, aliquots were prepared for microbiome analysis and sequenced using Oxford Nanopore's GridION™.

Results: The probability of detection (POD; number positive/number tested) was calculated for each treatment. Multiple regression of the entire data set (150 total analyses) showed that only enrichment time had a statistically significant effect on POD ($P < 0.01$). All inoculated samples tested positive on both platforms after 8 h enrichment. After 6 h of enrichment, cold stress reduced sensitivity of the two platforms at the lowest inoculum level. Microbiome analysis showed differences between enrichments of cold-stressed and unstressed samples but these did not affect STEC detection by rtPCR.

Significance: These data suggest that cold stress has an effect on the diversity of the lettuce microbiome, but that rtPCR detection sensitivity is unchanged.

P2-118 Evaluation of the Phagedx™ *Salmonella* Assay for the Detection of *Salmonella* in Lettuce

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Introduction: The PhageDx™ *Salmonella* Assay is a reporter-phage based method that detects viable *Salmonella* 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* PCR Assay (Hygiena).

Methods: Batches of fresh bagged romaine lettuce were inoculated with a 3-strain *Salmonella* cocktail at three levels: 20 samples at (0.2-2 CFU/test portion (low level) likely to give fractional positive results), 5 samples at (2-5 CFU/test portion (high level)), and 5 un-inoculated control samples (0 CFU/test portion). After a 48h cold stress acclimatization period, 25g portions were homogenized with 225mL of pre-warmed BPW, incubated at 41 ˚C and tested by the PhageDx™ assay at 7h and 18h of incubation. A second batch of 25g portions were enriched in 225 mL BPW with added supplement, incubated at 41˚C and tested by the SPT Assay at 18h. A third batch of 25g portions was enriched in 225 mL BPW, incubated at 35 ˚C and analyzed by the BAX *Salmonella* PCR Assay at 22-26h. All PhageDx™, SPT, and BAX PCR assay analytical outcomes were confirmed by the MFHPB-20 reference method.

Results: Statistical analysis of fractional positive results was performed according to the probability of detection AOAC model (AOAC PTM #121904). No statistically significant differences were observed between the PhageDx™ assay tested at 7h or 18h of incubation and the reference method MFHPB-20, nor between the PhageDx™ assay and the VIDAS SPT or BAX PCR Assays.

Significance: The PhageDx™ *Salmonella* Assay is a suitable assay for the rapid detection of *Salmonella* in lettuce (25g portions) after 7-18h of incubation. While equivalent in performance, the PhageDx™ required a shorter incubation time than the BAX *Salmonella* PCR Assay and the VIDAS SPT Assay.

P2-119 Comparison of RNA Isolation Methods for *Escherichia coli* O157:H7 Inoculated on Fresh-Cut Romaine Lettuce

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Introduction: Transcriptome analysis using RNA sequencing is an important tool that can aid understanding of the molecular mechanisms mediating the growth of STEC on leafy vegetables. However, isolation of sufficient quantity and high-quality bacterial RNA from inoculated leafy greens has proven challenging.

Purpose: To determine a suitable RNA isolation protocol for STEC inoculated on Romaine lettuce.

Methods: Freshly cut Romaine lettuce was inoculated with STEC CFSAN101716 at 10^8 CFU/g. After drying inside a biosafety cabinet for 1 h, inoculated samples were transferred into 710-ml Whirl-Pak bag (30g per bag) and 80 ml of selected diluent (ice-cold water, 30% ethanol, room-temperature sterile water, or *RNALater* solution) were added to the leaves. The samples were hand-massaged for 30 s, the rinsates transferred into multiple 50-ml centrifuge tubes, and bacterial cells were pelletized by centrifugation. A comparative analysis of the RNA quality and quantity from bacterial cells with the diluent used was performed using three RNA isolation methods. Ambion® Trizol™ Max Bacterial RNA isolation kit and RiboPure™ RNA purification kit were used according to the manufacturer's instructions. The third method was a phenol-chloroform extraction followed by Promega® SV total RNA isolation system.

Results: Total RNA concentration ranged from 2.6 (Trizol™/*RNALater*) to 14.7µg (Promega/ethanol) per 30g of sample. The yields were sufficient for downstream RNA sequencing regardless of RNA extraction method and diluent combination. The quality of RNA isolated with Trizol™ and RiboPure™ kit, however, was poor (RNA Integrity Number < 6) due to excessive degradation. Phenol-Chloroform with SV total RNA isolation resulted in high quality RNA (RIN > 7), with samples processed in ethanol diluent having the best RNA quality.

Significance: The development of a suitable bacterial RNA isolation protocol will permit analysis of the transcriptional responses of STEC on leafy vegetables which will provide important insights that could improve food safety.

P2-120 Determination of Antifouling Capabilities of Silane-Treated Wood

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❖ ***Developing Scientist Entrant***

Introduction: Wood is commonly used in the production of fresh fruits and vegetables, especially by smaller commercial growers. Research has shown silanes can usefully confer antifouling properties to the surfaces of porous organic food-contact surfaces, including wood.

Purpose: The purpose of this study is to determine the antifouling capabilities of silane-treated wood for use during fresh produce harvest against bacterial foodborne pathogens.

Methods: Pine and oak boards were cut into 4 cm^2 coupons. Coupons were then treated with 1% (w/w) heptadecafluoro-1,1,2,2-tetrahydrodecyl trichlorosilane or left untreated (control). Pine and oak coupons, treated and control, were then placed in a multi-well plate in order to measure pathogen attachment to wood surfaces after 1, 4, and 8 hours post-pathogen application. All coupons were inoculated with 100 µL of bacterial inoculum with a target of 9 Log CFU/mL suspended in phosphate buffered saline (PBS) of either *Salmonella enterica* or *Listeria monocytogenes*. At each sampling time, each coupon was rinsed using sterile PBS and subsequently collected. The rinsate was serially diluted and recovered cells enumerated following 24-48 hours incubation at 35 °C. Bacterial attachment was calculated by difference from starting inoculum level.

Results: Silane treatment for both woods produced significant reductions in pathogen attachment compared to controls for both pathogens. After allowing to attach for 1 hour, silane treatment to pine produced a mean *Salmonella* attachment reduction of 61.44% (95% CI: ± 15.84%); *L. monocytogenes* attachment reduction (72.06%; 95% CI: ± 14.01%) was consistent with that of *Salmonella.* Treatment of oak produced similar reductions in attachment (*Salmonella* 50.02 ± 32.75% & *Listeria* 60.17 ± 16.90% [95% CI for both]). Non-treatment produced <1% reduction in both bacterial species' attachment to wood surfaces.

Significance: These data suggest silane-treatment of wood surfaces effectively helps to prevent pathogenic bacterial attachment to hard woods used in fruit and vegetable harvest following pathogen contact with wood surfaces.

P2-121 Evaluation of Hygiena's BAX® Real-Time PCR Assays for Detection of *Salmonella* spp. and STEC in Cannabis Flower and Hemp Flower for AOAC Research Institute's *Performance Tested Methods*SM Certification

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Introduction: The introduction of the hemp pilot program in the 2014 Farm Bill has led to the authorization of hemp production through the 2018 Farm Bill and the increased, state-level legalization of cannabis for medicinal and recreational uses. Due to increasing popularity to inhale, ingest and topically apply these products, a vital need exists to test these products for pathogens.

Purpose: This study assessed real-time PCR assays for the detection of *Salmonella* and STEC in dried cannabis [> 0.3% delta 9-tertahydrocannabinol (THC)] and hemp flowers (≤ 0.3% THC). Since there are no federal reference methods for pathogen testing in these matrices, the guidance for confirmation in AOAC SMPR 2020.002 and 2020.012, were used to evaluate the methods' effectiveness.

Methods: Ten gram portions of dried cannabis flower (n=20) and dried hemp flower (n=20) were artificially inoculated with fractional levels of one *Salmonella* sp. and one STEC strain and held at room temperature for 2 weeks. Five uninoculated and five high level (10X fractional) portions per matrix, were also inoculated. Portions were enriched with 90 mL of prewarmed (37-42°C) Buffered Peptone water (BPW) and incubated at 42°C for 22-26 hours. All results were culture-confirmed.

Results: The real-time PCR Assays successfully detected *Salmonella* and the target STEC species in dried cannabis flower and dried hemp flower in a 10 g sample size. Difference in POD analysis for the presumptive versus confirmed positives showed no statistically significant differences, with all ranges of the 95% confidence intervals containing the zero point.

Significance: The BAX® Real-Time PCR assays for *Salmonella* , STEC, and *E. coli* O157:H7 Exact have successfully supported their claim for detection of the target pathogens from a single enrichment. These assays allow for accurate, rapid results for the testing of hemp and cannabis flowers, while preserving the ease, accuracy, and reliability of the method.

P2-122 A Comparison of Methods for Recovery of Shiga Toxin-Producing *Escherichia coli* from Agricultural Soils

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are known to survive in soils which may lead to contamination of produce. The density and diversity of the soil microbiome has made isolation of STEC from this matrix a challenge.

Purpose: To systematically evaluate two (*E. coli* O157:H7) or one (STEC) enrichment methods against Food and Drug Administration (FDA) reference methods.

Methods: To test for significant differences between methods, the Relative Limit of Detection (RLOD) in an unpaired study (ISO 16140) was used. A 3-strain cocktail of rifampicin-resistant (rifR) *E. coli* O157:H7 or STEC (serovars O104, O145 and O111) was inoculated into Salinas-valley soil at 0 (control), ~1 (low), or ~10 (high) CFU/25 g and then stored at 4°C for 3 days. Control and high (5) and low inoculum samples (20) were each processed using the selected enrichment methods through to colony isolation and confirmation by real-time PCR for the presence of Shiga toxin genes. Shotgun metagenomic sequencing of enrichment broth was performed followed by bioinformatic analyses for taxonomic identification and molecular serotyping/virulence gene determination using custom programs and BLAST with public and privately curated databases.

Results: The RLODs were 1.36 (95% CI 0.47-3.94) and 1.00 (95% CI 0.38-2.66) for O157:H7 and 1.64 (95% CI 0.54-5.00) for STEC, indicating that the test methods were as sensitive as the FDA method (RLOD < 2.5). True positives, true negatives, and false negatives were identified with all the methods for soil inoculated at the low level (0.93 MPN/25 g). The test and FDA methods exhibited strikingly different bacterial communities at endpoint, with the FDA method resulting in a higher relative abundance of *Enterobacteriaceae* and a greater proportion of the inoculated STEC, but not O157, within the overall *E. coli*population.

Significance: Systematic comparison coupled with bioinformatic analysis of endpoint enrichment can be used to inform methods selection for complex matrixes.

P2-123 A Comparison of Methods for Recovery of *Salmonella* from Agricultural Soils

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Introduction: *Salmonella* is known to survive in soils for extended periods which may lead to contamination of produce. The density and diversity of the soil microbiome can impact recovery of *Salmonella* from this matrix.

Purpose: To systematically evaluate two *Salmonella* enrichment methods (A and B) against a Food and Drug Administration (FDA) reference method.

Methods: The Relative Limit of Detection (RLOD) in an unpaired study (ISO 16140) was used to test for significant differences between methods. A 3-strain cocktail of rifampicin-resistant (rifR) *Salmonella* was inoculated into Salinas-Valley soil at 0 (control), ~1 (low), or ~10 (high) CFU/25 g and then stored at 4°C for 3 days. Control and high (5) and low inoculum samples (20) were each processed through the selected enrichment methods to colony isolation and confirmation by real-time PCR for the presence of *invA* gene. Shotgun metagenomic sequencing was performed on a subset of samples at each culture incubation endpoint followed by bioinformatic analyses for taxonomic identification, relative abundance, and molecular serotyping.

Results: Eight (FDA), 7 (method A), or 11 (method B) low inoculum samples were positive. RLOD was 1.19 (95% CI 0.42-3.37) for method A and 0.64 (95% CI 0.25-1.65) for method B indicating that the test methods were as sensitive as the FDA method (target RLOD <2.5). False negative results were identified in 1 (FDA and method B) and 5 (method A) of 20 samples. Following Rappaport-Vassiliadis (RV) selective enrichment, method A and FDA had highest levels of *Salmonella* relative abundances, while tetrathionate (TT) enrichment endpoints yielded lower and more variable relative abundances of *Salmonella,*but more serovar diversity compared to RV.

Significance: Systematic comparison coupled with bioinformatic analysis of endpoint enrichment can be used to inform selection of methods for isolation of pathogens from complex matrixes.

P2-124 Identification of Animal and Plant Species in Food-Based Products Using Next Generation Sequencing: Results from an Interlaboratory Study

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Introduction: The complexity of the food supply chain is challenging the abilities of analytical tools used for traceability of ingredients for food production. Although there is no reference method for food authenticity analysis, the introduction of Next Generation Sequencing (NGS) in recent years has demonstrated the suitability of this method to verify species composition of food products.

Purpose: An interlaboratory study involving 11 European laboratories from eight countries was conducted to support the implementation of NGS for routine food authenticity analysis**.** In this study the Thermo Scientific™ NGS Food Authenticity Workflow, was used to determine the species composition in a range of different samples

Methods: A total of 72 samples were received by each participant. The targets included meat, fish, and plant. All the experiments were carried out in duplicate. Each participant used the Thermo Scientific NGS Food Authenticity Workflow using the Ion Chef and Ion GeneStudio™ S5 instruments and sequence data analysis was done with the SGS® All Species ID software. Then, the performance of each participant was scored, and the robustness and reliability of the workflow was evaluated.

Results: The overall scores calculated ranged from 84.4% to 100% for fish samples, 77.8% to 97.8% for meat samples and 80.8% to 98.1% for plant samples. The real food samples produced the most variable results which can be explained by the possible heterogeneity of the samples. Among artificial DNA mixtures, a total of 10 meat species, 15 fish species and 18 plant species were successfully identified. Some of the species were identified at low concentration levels (1%).

Significance: This is the largest NGS interlaboratory study performed that included meat, fish and plants. The results obtained demonstrated the high performance and robustness of the Workflow. This study, together with recent developments at ISO and AOAC about the use of NGS for animal and plant species identification, supports the routine implementation of NGS for food authenticity analysis.

P2-125 Detection of *Listeria monocytogenes* and *Salmonella* spp. in Plant-Based Foods

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Introduction: As commercial markets grow for plant-based substitutes for foods such as meats and dairy products, producers need reliable methods for the enrichment and detection of *Listeria monocytogenes* and *Salmonella spp*.

Purpose: In this study, iQ-Check *Listeria monocytogenes II* and *Salmonella spp.* II PCR Kits were evaluated for the detection of target bacteria in plant-based alternative food products and compared to the FDA BAM reference method.

Methods: A plant-based alternative to ground beef was inoculated with *L. monocytogenes* or *S.* Typhimurium from lyophilized pellets, at high and fractional inoculum levels or left uninoculated. After a food stabilization period, 25 g test portions (20 fractional, 5 high, and 5 uninoculated) were enriched at 1:10 in *Listeria* Special Broth for *L. monocytogenes* at 30°C for 24 hr and Buffered Peptone Water Standard for *Salmonella* at 37°C for 20 hr while FDA BAM reference samples (20 fractional, 5 high, and 5 uninoculated at 25 g as well as 5 g, 10 g and 50 g portions for MPNs) were enriched in Buffered *Listeria* Enrichment Broth for *L. monocytogenes* and Lactose Broth for *Salmonella* using FDA BAM protocols. Test method samples were analyzed using iQ-Check PCR Kits and all samples were culture confirmed using FDA BAM protocols

Results: PCR results for *L. monocytogenes* and *Salmonella* test method samples were identical to culture confirmation. When compared to the reference method, the POD was found to be identical for the *Salmonella* test method and better for the *L. monocytogenes* test method.

Significance: The results of this study show test method performance equal or better than FDA BAM reference method for the detection of *L. monocytogenes* and *Salmonella* in plant-based food substitutes.

P2-126 Crystal Diagnostics XpressTM E7 STEC Test Kit AOAC Performance Tested MethodSM (PTM 011502) for *Escherichia coli* Big Six, and O157 in Fresh Raw Ground Beef, Fresh Raw Beef Trim, Raw Spinach, Romaine Lettuce, and Spring Mix Greens

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Introduction: The Crystal Diagnostics Xpress (CDx) E7 STEC test kit was certified for detection of *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 in fresh raw ground beef, fresh raw beef trim, raw spinach, romaine lettuce, and spring mix greens using with the automated AutoXpress platform and CDx patented Liquid Crystal technology with antibody-coated microspheres.

Purpose: To evaluate the Crystal Diagnostics Xpress™ AXSTEC Test Kit for AOAC PTM certification.

Methods: The AutoXpress AXSTEC test kit was examined for inclusivity, exclusivity, and then compared to the United States Department of Agriculture (USDA) reference method for the rapid detection of *E. coli* O45 in fresh raw beef trim.

Results: The Crystal Diagnostics AutoXpress AXSTEC detected 57 of 57 STEC isolates tested and did not detect 47 different exclusivity bacteria tested. The matrix test with fresh raw beef trim was 96.7% percent accurate in the expeditious detection of E. coli O45 per 325 g of fresh raw beef trim with 9.5 h enrichment. There were no significant differences between the CDx and reference methods.

Significance: The CDx AX STEC test kit is highly selective for the pathogens of interest based on the inclusivity and exclusivity results. It demonstrated comparable results to the USDA MLG 5C.01 (for meat product) reference method, is fully automated, and requires less than half of the time to results, which is very suitable for testing meat samples with high fat content and complex background flora.

P2-127 Comparative Evaluation of GENE-UP® *Campylobacter* Method for the Detection of *Campylobacter* Species in Select Poultry Matrices

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Introduction: Campylobacteriosis remains the second highest cause for hospitalization from food borne illness in the US. Based on attribution models, testing of raw poultry remains an effective strategy to surveil the incidence and measure the effectiveness of the interventions for *Campylobacter sp.*. The GENE-UP® *Campylobacter* assay (CAM) is a real-Time PCR assay that provides simultaneous multi-target detection of three *Campylobacter* strains of interest (*C. jejuni, C. coli, and C. lari,)* in raw poultry products and carcass sponges.

Purpose: To conduct a method validation of the candidate method according to AOAC Research Institute *Performance Tested Method*℠ (PTM) Guidelines versus the USDA FSIS MLG 41.06 reference method.

Methods: The CAM method was evaluated for the detection of *Campylobacter* species in raw ground chicken (25 g), raw poultry parts rinsate (30 mL), chicken carcass rinsate (30 mL) and turkey carcass sponge transfer media (25 ml). Three contamination levels were evaluated per AOAC guidelines. Samples were enriched with 2X Bolton and incubated at 42°C for 22-24h under normal atmospheric conditions. All presumptive results were alternatively confirmed by plating to CAMPYFOOD™ Agar, Campy-CEFEX and mCCDA. Colony PCR was performed on all positives from Campy-CEFEX agar and Campy-Food agar.

Results: Statistical analysis was performed using the Probability of Detection model. The $dPOD_c$ values with a 95% confidence interval using either the alternative or reference confirmation indicated no statistically significant difference between presumptive and confirmed results for the CAM method or between the CAM and reference method for all matrixes evaluated. These data demonstrate that the CAM method is an acceptable rapid screening method for the presence of *Campylobacter* spp. in select poultry matrices. Colony PCR of positives also indicates that the CAM is an acceptable confirmation method from the indicated media.

Significance: These data support the validation of the CAM assay as a AOAC PTM method.

P2-128 An Evaluation of the GENE-UP® Pathogenic *E. coli* (PEC) Method to Detect Pathogenic *E. coli* Species in Ground Beef (375g), Beef Trim (375g), Bagged Romaine Lettuce (375g) and Microtally™ Sampling Cloths (200ml)

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Introduction: EHEC (Enterohemorrhagic *Escherichia coli*) are highly pathogenic E. coli harboring *stx* and *eae* genes which can cause clinical manifestations ranging from severe diarrhea to hemolytic uremic syndrome. PCR screenings for EHEC falter when distinguishing between *E. coli* carrying both *stx* and *eae* versus those carrying only a single gene which are considered non-pathogenic per the USDA MLG. The GENE-UP® Pathogenic *E coli* (PEC) assay is a real-time PCR that provides multi-target detection of unique pair of pathogenicity genes shown to be highly correlated (>99%) with the presence of *stx* and *eae*. Detection of these additional genetic markers can appreciably improve the detection of EHECs at the screening step.

Purpose: To evaluate the performance of the PEC method against the USDA/FSIS MLG 5C.02 or FDA BAM Chapter 4A reference methods, as applicable using AOAC Research Institute PTM guidelines.

Methods: The PEC method was evaluated both with and without the use of immuno-concentration via VIDAS® ESPT (*E coli* Serogroups) as compared to the applicable reference method. Each matrix was tested at three contamination levels per AOAC PTM guidelines.

Results: Statistical analysis was performed using the Probability of Detection model. The dPODC values with a 95% confidence interval showed no statistically significant difference between the data using direct lysis, immunoconcentration, and the pertinent reference method confirmations on all analyzed matrices.

Significance: The PEC method demonstrated reliability as a rapid method for the detection of pathogenic *E coli* in the tested matrices. These data support the certification of PEC as a PTM method.

P2-129 Next Generation Enrichment for Accelerated, Same-Day Pathogen Detection in Produce and Trim

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Introduction: In the current laboratory workflow for the detection of Salmonella and STEC in meats and produce, a 1– 2 hour detection method of PCR or ELISA is preceded by a lengthy microbial enrichment process of 10 – 22 hours – the bottleneck in accelerating the time to results for pathogen detection.

Purpose: The aim of this study is to develop a high-throughput enrichment method capable of integrating into the standard laboratory pathogen testing workflow that will enable same-day pathogen detection results, namely for 1 CFU/375 g in < 8 hours.

Methods: Sample matrices of beef trim (*n* = 10; 375 g) and romaine lettuce (*n* = 10; 375 g) were inoculated with fractional levels (0.3 – 0.8 CFU) of *Salmonella*, *E. coli* O157:H7, or STEC and incubated for 4 - 6 hours in novel sample bags. After incubation, a 10-minute concentration step was performed to extract aliquots of bacteria suitable for a variety of detection methods. All samples were also allowed to incubate for 18 hours for further confirmatory testing. This method was performed using a variety of detection methods, including multiple qPCR systems and selective agar plating.

Results: We were able to demonstrate the compatibility of our enrichment and extraction method with Bio-Rad's iQ-Check PCR detection systems, Hygiena's BAX system PCR assays, and selective agar plating. Detection of single CFU of Salmonella, STEC, and E. coli O157:H7 was possible in 375 g of produce and beef when using a 5-hour incubation time coupled with our newly developed 10-minute enrichment step.

Significance: Pathotrak's Next Generation Enrichment accelerates the current workflow for pathogen testing to yield same-day results (< 8 h time-to-results) and has proven to be compatible with a variety of matrices and detection methods.

P2-130 Detection of *Listeria monocytogenes* in Raw Dairy, Meat and Seafood Commodities Utilizing Alternative Proprietary Selective Enrichment Media and Loop-Mediate Isothermal Amplification (LAMP)-Bioluminescent Assay

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Introduction: *Listeria monocytogenes* is a pathogenic microorganism that can be found in a variety of foods, its recovery in raw food commodities can be challenging due to high levels of background microbiota, often requiring the use of a secondary enrichment or higher sample dilution.

Purpose: To compare the recovery of *Listeria monocytogenes* from raw food commodities between ISO 11290-1:2017 and an alternative method using Actero (FoodCheck™) media and a *Listeria monocytogenes* LAMP-Bioluminescent assay.

Methods: A minimum of 60 different foods were tested for each raw commodity (dairy, seafood and meat). For each panel sixty 25g test portions were tested following ISO 11290-1:2017, the other sixty 25g portions were enriched with 225mL of pre-warmed Actero media and incubated at 30°C for 28-30h for raw-dairy and at 37°C for 28-30h for raw-meat and seafood. Method comparison and inclusivity was done following ISO 16140-2 guidelines. All foods were artificially inoculated with 1-1.8 CFU/test portion with a variety of *Listeria monocytogenes* strains previously isolated from raw foods. All foods were stored refrigerated for 48 h before processing. The study was repeated in two geographical locations to consider variability in natural microflora.

Results: Sensitivity of the alternative method for all three categories was 84%,71% and 82% for raw meat, fish and dairy respectively and 65%, 71% and 50.7% respectively for the reference method. Based on ISO 16140-2 the difference between negative and positive deviations in an unpaired study for a single category, showed that the alternative method met the acceptability limits to be equivalent to the reference method in the performed study.

Significance: The use of proprietary Actero media and LAMP may offer a next day result with a single enrichment step to recover *Listeria monocytogenes* in foods from raw categories enabling food processor faster testing of raw foods.

P2-131 Rapid Detection of *Salmonella* spp. in Alkaline Primary Production Boot Swabs Using the Loop-Mediated Isothermal Amplification (LAMP) Assay – Bioluminescent

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Introduction: Brazil is one of the world's largest exporters of poultry products. To maintain safe production, sanitary measures are necessary from farm to processor. A main measure is the analysis of broiler litter for monitoring and controlling *Salmonella* spp.. These highly alkalinized samples are collected by dragging boot swabs moistened with BPW ISO in the environment. Choosing a rapid and accurate *Salmonella* spp. detection method in primary production samples is important for monitoring effectiveness and for taking action to avoid product contamination.

Purpose: Determine the sensitivity, specificity, relative trueness (RT), relative limit of detection (RLOD), and acceptability limit (AL) of the *Salmonella* LAMP-Bioluminescent assay in primary production boot swabs compared to cultural method confirmation.

Methods: Boot swabs samples (n=45) composed of alkaline broiler litter collected from farms (e.g., soil, feathers, feces, wooden shavings, and lime powder) and sterile moistened boot swabs were spiked in groups of 7 samples with 6 levels of *Salmonella* ATCC 14028 adapted to the condition, with fractional inoculation from 0.5 to 10^4 CFU/ test portion (n=42) and a blank sample group (n=3). The samples were pre-enriched in 225 mL of BPW ISO and incubated at 37 °C for 24 hours. After measuring pH, the enrichment was diluted 1:10 in BWP, and then analyzed with LAMP-Bioluminescent assay. All enrichments were confirmed by an adaptation of ISO 6579-1:2017. Parameters required by ISO 16140-2:2016 for method comparison were determined. Naturally occurring microflora competitors (mesophilic aerobics, *Enterobacteriaceae*, coliforms and *Escherichia coli)* were evaluated by enumeration.

Results: Compared to the traditional method, the alternative LAMP-Bioluminescent method sensitivity, specificity, RT, and RLOD were 93.9%, 100.0%, 95.6%, 1.09, respectively. The AL was fit for purpose.

Significance: The alternative LAMP-bioluminescent method enabled reliable and rapid detection of *Salmonella* spp. in highly alkaline primary production boot swabs.

P2-132 Evaluation of a Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay for Rapid Detection of *Salmonella* in Protein Industry as Compared to the Cultural Method

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Introduction: For the selective enrichment of *Salmonella* GB method 4789.4-2016 uses Tetrathionate broth (TTB) and Selenite Cystine broth (SC) and ISO 6579-1:2017 uses Rappaport-Vassiliadis-Soya broth (RVS). These different selective enrichments can affect the recovery of the target pathogen which may lead to a false negative result associated with the detection process. There is a higher sensitivity in the use of DNA molecular detection methods compared to traditional culture method. The combination of a selective enrichment and LAMP-bioluminescent DNA detection can be used for rapid and accurate detection of *Salmonella* spp.

Purpose: To determine performance of different selective enrichments with a LAMP-Bioluminescent assay for the detection of *Salmonella* in samples rich in protein and poultry environmental samples.

Methods: Five matrices, carcass sponge (n=5), boots swabs (n=5), poultry liver (n=5), poultry fecal swab (n=5) and protein powder processing samples (n=5), were enriched for 8 hours with BPW-ISO at 36°C. After primary enrichment, samples were randomly selected for secondary enrichment with SC (n=17), TTB (n=25) and RVS (n=25); all samples were analyzed for *Salmonella* detection through culture. Additionally, their primary enrichments (n=21, BPW-ISO enrichment), TTB (n=12) and RVS (n=19) were also analyzed via the LAMP method. All assays were characterized with respect to specificity, sensitivity and accuracy.

Results: Sensitivity of analyses completed following enrichment in the BPW-ISO broth, SC, TTB and RVS broths were 77.8%, 90.0%, 63.6% and 90.9%. In all cases, the specificity was 100%. Sensitivity of LAMP assay with TTB and RVS broths were 85.7% and 100% while specificity for all was 100%. No significant differences were observed using LAMP test with RVS broth.

Significance: The LAMP-bioluminescent DNA detection method performs well with RVS broth compared with other selective enrichment broths and it is a reliable method for the rapid and specific detection of *Salmonella* spp. in protein-related matrices.

P2-133 Development and Validation of High-Resolution Melting Assays for the Detection of Potentially Virulent Strains of *Escherichia coli* O103 and O121

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❖ ***Developing Scientist Entrant***

Introduction: Infection by Shiga toxin-producing *Escherichia coli* (STEC) strains results in bloody diarrhea, hemolytic uremic syndrome, and renal failure. According to the CDC, the O103 and O121 strains are responsible for 15.6% and 4.7% of STEC cases, respectively. Presence of avirulent strains of O103 and O121, which lack crucial virulence genes, can interfere with the interpretation of STEC detection assays, causing product hold-up and financial losses to the red meat industry.

Purpose: To develop high-resolution melting (HRM) real-time PCR assays to specifically detect *E. coli* O103 and O121 strains and classify detected strains as STEC or non-STEC.

Methods: Single-nucleotide polymorphisms (SNPs) in serogroup-specific genes conserved among the STEC O103 and O121 strains were targeted. Primers targeting the O103 *wbtD* and O121 *vioA* genes were designed. The two HRM assays were validated using DNA from bacterial strains (n=215), inoculated beef (n=48) and spinach (n=24) samples, and meat enrichments from a federal red meat surveillance program (n=168). The proportion of serogroup detection by the reference method (MLG Chapter 5C.01) versus the two HRM assays were compared using Fisher exact test ($P < 0.05$).

Results: Serogroup-specific O103 and O121 primers showed 100% specificity for pure culture strains. Virulent (STEC) and avirulent strains of serogroups O103 and O121 formed distinct melt profiles in normalized and differential melting curves plots. The 103 HRM assay demonstrated the ability to differentiate between STEC O103:H2 and STEC O103:H25 strains. The assays were able to accurately detect all inoculated strains following 15 h enrichment. Further, the O103 and O121 HRM assays were equally effective when compared to real-time PCR used in the MLG 5C.01.

Significance: The assay developed in this study specifically identifies STEC strains of O103 and O121 serogroups and differentiates them from non-STEC strains. The assay can be used to reduce the number of potential-positive results caused by the presence of mixed strains in red meat.

P2-134 A Novel Real-Time PCR Approach for Specific Detection and Estimation of *Salmonella* in Poultry Rinse

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Introduction: *Salmonella* is not considered an adulterant in raw poultry and hence does not require mandatory testing. Therefore, *Salmonella*-tainted food products may end up in commerce. Current *Salmonella* testing approaches rely on "presence/absence" testing of food enrichments and ignore the *Salmonella* concentration in contaminated samples. A food sample that is positive for *Salmonella* or any other foodborne pathogen by PCR may contain anywhere from one to a million or more cells of the pathogen.

Purpose: To develop a reliable, multiplex real-time TaqMan PCR assay for the specific detection and simultaneous estimation of *Salmonella* contamination levels in food samples.

Methods: Our method developed in this study takes advantage of varying PCR amplification efficiencies of three *Salmonella*-specific primers, facilitating the detection of three contamination levels i.e., low (≤1log CFU), medium (≤2log CFU) and high (≤3log CFU) levels. A multiplex TaqMan assay was standardized, targeting three *Salmonella*-specific genes (*inv*A, *fim*A, *stn*) with an internal amplification control. The assay was validated with chicken rinses spiked with 1-5 log CFU/30 mL *Salmonella* strains, stressed at 4°C for 48h, and enriched with 15mL 2× buffered peptone water.

Results: The *inv*A primers produced highest amplification efficiency and detected *Salmonella* between 1-7 log CFU/mL; the *fim*A primers worked in the range of 2.7-7 log CFU/mL; and *stn* primers amplified above 3.7 log CFU/mL. During validation with spiked poultry rinse, the *inv*A primer detected *Salmonella* in chicken rinses inoculated between 1.4-4.4 log CFU/30 mL; the *fim*A primer detected samples spiked at 2.4-4.4 log CFU/30 mL; and *stn* primer detected 3.4-4.4 log CFU/30 mL after 6h enrichment.

Significance: This standardized *Salmonella* assay can be used to precisely detect and estimate *Salmonella* contamination levels. Further, *Salmonella* levels can be refined by plotting Cq values on the *inv*A standard curve.

P2-135 Validation of Reduced Time to Detection of Shiga-Toxigenic *Escherichia coli* in Beef Trim with an Improved Sampling Device

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Introduction: The beef industry needs faster results from Shiga-toxigenic *Escherichia coli* testing to make safe decisions on product release, which pushes the limits of current sampling methods and detection technologies.

Purpose: To validate shorter detection times with qPCR when replacing a standard carcass sampling cloth with a MicroTally™ cloth.

Methods: Fifty portions of beef trim were swabbed using MicroTally™ cloths following manufacturer directions, to simulate the sampling process. Cloths were inoculated with 100 µL of dilutions of an overnight culture of *E. coli* O111 in modified tryptone soy broth (mTSB) to deliver 0.2, 2.0 and 41 CFU/cloth for 20, 20 and 5 cloths respectively. Five more cloths received 100 µL of sterile mTSB as negative controls. Inoculated cloths were held at 4°C for 48 h and then enriched at 41.5°C in 200 mL of mTSB. After 8, 10 and 24 h, 30 µL aliquots were drawn for testing using Eurofins Technologies BAC*Gene*™ Mplex STEC screening kit. Presumptive positives were confirmed using the BAC*Gene* Mplex STEC SER*Otype*1 kit, and O111 latex agglutination of colonies from rainbow agar.

Results: Presumptively positive results were 7/20 at 0.2 CFU/cloth, 20/20 at 2 CFU/cloth, 5/5 at 41 CFU/cloth and 0/5 for negative controls, in all cases at 8, 10 and 24 h. All results were identical when retested using the STEC SER*Otype*1 assay. Only fractional-positive and negative-control results were retested by O111 latex agglutination and were again identical. Results were not significantly different between enrichment times when compared following the statistics for method validation in AOAC "Appendix J".

Significance: Results demonstrate that a 2-hour reduction from the 10 h of enrichment validated with regular carcass cloths to 8 h of enrichment when using MicroTally cloths is possible. This may provide shorter product hold-times pending test results.

P2-136 Performance Evaluation of a Loop-Mediated Isothermal Amplification (LAMP) - Bioluminescent Assay for Rapid Detection of Shiga Toxin-Producing *E. coli* (STEC) in Raw Beef from Brazilian Meat Processing Facility

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Introduction: STECs are characterized by the production of Shiga toxins and intimin adhesin. This group includes the top seven *Escherichia coli;* serotype O157:H7 and serogroups O26, O45, O103, O111, O121, and 0145 which are often associated with foodborne outbreaks globally. STEC transmission occurs through the consumption of contaminated food or water and can cause human disease such as gastroenteritis, bloody diarrhea and the development of hemolytic uremic syndrome (HUS).

Purpose: To determine the specificity, sensitivity, relative trueness (RT) and relative limit of detection (RLOD) of a LAMP-Bioluminescent Assay for STECs detection in raw beef (AOAC PTM 071902) compared to a RT-PCR method (AOAC PTM 091301).

Methods: Beef samples (n=40) were analyzed in an unpaired study. Samples were artificially contaminated with a cocktail of strains: *E. coli* O103 CDC 06-3008, 0145 CDC 99-3311, O26 CDC 03-3014 and O45 CDC 00-3039 targeting 2CFU/25g and with *E. coli* ATCC 25922 as an interferent microorganism targeting 100CFU/25g. Samples were enriched in 225mL of mTSB, incubated at 42°C for 16h (n=20) or enriched in 225mL of BPW-ISO, incubated at 41°C for 16h (n=20), for the PCR and LAMP methods, respectively. Presumptive positive samples were confirmed using a culture-based method. Sensitivity, specificity, false positive, false negative, RT and RLOD were determined.

Results: The LAMP-Bioluminescent assay detected STEC genes *stx* and *eae* in all samples. Compared to the RT-PCR method, the overall sensitivity and specificity of the LAMP-Bioluminescent Assay were both 100%. The LAMP-Bioluminescent assay had no false positives nor false negatives. The RLOD of the alternative method was 1 with a 90% confidence. These results indicate that there are no significant differences between the methods.

Significance: The alternative LAMP-Bioluminescent molecular method enabled rapid detection of the top seven STEC in beef samples and offers beef producers and reference laboratories a rapid method to detect these relevant pathogens.

P2-137 Evaluation of GENE-UP® EHEC Method on Environmental and Beef Samples: A Study Comprising Brazilian Samples

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Introduction: Shiga-toxin producing *Escherichia coli* (STEC) are a major concern on specific food matrices, once they are able to trigger toxinfection that might lead to severe illness. However, detection of STEC relies on specific serotypes detection, and commonly food samples present a mixture of several *E. coli* serotypes that independently contribute with genetic markers (*stx*, *eae* and serogroup marker), leading to imprecise screening process.

Purpose: Performance assessment and comparison of current screening methods with GENE-UP® EHEC method and assessment of the impact of immunoconcentration technique on beef samples showing one or both virulence markers.

Methods: 42 artificially contaminated samples (environmental or 375g trimmed/ground raw beef) were used to assess GENE-UP® EHEC performance as per Brazilian guideline. Artificial contamination gathered all STEC serogroups ranging between 1-6 CFU/sample. Also, 132 25g or 375g routine raw beef samples presenting one (negative) or both (presumptive positive) STEC genetic markers and 15 controls were analyzed by current methods and by GENE-UP® EHEC after the same mTSB enrichment, as stated by the methods.

Results: Method performance on the 42 samples group: 100% for sensitivity, specificity and precision; 0% for false positive and false negative rates. On the 147 samples group, 100% of concordance has been obtained on the controls group. One positive sample has been satisfactorily detected by methods in comparison. Heterogeneity was demonstrated by Chi-square test (95% of confidence interval) when comparing samples analyzed by current methods and GENE-UP® EHEC.

Significance: Immunoconcentration step was beneficial as it diminished the number of presumptive positive samples maintaining satisfactory performance of GENE-UP® EHEC method. Thus, the GENE-UP® EHEC method offers a specific and assertive approach for detection of STEC in selected food samples, providing rapid results to evaluate the microbiological safety of the products and preventing losses related to imprecise STEC screening.

P2-138 Development and Verification of *Salmonella* Quantification on Beef Lymph Nodes Utilizing the Hygiena's BAX® System Salquant™

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❖ ***Developing Scientist Entrant***

Introduction: Because beef lymph nodes are a well-known source of *Salmonella* and when not extracted from trim, ground beef can often become a public health concern, quantification of *Salmonella* utilizing BAX® System SalQuant™ can provide rapid, actionable results to determine *Salmonella* loads in beef lymph nodes prior to product release.

Purpose: The purpose of this study was to develop and assess the performance of a rapid enumeration method for *Salmonella* in beef lymph nodes utilizing BAX® System SalQuant™.

Methods: Beef lymph nodes (N=100) were procured from a commercial beef processing facility and pre-screened for *Salmonella.* Negative *Salmonella* lymph nodes (n=38; small: 0-10 g, large: >10 g) were weighed, trimmed, sterilized for 3-5 seconds, pulverized with a rubber mallet, and spiked with 0.00 – 5.00 LogCFU/Lymph Node of *Salmonella* Typhimurium with three biological replicates per level. Primary enrichment was conducted by adding BAX® MP media (small: 40 mL; large: 80 mL) to the samples homogenized and incubated at 42˚C for 6, 8, and 10 hours for recovery, then each sample was tested in quintuplet using BAX® System Real-Time *Salmonella* Assay and linear fit equations were created at each timepoint utilizing Cycle Threshold (CT) values to estimate pre-enrichment levels of *Salmonella*. The timepoint utilized for BAX® System SalQuant™ was determined through R^2 (≥ 0.75) and Log RMSE (≤ 0.40) using JMP® v. 15. Estimations were compared to MPN through 95% confidence intervals.

Results: The 6-hour linear fit equation met statistical parameters for small and large lymph nodes with R^2 of 0.84 and 0.85 with a Log RMSE of 0.51 and 0.30 for small and large lymph nodes, respectively. SalQuant™ and MPN estimations were not statistically different.

Significance: These results indicate that the BAX® System SalQuant™ can be considered a useful data-driven tool for beef processing facilities to determine loads of *Salmonella* in beef lymph nodes prior to ground product formulation and release, ultimately reducing risk of final product and improving food safety.

P2-139 Development and Verification of a *Salmonella* Quantification Methodology for Beef Ceca Swabs and Ceca Contents Utilizing Hygiena's BAX® System Salquant™

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Introduction: Beef ceca has been found to be a reservoir for *Salmonella*, but the actual contribution of ceca contents to carcass and trim contamination can only be evaluated if the load of *Salmonella* is determined by novel methodologies such as the BAX® system SalQuant™, which can serve to make actionable decisions to reduce *Salmonella* levels before product reaches the consumer.

Purpose: The aim of this study was to develop and assess the performance of a rapid enumeration method for *Salmonella* in beef ceca swabs and ceca contents utilizing the BAX® system SalQuant™.

Methods: Beef cecal swabs and ceca contents were obtained from a commercial beef processing facility and pre-screened for *Salmonella*. Swabs (n=16) were spiked with 0.00 to 4.00 LogCFU/mL and ceca content samples (n=28) with 0.00 to 8.00 LogCFU/mL of *Salmonella* Typhimurium. Swab processing used 50 mL BAX® MP media; ceca content samples combined 10 mL aliquot of primary enrichment and 10 mL BAX® MP. Swabs and cecal content samples (low level) were incubated at 42˚C for 6, 8, and 10 hours of recovery; five technical replicates were tested using BAX® System Real-Time *Salmonella* Assay. Linear fit equations were created utilizing Cycle Threshold (CT) values to estimate pre-enrichment levels of *Salmonella*. The BAX® System SalQuant™ timepoint was determined with R^2 (≥ 0.70) using JMP® v. 15. Estimations were compared to MPN through 95% confidence intervals.

Results: Linear-fit equations for both sample types met statistical parameters with R^2 from 0.72 – 0.85 and Log RMSE from 0.47 to 0.61. SalQuant™ and MPN estimations were not statistically different in inoculated samples ($p \leq 0.05$).

Significance: Results show that the BAX® system SalQuant™ is a potential tool for beef processing facilities to estimate loads of *Salmonella* in beef ceca swabs and ceca contents, which can help reduce risk in final product and guide data-driven food safety decisions with pre-harvest implications.

P2-140 Development and Verification of *Salmonella, Vibrio, Campylobacter, Escherichia coli, and Listeria spp.* Pure Culture Estimations Utilizing Hygiena's PCR-Based Quantification Methodologies

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Introduction: Inoculation level evaluation is needed prior to microbial challenge studies; however, traditional methods for enumerating inoculation levels, such as plate counts, take 24 – 48 hours for results. There is a need for alternative quantification methodologies with accurate results for pure culture estimations.

Purpose: The aim of this study was to develop a RT-PCR enumeration method to efficiently estimate the levels of 7 *Salmonella* serotypes, 3 *Vibrio* serotypes, 3 *Campylobacter* serotypes, *Escherichia coli* O157:H7, *Listeria* genus and *L. monocytogenes*, respectively, in pure culture preparations.

Methods: Pure cultures of *Salmonella* Braenderup, *S.* Dublin, *S.* Enteritidis, *S.* Heidelberg, *S.* Newport, *S.* Reading, and *S.* Typhimurium, *Vibrio vulnificus*, *V. cholerae*, *V. parahaemolyticus*, *Campylobacter jejuni*, *C. coli*, and *C. lari*, *Escherichia coli* O157:H7, *Listeria* genus and *L. monocytogenes* were grown in TSB (approximately 1×10^9 CFU/mL) under individual growth specifications with three replicates per serotype. Dilutions, 1 mL into 9 mL BPW, were performed on each culture and 10^9 to 10^5 on 1 Log CFU/mL incremental dilutions were evaluated in quintuplet using the BAX® System assays for *Salmonella, Vibrio, Campylobacter, E.coli* Exact*, Genus Listeria* and *Listeria monocytogenes*. Cycle threshold (CT) values were grouped by genus and a linear-fit equation utilized for quantification was created using JMP® v. 15.

Results: The system detected and enumerated 10^9 to 10^5 Log CFU/mL for *Salmonella* (SalQuant™) *Vibrio* (VibrioQuant), *Campylobacter* (CampyQuant™) *E. coli* O157:H7 (E.coliQuant), and *Listeria* (ListeriaQuant) in pure cultures. All linear fit equation estimations met statistical parameters (R^2 from 0.90 – 0.98 and Log RMSE from 0.14 to 0.39) and statistically compared to plate counts on a 95% confidence interval.

Poster

Significance: A rapid PCR-based enumerative method with pure culture testing capabilities provides the microbiological and the food industry with a tool to reduce the time-to-result to confirm inoculation levels and less variation when conducting challenge studies and making data-driven food safety decisions.

P2-141 *Salmonella* Quantification (SalQuant™) Utilizing the BAX® System for Pork Primary Production Boot Cover Samples

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❖ ***Developing Scientist Entrant***

Introduction: Personnel involved in the rearing of swine routinely wear boot covers, which can be used for quantifying *Salmonella* in a swine herd, as *Salmonella* is a natural inhabitant of the swine gastrointestinal tract and can be found in the environment of swine farms.

Purpose: This study was conducted to develop and validate SalQuant™ as a rapid PCR quantification for *Salmonella* in boot cover samples from the pork primary production setting.

Methods: Boot covers were worn in a pork primary production environment and used as samples to represent background flora and debris. Samples were screened for *Salmonella* and negative samples were inoculated with *Salmonella* Typhimurium (ATCC 14028) at concentrations of 0.00 – 4.00 $\log_{10}$ CFU/mL. Samples were enriched with BAX® MP with Quant solution for 6, 8, or 10h at 42°C and then quantified using the BAX® System Real-Time Assay for *Salmonella*. A 3 × 5 MPN was also conducted, and the two quantification procedures were compared using GraphPad Prism 9.

Results: Incubating the boot cover samples for 8 hours at 42°C resulted in a linear fit equation with an R^2 of 0.91 and a log RMSE of 0.46, which was the best linear fit equation produced by the three incubation times investigated. A statistical difference was not observed for the MPN and SalQuant™ methods (P>0.05).

Significance: According to the results of this experiment, BAX® System SalQuant™ can accurately quantify *Salmonella* from pork primary production boot covers after an 8-hour incubation at 42°C. This provides the pork industry with a rapid approach to quantifying *Salmonella* populations in the primary pork production environment.

P2-142 Evaluation of Lamp-Based Amplification and Bioluminescence Detection Method for Detection of *Salmonella* spp. in Raw Pork Meat

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Introduction: Pork meat is a common vehicle for *Salmonella* around the world. Therefore, the European Food Safety Authority (EFSA) considers as public health hazards specific *Salmonella* serovars isolated from pigs, including Choleraesuis, Enteritidis and Typhimurium. Rapid pathogen detection methods are important verification tools for food processors.

Purpose: To evaluate a LAMP-based method for the detection of Salmonella spp on raw pork meat compared to the to USDA- FSIS MLG 4.11 method.

Methods: Raw pork parts were obtained from a local butchershop in Irapuato México. A total of 40 samples were analyzed. Twenty samples were spiked with 5-6 cells of *Salmonella* Typhimurium ATCC 14028 and 125-128 cells of generic *E. coli* ATCC 25922. Twenty samples were spiked only with 125-128 cells of *E. coli*. Each sample (25g) were enriched with 75 mL of mTBS and incubated at 42°C by 24h. After incubation 20 µL aliquots were analyzed using a LAMP-based DNA amplification method. All samples were culture confirmed by following USDA-FSIS MLG 4.11.

Results: All spiked samples with *Salmonella* yielded as positive by molecular method and confirmed as positive by culture, regardless presence of *E. coli* as competitor. Twenty out twenty samples spiked only with *E. coli* were reported as negative by molecular method and confirm as negative by culture. No false positive or negative were found with the LAMP-based DNA method.

Significance: The use of a LAMP-based DNA amplification method provided an accurate *Salmonella* detection and faster time to result which may enable pork meat manufacturers and inspection agencies the use of rapid tools to verify effectiveness of Salmonella control measures to prevent of illness.

P2-143 *Salmonella* Quantification in Pork Lymph Nodes Using Different Methodologies

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❖ ***Developing Scientist Entrant***

Introduction: Because Salmonella is harbored in the lymph nodes (LNs) of market swine and may contaminate ground meat products, the pork industry could benefit from the development of novel pathogen quantification methodologies that provide rapid and sensible results that can lead to actionable decisions and support efforts to comply with incoming performance standards.

Purpose: To evaluate the performance of a novel pathogen quantification methodology known as SalQuant® *Salmonella* enumeration assay for pork LN matrices as compared to published methods that include a modified Most Probable Number (MN) technique, and a direct plating methodology.

Methods: Pork LNs (n = 26) were trimmed, sterilized, pulverized, spiked with 0.00–5.00 Log CFU/LN using *Salmonella* Typhimurium (ATCC-14028), and then homogenized with 80ml of BAX-MP (LN homogenate [LNH]). From each LNH, samples were enumerated by 1) a direct-plating method on Enterobacteriaceae (EB) petrifilms® that were replica-plated onto XLD, 2) a 3x3 MPN, and 3) the BAX®-System-SalQuant™ methodology for pork LNs. Linear regression analysis was used to evaluate the relationship between counts obtained from different enumeration technologies when compare with MPN.

Results: The slope for both alternative enumeration methods when compared with MPN were 1.25 (P >0.05) and 1.02 (P < 0.05) with an adjusted-r-square of 0.93 and 0.75 for Petrifilm and SalQuant™ methodology, respectively. The intercept value was -1.003 for Petrifilm methodology (P <0.001) and -0.333 for SalQuant methodology (P >0.05) when compared against the MPN method.

Significance: The development and validation of more rapid and sensible quantification methodologies when compare with actual methodologies can help the meat industry to conduct contamination risk assessments and make decisions for receiving and processing operations, as well as release of pork products based on quantifiable data.

P2-144 Evaluation of an Alternative Method for Enumeration of the Total Viable Count in Cooked Sausages

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Introduction: Microbial spoilage is the major reason for the loss of consumer trust in cooked meat products. In China, cooked sausage is one of the most important meat products. A rapid method for the enumeration of total viable count (TVC) will enable producers to access quality products and provide a basis for the quick release of products.

Purpose: To determine the performance of an alternative method, the 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plate in comparison to the China National Standard (GB) agar method 4789.2-2016.

Methods: Naturally contaminated and spiked samples of 14 types of cooked sausages (N=100) were analyzed with 3M Petrifilm RAC Count Plate and GB 4789.2-2016. Twelve samples of cooked sausage had natural microbial flora and 88 samples were inculcated with quality-control strains, which included

Poster

Escherichia coli ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 10031, *Citrobacter freundii* ATCC 8090, and *Bacillus cereus* DM 423. The 3M Petrifilm RAC Plates were incubated at 36°C for 24h, and the Plate Count Agar of the GB method was incubated at 36°C for 48h. The colonies were enumerated according to each method and used to determine the correlation coefficient between the alternative and reference method.

Results: No statistical differences were observed between the 3M Petrifilm plate method and the GB method when enumerating TVC in cooked sausages. The correlation coefficient was equal to 0.97 between the two methods.

Significance: The 3M Petrifilm RAC Plates were shown to be a reliable rapid and accurate alternative to the GB method for the TVC enumeration in cooked sausages.

P2-145 Comparative Study on *E. coli* Tests between Alternative Method, TBX Agar and Japan's Fecal Coliform Test in Raw Meat Samples

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Introduction: The *E. coli* test is generally conducted using chromogenic agar such as TBX agar (e.g. ISO 16649-2:2001). On the other hand, fecal coliform tests are often used as an alternative method to *E. coli* testing, as self-testing in Japan. Both methods are more time-consuming and labor-intensive than alternative methods such as 3M™ Petrifilm™.

Purpose: The study evaluated the 3M™ Petrifilm™ Rapid *E.coli*/Coliform Count Plate [REC plate] in comparison to fecal coliform tests using *E. coli* fermentation tubes and *E. coli* tests using TBX agar in naturally contaminated raw meat samples, sourced from Japan.

Methods: Test samples from 110 various types of ground meat. 1mL of subsequent 1:10 dilutions were inoculated to REC plates, TBX agar and EC fermentation tubes (n=3). REC plates were incubated at 42℃ for 24 hours, TBX agar plates were incubated at 44℃ for 24 hours and EC fermentation tubes incubated at 44.5℃ for 24 hours. Confirmation tests using EMB medium and lactose broth were used if gas was detected in the EC fermentation tubes for the fecal coliform tests. Positive colonies detected by REC plates were confirmed by 16s sequencing.

Results: The highest positivity rate was observed in REC plates (41.8%), followed by TBX agar (35.5%) and fecal coliform tests (29.1%). Positive colonies detected by REC Plates were identified as *Escherichia coli* except for colonies detected from one sample.

Significance: It was confirmed that an equivalent or more appropriate test results could be obtained by REC plates compared to TBX agar or Japan's fecal coliform test in naturally contaminated raw meat samples. Alternative methods such as the REC plate is less time-consuming and labor-intensive than conventional methods and this study has shown that E. coli can be detected appropriately by the alternative method. The REC Plate has be shown to provide a very useful option for the field of food microbiology.

P2-146 Evaluation of Hygiena BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact in Raw Meats

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Introduction: Due to the adulteration of *E.* coli O157:H7 and risk to consumers from raw meat products, it is imperative to provide the industry with rapid assays that have proven performance claims with enhanced sensitivity and specificity.

Purpose: The performance of the alternative Real-Time PCR Assay for *E. coli* O157:H7, was compared to the Canadian culture-based reference method MFHPB-10 and evaluated according to Health Canada, Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods for consideration as a laboratory procedure (MFLP status).

Methods: Raw Meats (including frozen, processed and unprocessed raw meats at 65g and 375g portion size), were each inoculated at three inoculum 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. Unpaired samples were spiked with *E.coli* O157:H7 and co-inoculated with 10 times L_1 with generic *E.coli* as an interference organism. Alternative samples were enriched in MP media (Hygiena), incubated at 42˚C, and tested at 8h (Frozen Meat-375g was tested at 10h) and 24h of incubation, whereas reference samples were enriched in m-TSB supplemented with 20 g/ml Novobiocin for 18-24h at 42˚C. All analytical outcomes were compared and culturally confirmed by the reference method

Results: Collectively from the analysis of 405 samples, a probability of detection (POD) statistical model determined the alternative method exceeded the criteria outlined by the MMC which requires a relative sensitivity of ≥98%, a relative specificity of ≥90.4%, a false positive rate of <9.6%, a false negative rate of <2% and a test efficacy of ≥94%.

Significance: The Hygiena BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact is a suitable method for detection of *E. coli* O157 (including H7) in raw meats and significantly reduces total time to presumptive results compared to the reference method.

P2-147 A Rapid Screening Method for ß-Adrenergic Agonist Residues Incurred in Animal Urine Using Direct Analysis in Real Time Mass Spectrometry (DART-MS)

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Introduction: Direct analysis in real time mass spectrometry (DART-MS) works at atmospheric pressure without lengthy, laborintensive sample preparation or chromatographic separation of analytes. Consequently, DART-MS reduces labor, most laboratory supply costs, and overall time of analysis.

Purpose: To determine the usefulness of DART-MS for the rapid detection (screening) and semi-quantitation of clenbuterol, ractopamine, salbutamol and zilpaterol, ß-agonists used as feed additives or veterinary therapeutics, in cow, horse, and sheep urine.

Methods: Samples were mixed with 10% sodium carbonate, extracted with ethyl acetate, and applied to a 96-grid DART mesh prior to DART-MS analyses. Matrix matched calibration curves were used to extrapolate the ß-agonist concentrations in incurred urine samples.

Results: The coefficient of determination of standard curves for the compounds in cow, horse and sheep urine ranged from 0.9930 to 0.9986, 0.9829 to 0.9990, and 0.9925 to 0.9997, respectively. Limits of quantitation for clenbuterol, ractopamine, salbutamol and zilpaterol ranged from 4.0 to 239.7, 29.5 to 551.3 and 6.9 to 205.2 ng/mL in cow, horse, and sheep urine, respectively. The inter-day relative standard deviations of standards fortified in cow, horse and sheep urine were 26.3 to 33.4, 23.9 to 41.1 and 30.7 to 37.0%, respectively. Moderate correlations were measured between DART-MS and LC-MS/MS data for incurred zilpaterol residues in horse (R^2 = 0.73) and sheep urine (R^2 = 0.59).

Significance: The study demonstrates the utility of DART-MS for rapidly screening four commonly screened ß-agonists in urine matrices with very little sample preparation.

Poster

P2-148 Clear SafetyTM *Salmonella*: Automated Targeted NGS Detection and Serotyping from Sample Enrichments

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Introduction: *Salmonella* serotyping is an important tool to help identify strains of concern for implementation of risk-based mitigation strategies. Clear Safety is an automated NGS platform that can analyze up to 196 samples to detect *Salmonella* and identify the 63 most common serotypes from sample enrichments, avoiding the laborious and time-consuming steps of isolating *Salmonella* colonies that other serotyping methods require.

Purpose: The purpose of this study is to compare an NGS based system of *Salmonella* detection and serotyping to the USDA National Poultry Improvement Plan (NPIP) Program Standard culture-based method.

Methods: A total of 363 poultry environmental samples (boot swabs, dust swabs, and hatchery waste) were collected from primary production houses. Samples were analyzed according to the NPIP Program Standard (enrichment in Tetrathionate Broth at 1:10 sample to media ratio for 24h at 35°C, spotted on MSRV incubated at 35°C for 24h, plated on BGN and XLT4 agar incubated at 35°C for 24h, and traditional phenotypic serotyping was performed using the standard agglutination method). A portion of the tetrathionate enrichments was also analyzed by the automated targeted NGS system for *Salmonella* detection and serotyping.

Results: For the 363 poultry environmental samples, *Salmonella* was detected in 118 samples by the NPIP culture method, and 116 samples by the targeted NGS platform, demonstrating a strong agreement with a Cohen's kappa coefficient of 0.95. Serotyping of enrichments by targeted NGS agreed with traditional serotyping methods of isolates for 86% of the positive samples.

Significance: Current methods (WGS, Micro-bead based array, DNA arrays, traditional anti-sera) for serotype identification require isolated colonies. The automated NGS platform can accurately detect *Salmonella* and provide serotype information by analyzing sample enrichments, reducing the time to serotype results from >72h to 36h.

P2-149 Reduced Enrichment Time and Threshold Testing for *Salmonella* spp.

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Introduction: When testing raw meats and poultry with limited shelf life, food producers need rapid results to make food safety and processing decisions with increasing importance placed on quantification of *Salmonella* contamination.

Purpose: This study demonstrates methods to reduce enrichment times for the detection of *Salmonella* spp. along with evaluating the enrichment periods necessary to detect *Salmonella* at the LOD 1 (1 CFU/g) and LOD 10 (10 CFU/g) contamination thresholds.

Methods: Ground turkey samples were artificially contaminated with a cold-adapted cocktail of *Salmonella* Enteritidis, Typhimurium, Heidelberg, Kentucky and Newport before enrichment. Ground beef and beef trim samples were inoculated with either cold-adapted cocktails or individual strains of *Salmonella* Dublin, Typhimurium, and Montevideo before enrichment. Samples were inoculated at approximately 1 or 10 CFU/g, enriched at a 1:3 ratio with 2x Buffered Peptone Water prewarmed to 48°C, incubated at 42°C, and tested using the iQ-Check *Salmonella* II test kit with Easy II extraction.

Results: Results demonstrated the detection of *Salmonella* in 3-4 hr for LOD 10 and 4-5 hr for LOD 1 (depending on the food matrix and strain tested).

Significance: These data demonstrate the ability to reduce the incubation time necessary for the detection of *Salmonella spp*. This also shows that incubation times can be combined with the test method limit of detection to estimate original contamination levels of a food matrix.

P2-150 Rapid Detection of *Listeria monocytogenes* in Poultry Matrices Using Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay

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Introduction: Poultry is one of the most consumed sources of protein globally. *Listeria monocytogenes* is one of the most important foodborne pathogens causing listeriosis through contaminated food including undercooked raw poultry. Early detection of *Listeria monocytogenes* may help to minimize release of contaminated foods. Detection via traditional methods requires up to 4-5 days for final confirmed results. The use of rapid methods based on DNA amplification may provide a faster time to result with equivalent or better accuracy.

Purpose: The purpose of this study was to evaluate LAMP- and PCR-based methods and compare results with culture ISO method for recovery of *Listeria monocytogenes* in a variety of poultry matrices.

Methods: A total of 60 non-spiked samples were evaluated. The samples included mechanically separated chicken (n=4), cooked chicken (n=6), nuggets (n=6) and poultry parts (n=44). Each sample (25g) was enriched with Half Fraser Broth (1:1O) for *L. monocytogenes* and incubated at 37°C for 24h. After incubation, aliquots were tested by LAMP- or PCR- based methods. A second test portion of each of the 60 foods was analyzed (unpaired sample) following ISO 11290-1:2017. All enriched samples were culture confirmed according to the ISO 11290-1:2017.

Results: Detection of *L. monocytogenes* using the LAMP method and the PCR method were not significantly different ($p<0.05$). For the LAMP-method, seven out of sixty samples were presumptively positive; all were confirmed through culture. No false positive samples were found. For PCR-method, ten samples were presumptively positive; nine were confirmed through culture. No false negatives were found by either method.

Significance: The LAMP method is a rapid and accurate solution for *Listeria monocytogenes* in poultry samples. The LAMP-bioluminescent method is easy to use and offers poultry producers and reference laboratories a rapid method that may enable faster release of raw food products.

P2-151 A Nonculture Technique for Rapid Screening of *Salmonella* Typhimurium Flagellar Antigens in Raw Chicken Meat

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Introduction: *Salmonella* contaminated poultry products are important sources of food poisoning. Rapid and efficient approaches to identify the contaminated products are urgently needed to prevent the outbreaks of foodborne illnesses. Traditional methods for detection of *Salmonella* involve many time-consuming and laborious steps. Recent developments in immunomagnetic-nanoparticle separation have enabled the design of concentration and purification procedures to detect flagellar antigens of *Salmonella* directly from the contaminated foods without conventional culture methods.

Purpose: The purpose of this study was to develop a nonculture method for rapid screening of *Salmonella* Typhimurium flagellar antigens in raw chicken products.

Methods: Chicken drumsticks were artificially contaminated with *S*. Typhimurium at various levels and washed with deionized water. Flagellar antigens were extracted from the sediments of the chicken rinse after centrifugation. Immunomagnetic nanoparticles functionalized with monoclonal antibody (MAb 1C8) specific to flagellar antigens were prepared and incubated with the sample extract. The antigen-antibody nanoparticle complexes formed after incubation were concentrated and purified using a magnetic column. The purified antigen-antibody nanoparticle complexes were then reacted with another biotin-labeled MAb 1E10 specific to flagellar antigens and subsequently detected by an avidin-enzyme conjugate in the format of a sandwich immunoassay.

Results: The developed assay was highly sensitive and can be successfully performed within 2.5 hours. The assay showed a log-linear correlation for the concentration of *S*. Typhimurium in the range of 10^1-10^6 CFU/g, with an R^2 value of 0.9933. The detection limit of the assay was determined as low as 10 CFU/g. The results suggested that it can be further developed into small portable measuring devices in order to facilitate preliminary screening tests.

Significance: The flagellar antigens of *S*. Typhimurium can be detected directly from the contaminated foods without conventional culture methods. This assay allows the specific contamination sources to be traced along the processing and distribution lines in a timely and economic manner.

P2-152 Validation of a Cultural-Based Detection System for the Isolation of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* in Raw Ground Poultry

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Introduction: *Arcobacter* species are emerging foodborne pathogens whose prevalence is currently being underestimated due to limitations in current isolation procedures.
Purpose: The objective of this study was to evaluate the limit of detection, sensitivity, and specificity of two *Arcobacter* culture-dependent isolation detection systems: (1) the *Nguyen-Restiano-Juárez Arcobacter* detection method (NRJ-B/M) and (2) enrichment in *Houf* broth (HB) followed by isolation on modified Charcoal Agar (mCCDA+CAT).
Methods: *Arcobacter butzleri* (Ab), *Arcobacter cryaerophilus* (Ac), and *Arcobacter skirrowii* (As) were artificially inoculated in 25-g ground chicken samples (n = 780) at 0.1 and 1.0-2.0 CFU/g to determine method sensitivity. Inoculated samples were enriched (1:10 dilution factor) in either HB or NRJ-B and incubated aerobically at 30ºC for 48 h. HB samples were streaked onto mCCDA+CAT and incubated aerobically at 30ºC for 48 h; whereas, NRJ-B samples were streaked onto NRJ-M and incubated aerobically at 30ºC for 72 h. Isolates were confirmed as *Arcobacter* spp. using Gram-strain, catalase, oxidase, and wet mount microscopy. Method specificity for NRJ-B/M and HB/mCCDA+CAT was evaluated using un-inoculated 25-g ground chicken samples (n = 40).
Results: No significant difference ($p > 0.05$) was observed when isolating *A. butzleri* for NRJ-B/M (>90%, Ab) and HB/mCCDA+CAT(>90%, Ab) at the 0.1 and 1.0-2.0 CFU/g. However, the sensitivity was significantly higher ($p < 0.05$) for NRJ-B/M (78.0%, Ac; 25.3%, As) compared to HB/mCCDA+CAT (34.1%, Ac; 13.8%, As) for the isolation of *A. cryaerophilus* and *A. skirrowii* at the 0.1 CFU/g. At the 1.0-2.0 CFU/g, NRJ-B/M also yielded a higher sensitivity (95.1%, Ac; 40.7%, As) compared to HB/mCCDA_CAT (51.2%, Ac; 22.0%, As) for *A. cryaerophilus* and *A. skirrowii* isolation. Moreover, the specificity for HB/mCCDA+CAT (30.0%) was noticeably lower ($p < 0.05$) than NRJ-B (90.0%) using un-inoculated ground poultry samples.
Significance: This study demonstrates NRJ-B/M was superior to HB/mCCDA+CAT in isolating *Arcobacter* spp. from ground poultry. Here, we strongly recommend its use in future prevalence studies for different food matrixes.

P2-153 Evaluating the Shelf-Life of Vacuum-Packaged Chicken Using an Accelerated Shelf-Life Method

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Introduction: The poultry industry uses various antimicrobials to extend the shelf-life of various products. However, shelf-life testing can be time-consuming and expensive, especially for products with longer shelf-life. An accelerated shelf-life method can be used to evaluate antimicrobial shelf-life extension in a shorter time with accuracy.
Purpose: To determine the shelf-life of chicken breast using an accelerated shelf-life testing method at different temperatures.
Methods: Raw, boneless, and skinless chicken breasts were cut manually into cubes (5 x 5 cm) and inoculated with a four-strain cocktail of lactic acid bacteria (LAB) to achieve a population of ca. 1.2 log CFU/g. Post-inoculation, the cubes were held for 30 min for bacterial attachment. For treatment 1 (T1), chicken cubes were left untreated, and for treatment 2 (T2), chicken cubes were treated by dipping in 10% Verdad® N100, a novel, vinegar-based antimicrobial solution for 30 s. Samples were permitted to air dry for 5 min, vacuum packaged, and stored at three temperatures (4, 10, 16°C). For enumeration, the chicken cubes were removed on different days, transferred to a sterile bag, diluted, stomached, and spread plated on de Man, Rogosa, and Sharpe agar (30°C for 48 h).
Results: Temperature and treatment showed a significant effect ($p<0.05$) on the growth rate of LAB. For T1 and T2, as the temperature increased from 4 to 10°C (x2.5), the growth rate increased by ca. 2.5 times from 0.547 to 1.692 and 0.255 to 0.630 log (CFU/g) $days^{-1}$, respectively. Also, the shelf-life of products T1 and T2 decreased by ca. 2.5 times as the temperature increased from 4 to 10°C.
Significance: While further data generation into accelerated testing is warranted, the identification of an acceleration factor (x2.5 at 10°C) may allow quicker testing of different antimicrobials in a shorter period of time.

P2-154 Shelf-Life Extension of Vacuum-Packaged Raw Ground Turkey Using Newly Developed Antimicrobials

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Introduction: Ground turkey is popular among consumers worldwide due to its nutritional characteristics. Currently, meat processors are looking for solutions to extend the shelf-life of ground turkey. Two novel antimicrobial solutions, BV (low-sodium buffered vinegar) and PF (product of fermentation) have been developed to extend the shelf-life of ground turkey.
Purpose: The aim of this study was to extend the shelf-life of ground turkey by the application of newly developed antimicrobials.
Methods: The following treatments were evaluated in this study: control (non-treated), 1% BV, and 1% PF. Uninoculated raw ground turkey was mixed with the desired amount of treatment, vacuum packaged in 50 g portions, and stored at 4.4°C for 21 d. Samples were plated on different days, diluted with buffered peptone water, stomached for 30 s at 200 rpm. The dispersions were serially diluted with Butterfield's buffer and spread plated onto tryptic soy agar for aerobic plate count (APC; 35°C for 24 h) and psychrotrophs (7°C for 10 d), de Man, Rogosa, and Sharpe agar for lactic acid bacteria (LAB; 30°C for 72 h), and violet red bile glucose agar for Enterobacteriaceae (EB; 35°C for 24h).
Results: LAB and APC counts in control turkey samples reached 6 log by day 4, while BV- and PF-treated samples reached 6 log CFU/g by day 14. On day 21, EB population in the control sample (6.0 log) was significantly ($p<0.05$) greater than both BV- and PF-treated samples (<1.0 log). Similarly, psychrotroph counts in control samples (8.2 log) were also significantly greater than treated samples (6.1 log) on day 14.
Significance: Newly developed antimicrobials (BV and PF) were effective in increasing the shelf-life of ground turkey by additional 10 days when compared to the control sample.

P2-155 Low Temperature and Copper Exposure Enhances *Listeria monocytogenes* Biofilm Formation

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Introduction: *Listeria monocytogenes* (*Lm*) is a foodborne pathogen that causes listeriosis, a very serious and lethal disease. *Lm* can resist diverse stress factors utilized in the food industry to prevent bacterial growth, such as low temperatures and copper surfaces. *Lm* can attach to abiotic surfaces and form biofilms, which allows it to persist in food processing equipment.
Purpose: To evaluate the effect of low temperature and copper exposure on *Lm* biofilm formation and its transcriptional response.
Methods: *Lm* isolates (n=22) were evaluated for their biofilm production at 8°C, 37°C, and in the presence of sub-lethal copper concentrations (0.5 mM). At 48 h, biofilm formation was measured with the crystal violet method. Isolates were ranked according to their biofilm formation abilities at 37°C, and five isolates (high and low biofilm producers) were selected to evaluate the transcriptional response and motility under the previous conditions.
Results: Biofilm assays showed that 8°C and copper, separately, decreased biofilm formation compared to 37°C. However, simultaneous stress of low temperature + copper significantly enhances biofilm formation. Transcriptional studies showed that isolates increased the expression of the copper-homeostasis gene *csoR* in the presence of copper at 8° and 37°C. Only high biofilm producers (HBP) showed an increased expression of *motA*, *agrD,* and *luxS*

Poster

in biofilm formed at 8°C+Cu compared to 37°C. Motility assays showed that HBP were more motile at both temperatures than low biofilm producers. The addition of copper decreases motility at both temperatures.

Significance: This study provides novel information about *Lm* ability to adapt to simultaneous stressors such as low temperature and copper. This response was associated to a transcriptional change of biofilm-related genes which varies between isolates regarding their biofilm formation levels. Further studies should be performed to have a deeper understanding of these mechanisms to develop better control strategies by the food industry.

P2-156 DNA Extraction Method Comparison for the Detection of *Salmonella* in Seafood by qPCR

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Introduction: Blending is required for some food matrices like seafood in BAM *Salmonella* culture method. Blending may release PCR inhibitors and increase difficulties for DNA extraction from cloudy preenrichments. FDA qPCR has been validated as a rapid screening method using a 24 h preenrichment. Therefore, effective and efficient DNA extraction methods are needed for these types of matrices.

Purpose: To examine the effectiveness of various procedures for the extraction of DNA from fish samples.

Methods: Per FDA's Microbiological Method Validation Guidelines, thirty 25 g fish samples were inoculated with *Salmonella* inoculum at high, low and uninoculated levels and aged for two weeks at -20˚C. On the first day of sample analysis, 25 g samples were blended in 225 mL Universal Preenrichment Broth (UPB) for 2 min and then incubated at 35 ˚C for 24 h. Automatic and manual DNA extraction were performed from 24 h preenrichments. Four automatic DNA extraction methods (MagMax-Qiagen, MagMax-PrepSEQ, Kingfisher Apex-Qiagen and Maxwell-RSC culture cell kits) and two manual DNA extraction methods (direct boiling with purification and InstaGene boiling with purification) were compared for the detection of *Salmonella* using FDA's *Salmonella* qPCR method. All preenrichments were analyzed using the BAM *Salmonella* culture method.

Results: qPCR results from all the DNA extraction methods matched the cultural results. However, all automatic DNA extraction methods had lower C_T values than the manual DNA extraction procedures. Maxwell-RSC was the best automatic method in that it used the least preenriched culture media and generated the lowest C_T values. The InstaGene boiling DNA extraction method was more effective than direct boiling.

Significance: Multiple effective DNA extraction methods will provide flexibility for the application of qPCR as a rapid screening tool for FDA regulatory laboratories when processing different sample types or different sample sizes.

P2-157 Assessing the Efficacy of Addition of Bacteriophage in the Wheat Tempering Water to Reduce the *E. coli* O121 *and* O26 Load of Wheat

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Introduction: In recent years, food safety issues involving pathogenic *E. coli* – contaminated wheat flours have increased. This trend shows the need for incorporating antimicrobial interventions in the wheat milling process.

Purpose: The objective of this study was to determine the efficacy of bacteriophage treatment in reducing the *E. coli* O121/O26 load of wheat during the tempering step and evaluate its impact on wheat flour quality.

Methods: Pre-dried grains (200 g) were inoculated (~6.0 log CFU/g wheat) with the *E. coli* O121/O26 inoculum and equilibrated for 24 h at 25°C. Wheat grains were tempered to 16% moisture using the bacteriophage solution/ tempering water solution. The bacteriophage (BP) solution (Grain Protect™) was applied to the wheat at 8 log PFU/ g wheat (1% wheat basis; 2 ml BP + 14 ml water; 0.5% wheat basis; 1 ml BP + 15 ml water). The *E. coli* load of the wheat during tempering (0.5, 1, 2, 6, 12, 18, and 24 h) was evaluated by plating in MacConkey agar (100 µl, duplicates). The impact of the treatment on flour quality was assessed by analyzing the flour milling, physico-chemical properties and baking performance.

Results: The *E. coli* load of the inoculated wheat grains was significantly reduced ($P < 0.05$) at 1.0% treatment by 2.6 (± 0.1) and 2.8 (± 0.2) log CFU/g after 6 and 12 h of tempering respectively. Maximum reductions of 3.1 (± 0.2) log CFU/g was observed ($P < 0.05$) after 24 h of tempering. In terms of flour quality, no significant differences ($P > 0.05$) in the flour milling, physico-chemical, and baking qualities were observed between the control (water only) and bacteriophage treatment (1.0%).

Significance: The results show that bacteriophage treatment can be an effective, and natural antimicrobial treatment for wheat milling against *E. coli* O121/O26 contamination resulting in improved wheat flour food safety.

P2-158 The Impact of Drying on the Survival of *Escherichia coli*, *Listeria innocua* and *Bacillus cereus* on Sugar Kelp

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❖ ***Developing Scientist Entrant***

Introduction: Sugar kelp (*Saccharina latissima*) is a brown macroalga widely harvested in Asian and Western countries including Northeastern US. Sugar kelp is highly perishable. Drying can improve microbial quality and shelf life of sugar kelp by reducing water activity and moisture content. However, the effect of drying on the survival of bacterial pathogens is not well characterized.

Purpose: The purpose of this study was to investigate the effect of controlled drying conditions on the survival of pathogen surrogates *Listeria innocua, Bacillus cereus* and *Escherichia coli* inoculated on sugar kelp.

Methods: Fresh sugar kelp was washed and cut into blades of uniform size (15 cm length). Each blade was inoculated with 10^7 log CFU/g inoculum level of *L. innocua, B. cereus* and *E. coli* (separately) and dried in a convective dryer at various parameters: 40°C and 50°C, 25% and 50% relative humidity, to one of two target final water activities (0.3 and 0.5). Survival of pathogens on inoculated dried samples was assessed using cultural techniques. One-way ANOVA ($p<0.05$) was used to assess the effects of treatment temperature, relative humidity, and water activity on the survival of inoculated pathogens.

Results: Drying treatments at different drying conditions significantly reduced the population of *L. innocua*, *E. coli* and *B. cereus* on the inoculated sugar kelp. Drying at parameters 50 °C, 50%, 0.3 resulted in the maximum reduction of *E. coli* (4.24 ± 0.15 log CFU/g), and *L. innocua* (3.36 ± 0.94 log CFU/g). However, 40 °C, 25%, 0.5 parameters, resulted maximum reduction of *B. cereus* (4.64 ± 0.05 log CFU/g).

Significance: The results of this study indicated that drying can be a useful method to reduce the population of *L. innocua, B. cereus* and *E. coli* on the sugar-kelp, but that all pathogens have the capability to survive and are differently affected by process variables.

P2-159 Validation of a Multiplex PCR Workflow for the Detection of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* from Seafood

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Introduction: Vibriosis is responsible for an estimated 80,000 cases of illness annually, of which 100 result in death in the United States. Vibriosis is contracted mainly through consumption of raw and improperly cooked seafood. *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the predominant species of the *Vibrio* genus that cause vibriosis within humans.

Purpose: To evaluate the Thermo Scientific™ SureTect™ Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay (candidate method) for the detection of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* from seafood matrices according to the AOAC Performance Tested Methods℠ program.

Methods: Robustness, inclusivity/exclusivity and a matrix study consisting of 50 g raw tuna, 50 g raw mussels, 125 g cooked shrimp, 50 g salmon roll with cream cheese and 50 g Green Lipped mussel extract powder were conducted according to AOAC Appendix J. The candidate method was compared against the FDA BAM Chapter 9 reference method, which was modified to a detection principle. Raw mussels and raw tuna were also compared against the ISO 21872-1:2017 reference method. Product stability and consistency studies were conducted according to Appendix J.

Results: Stability and consistency testing data demonstrated that the method was robust and consistent across the specific time points and testing conditions. Inclusivity and exclusivity data showed the method was accurate and specific. Matrix testing data showed comparable performance between the candidate and reference method for all matrices and satisfied the requirements of Appendix J.

Significance: The data obtained shows that the candidate method uniquely provides rapid, reliable and accurate multiplex PCR detection of selected *Vibrio* Spp. from seafood, supported by data obtained during the AOAC validation studies.

P2-160 Impact of Environmental Stresses on the Viability State of *Listeria monocytogenes* and *Listeria innocua* Analyzed by Raman Microspectroscopy, Molecular Biology and Microbiology Techniques

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❖ ***Developing Scientist Entrant***

Introduction: Food-processing plants are a hostile environment for bacteria, which are exposed to various stresses, such as osmotic, nutritional, alkaline, acidic, thermal, chemical and oxidative. These stresses may cause some of the bacterial population to enter the VBNC state. Once in this metabolic state and became no detectable by the culture-based methods, which only target viable cultivable (VC) population. These VBNC bacteria retain their ability to become pathogenic again under favourable conditions. In recent years, Raman microspectroscopy has emerged as one of the most innovative tools in the research and characterization of bacteria. Coupled with deuterium isotope probing (Raman-DIP), this technique allows to measure the metabolism of a bacterium and to evaluate its viability.

Purpose: We evaluated the impact of different environmental stresses present in the seafood processing industry such as low food storage temperatures (4°C), salting step (20% of salt), and biocide procedures (P3-topactive DES at 2% v/v) on *L. monocytogenes* and *L. innocua* populations.

Methods: We treated the suspensions of *L. monocytogenes* and *L. innocua* with these different stresses, alone or combined. We analyzed their metabolism by Raman-DIP microspectroscopy and we quantified the total, viable and VC population respectively by qPCR, PMA-qPCR and plate count agar.

Results: After biocide treatment, the total population was in VBNC state for *L. monocytogenes* with a significantly lower C-D peak than VC bacteria observed by Raman microspectroscopy and the total population was dead for *L. innocua*. For salt and temperature treatment, the whole population was in VC state. For a combined stress mixing low incubation temperature and biocide treatment, we observed an antagonistic effect where bacterial population was more photoresistant to the laser beam of the Raman microspectroscope than when when these two stresses were applied separately.

Significance: We measure the impact of environmental stresses on the viability state of *L. monocytogenes* and *L. innocua* by Raman microspectroscopy with a better accuracy than molecular biology and microbiology techniques do.

P2-161 The Diversity of Fish Species Presents a Challenge with Universal Detection of Fish Residue – Can a Targeted Approach be Used to Improve Detection?

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❖ ***Developing Scientist Entrant***

Introduction: The detection and quantification of fish residues in food remains a challenge with currently available immunoassays due to the diversity of fish species. Thus, the development of an improved, sensitive, and robust fish ELISA method in detecting a wide range of commercially important fish species is essential.

Purpose: Two commercial fish ELISA kits were evaluated for their efficacy in detecting and quantifying fish residue from an incurred food matrix. Additionally, six anti-fish peptide polyclonal antibodies were assessed for their ability to detect proteins from nine diverse fish species of commercial importance.

Methods: Beef meatballs incurred with fish at different concentrations (0, 5, 10, and 100 ppm cod, salmon or tuna) were subjected to boiling, baking, frying, and high-pressure processing (HPP). Two commercial fish ELISA kits (Kit A & B) were evaluated for the recovery of fish residue from the incurred food matrix to serve as the baseline for current fish detection. Six peptides from fish aldolase, enolase, parvalbumin, and myosin proteins were selected using mass spectrometry and used to raise polyclonal antibodies in rabbits. Proteins extracted from nine commercially important fish species were used for antibody evaluation.

Results: Kit A performed better in recovering cod fish from incurred meatballs compared to salmon and tuna which were overestimated. Recovery was <10% with Kit B for all samples. Western blotting indicated that antibodies raised against peptides from myosin light proteins and aldolase recognized the extracts of all nine fish species, while eight fish species were recognized by antibodies raised against peptides from myosin heavy and parvalbumin proteins. These results indicate the robustness of the selected antibodies.

Significance: Currently available commercial fish ELISAs are limited in reliably detecting fish residue across multiple species. A targeted approach for selection of fish protein targets may aid in the improvement of fish protein detection across commercially important fish species.

P2-162 The Antibacterial Efficacy of Two Hemp (*Cannabis sp.*) Cultivars on *Listeria monocytogenes* and *Salmonella enterica*

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Introduction: Plants are an excellent source of bioactive components, hence some of their medicinal and natural antimicrobial attributes gain them much attention in the food and nutraceutical industry. Hemp (*Cannabis* spp), because of its well-known antibacterial activity, has gained interest in food safety applications. Although some work has been done in this area, the antibacterial efficacy of cultivars grown in Northern Alabama and their implication to food safety and quality is currently unknown.

Purpose: The purpose of this study was to evaluate the antibacterial potential of two Northern Alabama hemp (grown at the Winfred Thomas Agricultural Research Station, Hazel Green, AL) cultivars (CBD 5 and 17) as a whole (W) and defatted (DF) crude macerated ethanolic extracts in 96% ethanol.

Methods: The antibacterial activity against cocktails of enteric pathogens *Listeria monocytogenes* (LM- H7969 serotype 4b, H7962 serotype 4b, and Scott A NADC 2045 serotype 4b).), and *Salmonella enterica* (SE- ATCC13076, ATCC14802, and ATCC8324) were evaluated with a BioScreen C MBR (Oy Growth

Curves Ab Ltd, Finland), disc diffusion method, minimum inhibitory and bactericidal concentrations assay. The data treatments (n = 9) were analyzed using ANOVA. The statistical significance was evaluated based on P≤0.05, and all experiments were conducted in triplicates.

Results: The antibacterial results indicated that hemp extracts had a significantly ($p \leq 0.05$) lower optical density at the end of the 24-hour observation period compared to the negative controls (LM and SE) . Disc diffusion results ranged from 11.31 ± 2.75 mm (ethanol-positive control) to 12.77 ± 0.66 mm (CBD 5 DF) . CBD 5 and 17 extracts against LM and SE had minimum inhibitory concentrations of 11 mg/mL and 16 mg/mL and the minimum bactericidal concentrations of 16 mg/mL and 21 mg/mL, respectively.

Significance: The data suggests that the hemp extracts had significant antimicrobial efficacy, highlighting their ability to be used as an active antimicrobial film material.

P2-163 Predicting the Growth of *Salmonella* Typhimurium Inoculated on Chicken Breast Fillets during the Simulated Less-Than-Truckload Cyclic Temperature Abuse Conditions

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Introduction: Current less-than-truckload (LTL) shipping practices in the last mile of the cold chain allow for temperature abuse (TA) of perishable goods resulting in potential food safety issues at the retail level.

Purpose: The objective of this study was to develop models to predict the growth of *Salmonella* Typhimurium in simulated supply chain conditions to aid in retail decision making.

Methods: Commercially produced boneless skinless chicken breast were inoculated with *Salmonella* Typhimurium (35µg/mL nalidixic acid resistant) to approximately 10^3 CFU/mL. The breasts were placed in sterile whirl-pak bags which were then placed in an incubator programmed to alternate between 4°C (2 hours) and 25°C (2 hours) for 24 hours. Microbial sampling was performed every 6 hours with breast rinsates (n=3/trial x 3 trials) being serially diluted and plated on XLT4 agar. The temperature and microbial data from three trials were used along with a predictive model for chicken skin to develop a secondary model for the growth rate and lag time of *Salmonella* Typhimurium on cyclically TA chicken breast.

Results: The actual and predicted concentrations (CFU/mL) at 0, 6, 12, 18, and 24 hours were similar indicating the accuracy of the model to predict the pathogen growth. The lag time of *Salmonella* Typhimurium was predicted to be 15.75 hours or 0.66 days under our test conditions.

Significance: The study demonstrates that current LTL practices in cold chain could be impacting the food safety of perishable foods. Predictive models can be used in food supply chains to accurately predict the growth of *Salmonella* spp. and allow retailers to make the best decisions regarding their products.

P2-164 Assessment of Fresh Fish in Retail Markets for Contamination with Major Fecal Pathogens and Antibiotic-Resistant Organisms

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Introduction: Fresh fish sold at retail markets in developing countries are often contaminated with major foodborne pathogens including antibiotic resistant organisms.

Purpose: This study aimed to estimate the prevalence of fecal pathogens in two most consumed fresh fish, tilapia and pangas from retail markets in Dhaka city, Bangladesh.

Methods: A total of 249 tilapia and 251 pangas samples were collected in the morning and evening from the same fish-vendors in 32 wet-markets and 25 super-shops and tested for *E. coli*, extended spectrum beta-lactamase producing *E. coli* (ESBL-Ec), *Salmonella* spp., *Shigella* spp., *Vibrio cholerae* and *V. parahaemolyticus*. All fish vendors were interviewed for their vending practices.

Results: Of 500 samples, 92% were positive for *E. coli* with no significant difference between pangas (94%) and tilapia (89%) and between morning (92%) and evening (92%) collections. For both types of fish, significantly higher proportion of samples from wet markets (97%) were contaminated with *E. coli* compared to samples from super-shops (71%) ($p<0.001$). The estimated mean counts (SD) of *E. coli* in both fishes from wet markets and super-shops were 3.13 $\log_{10}$ CFU/g (0.92) and 2.82 $\log_{10}$ CFU/g (0.94), respectively ($p<0.001$) while, mean counts for ESBL-Ec were 2.80 $\log_{10}$ CFU/g (0.60) and 2.23 $\log_{10}$ CFU/g (0.29), respectively ($p<0.05$). Approximately 56%, 23% and 3% of samples were positive for *Salmonella* spp., *V. cholerae* and diarrheagenic *E. coli*, respectively. Among *E. coli* isolates, 53% were resistant to 3rd generation cephalosporin, 16% to ciprofloxacin and 46% were multi-drug resistant (MDR). Among other factors, washing of fish selling/cutting area for >3 times a day was found to be significantly associated with lower level of *E. coli*, ESBL-Ec and *Salmonella* spp. contamination in fish.

Significance: Fish at retail markets in Dhaka harbored MDR fecal pathogens, which is a significant public health concern and could be a major barrier to international trade.

P2-165 Understanding of and Compliance with Hazard Analysis and Critical Control Point (HACCP) in Selected Restaurants Along the East-West Corridor, Trinidad, West Indies

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Introduction: Restaurants and the fast-food industry are becoming more and more embedded in the cultural landscape of Trinidad and Tobago. Effective food safety practices are now critical to combat the potential for food borne illnesses and continue to build the confidence of consumers in the food that they purchase and eat at those restaurants.

Purpose: The purpose of this study was to determine the understanding of and compliance with HACCP in selected restaurants in the east-west corridor of Trinidad, West Indies by managers/owners and food handlers using a mixed methods research procedure.

Methods: A structured questionnaire initially designed by the Food Safety Authority of Ireland was adapted for use. Face-to-face interviews were conducted with 17 managers/owners and 48 food handlers. The questionnaires comprised of multiple choice and open-ended questions on general information on the restaurant; demographic information on the food handler; awareness of and compliance with HACCP; barriers to HACCP implementation and training. The raw food handler knowledge scores were converted to ranks and analyzed by Kruskal-Wallis one-way analysis.

Results: Most (58%) food handlers had heard of the term HACCP, while most (88%) restaurants were non-compliant with HACCP. These restaurants communicated that basic and Good Hygiene Practices (GHPs) are used as their food safety management system., whereas 42% said they did not. When the food handlers were asked about how their organization made them aware of food safety procedures, 65% stated through team meetings/briefings, 38% stated that posters and signage. Managers/owners and food handlers rated their understanding of HACCP as 47% 'poor' and 29% 'fair'. The average knowledge score by food handlers was 70%.

Significance: The research identified the barriers to HACCP implementation, food handlers' knowledge of GHPs, and training needs as perceived by managers and food handlers. A list of recommendations and further research studies were posited.

Poster

P2-166 Evaluating the Impact of Requiring Certified Food Protection Managers on Inspection Compliance

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❖ ***Developing Scientist Entrant***

Introduction: Certified food protection managers (CFPMs) have been shown to positively impact inspection performance and food safety compliance. In 2019, Ohio began requiring risk level 3 and 4 retail food establishments and foodservice operations to employ a CFPM. This provides a unique opportunity to assess the impact of this policy change on retail compliance with the Ohio Food Code.

Purpose: This study will evaluate the impact of the Ohio CFPM requirement on inspection compliance among establishments under the jurisdiction of Franklin County Public Health using historical inspection data collected before and after CFPMs became required.

Methods: Inspection records for all licensed food establishments during the study period (3/1/2018 – 2/28/2020) were obtained. Violations were categorized and totaled according to criticality and according to foodborne illness risk factors. Logistic regression was used to assess the association between the presence of a CFPM and compliance with individual inspection items.

Results: Approximately 8,351 inspections were conducted at 1,866 licensed establishments during the study period. Of these, 1,525 (81.7%) establishments were food service operations and 341 (18.3%) were retail food establishments. Establishments were fairly evenly distributed between risk level 3 (48%) and risk level 4 (52%). Initial analyses identified a violation for improper handwashing procedure (OAC 3717-1-02.2-b2) to be 0.306 (0.155 to 0.603) times more likely in establishments that also violated the CFPM requirement (OAC 3717-1-02-4a) (p= 0.0006). Further analyses will identify additional violations and violation categories (critical vs. non-critical, correspondence with the 5 foodborne illness risk factors) that are associated with violations of Ohio's CFPM requirement.

Significance: CFPMs have significant potential to improve establishment food safety performance and reduce the likelihood of a foodborne illness outbreak. A better understanding of how CFPMs impact foodborne illness risk factors in food service operations and retail food establishments could improve retail food safety, which is relevant to public health.

P2-167 Does Food Safety Education Matter? Perspectives of Hospitality Leaders

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Introduction: Food safety is an essential component of the hospitality management curriculum nationwide, however, it is noticed that students now have become reluctant to be enrolled in food safety classes or take the required tests and some programs tended to remove food safety courses from the curriculum.

Purpose: The purpose of this study was to obtain a comprehensive understanding of the food safety education needs from hospitality leaders' perspectives and better provide recommendations to hospitality educators regarding food safety education components.

Methods: Individual interviews were conducted with 20 leaders in the hospitality industry. All interviews were audio-recorded, transcribed verbatim, coded, and verified by researchers. The final interview transcripts were coded by following the six-stage framework analysis outline by Ritchie and Spencer (1994).

Results: Most respondents reported that ServSafe® certifications are required for supervisory or managerial staff but not entry-level employees due to the costs of training and high employee turnover rates, whereas all participants agreed that students should be trained about food safety before entering the job market. Motivational and experiential learning formats such as case studies and hands-on practices for students to apply food safety knowledge learned in class were recommended by interviewees regarding improving food safety education in hospitality management education programs. Improving students' awareness of the importance of food safety and how different culture shapes people's food handling behaviors is important in training the future food service workforce and preparing future leaders in the hospitality industry.

Significance: Results of this study serve as a solid foundation for hospitality management programs in the U.S. to revisit the current food safety education curriculum and improve it to better prepare students for the food service industry.

P2-168 Food Choice Behaviors of College Students with Food Allergies: A Comparison between On-Campus and Off-Campus Dining Options

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Introduction: Many college students reported having food allergies, however, limited studies have been conducted to examine factors that may influence their food selection behaviors.

Purpose: The Purpose of this study was to: 1) explore food choice decision among college students with food allergies and 2) compare their dining experiences in different settings, including dining halls, on-campus retail dining, and other types of dining facilities.

Methods: A qualitative approach was employed to achieve the research objectives. This study used a purposive, self-selecting, mixed sampling method to include a total of 26 participants with diverse demographic characteristics. Each participant was interviewed for 30 minutes using a semi-structured interview guide. All interviews were audio-recorded and transcribed verbatim. Transcriptions were coded independently by three researchers and then compared for consistently.

Results: In addition to safety, convenience, walking distance, food variety, nutritive values, and price of foods were identified as factors influencing the participants' food choice decisions. While on-campus dining facilities were convenient, accessible, have accommodating staff, the variety of food and business hours were limited. The participants felt that off-campus dining facilities offered a wider variety of food choices, at a lower price. Even so, some restaurant staff lacked food allergy knowledge and awareness. Food trucks were mentioned as an alternative dining option, but they did not usually display food allergen information, and there was also cross-contact concern with food trucks. The participants expected on-campus dining facilities to offer more variety of food choices and designate allergy-friendly dining areas for those in needs.

Significance: The results revealed food choice decision of college students with food allergies. This study also provided recommendations for the management of college dining services to meet the needs of college students with food allergies.

P2-169 Food Safety Attitudes, Knowledge, Self-Reported Practices and Observed Behaviour of Food-Service Employees: Triangulation of Findings in Published Research

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Introduction: Understanding food-handler food safety compliance and cognitive factors, influencing it, is essential to ensure consumer food safety. Previous research revealed that there is a lack of triangulation in food safety research. There is a need to triangulate research methods to have better understanding of cognitive and behavioural factors that influence food-handler compliance in food-service settings.

Purpose: To triangulate data across primary studies to gain deeper understanding of food-handler food safety behaviour and cognition.

Methods: A qualitative analysis of primary research studies (*n*=118) detailing food-handler food safety behaviour and cognition (e.g., knowledge, attitude, self-reports) was undertaken. Data detailing handwashing, preventing cross-contamination and temperature control were gathered. Collated data were triangulated using an interpretative approach.

Results: Triangulation of collated research (*n*=118) has demonstrated disparities between cognitive and behavioural data. For example, positive attitudes towards handwashing, adequate knowledge of handwashing procedure, and appropriate awareness of possible implications of incorrect handwashing among food-handlers were identified. Despite this, observational studies revealed improper and inconsistent handwashing behaviour. Studies demonstrated general awareness of cross-contamination risk when handling foods, but non-compliances were observed, such as wearing jewellery, using same utensils for raw and cooked foods and incorrect cleaning and sanitizing. Studies showed reasonable food-handler knowledge of refrigerating temperatures, but insufficient knowledge of 'danger zone' temperatures and use of sensory indicators for ensuring cooking adequacy. In addition, malpractices, such as incorrect thawing and refrigeration of foods were reported, however comparative behavioural data are lacking.

Significance: This study has determined potential discrepancies between cognitive and behavioural food-service food safety data, indicating that knowledge, attitude and self-reported practice may be subject to social-desirability bias and are not indicative of actual food safety performance. A lack of behavioural data detailing handwashing durations and temperature control has been identified among food-handlers. Future research based on triangulated research methods will provide an in-depth understanding of food-handler cognition and behaviour.

P2-170 Impact of Hand Washing or Alcohol-Based Hand Sanitizer on Bacterial Contamination of Hands and Surfaces in a Kitchen Environment: A Volunteer Study

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Introduction: In FDA Food Code regulated settings, alcohol-based hand sanitizers (ABHS) cannot replace soap and water [handwashing (HW)] for hand hygiene. Scientific evidence has demonstrated equivalency of some ABHS to HW for bacterial removal from hands, but these studies often did not: 1) consider food soils; 2) establish a baseline for bacterial contamination on hands; or 3) investigate efficacy during meal preparation.

Purpose: Using a kitchen environment with food handlers performing meal preparation, this study compared the effectiveness of HW vs. ABHS at removal of bacteria from hands as well as prevention of hand-mediated surface contamination.

Methods: Chicken tenderloins and ground beef were inoculated with a GFP-tagged *Escherichia coli* DH5α strain at 7-10 $\log_{10}$ CFU/meat item. Twenty volunteers with recent food handling experience were recruited per treatment group (control [no hand hygiene], HW, and ABHS). Volunteers were instructed to prepare 20 beef tacos, followed by 10 chicken salads while maintaining strict compliance with FDA Food Code guidelines. After meal preparation, whole hand sampling was performed, along with swabbing of the first five surfaces touched after handling contaminated meat. *E. coli* enumeration was performed on TSB-kanamycin plates.

Results: HW (1.4±0.5 $\log_{10}$) and ABHS (1.9±0.9 $\log_{10}$) displayed significantly lower *E. coli* counts on hands after meal preparation compared to the control group (4.0±1.3 $\log_{10}$; $p<0.05$) with 35%, 65% and 100% of samples found to be contaminated, respectively. Surface contamination was at rates of 73%, 15% and 8% for the control, HW and ABHS groups, ($p<0.05$), respectively. When positive, contaminated surfaces did not differ in total *E. coli* counts, regardless of intervention group ($p>0.05$).

Significance: HW and ABHS performed equivalently at reducing *E. coli* counts on hands. Additionally, HW resulted in lower prevalence of *E. coli* on hands, but ABHS resulted in lower prevalence of *E. coli* on surfaces. This study demonstrates the importance of hand hygiene interventions in reducing bacteria on hands and surfaces during meal preparation.

P2-171 A Study of Food Hygiene and Sanitation-Related *Journal of Food Protection* Articles from 2017–2021: Can This Evaluation Help Food Professionals Develop *Salmonella* Control Programs?

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Introduction: Salmonellosis is a global concern and is one that requires robust interventions from farm-to-fork, including sanitation controls. Food safety professionals require relevant and timely information that helps in the development of sanitation strategies used to meet *Salmonella* reduction goals. IAFP's Journal of Food Protection (JFP) can be one such reliable source of scientific research articles on food safety and sanitation.

Purpose: To provide food safety professionals with a summary of peer-reviewed information regarding food hygiene and sanitation, specifically in relation to *Salmonella*.

Methods: 223 selected articles published in JFP between 2017-2021 were classified using Zotero tagging software based on their: (1) country of study; (2) author(s) affiliation; (3) challenges/hazards of concern; (4) food sector represented; and (5) derived food hygiene and sanitation issue(s). The search was then refined to evaluate the *Salmonella*-related articles.

Results:

- Within the 223 articles selected, 43 countries were represented. The main contributors of the articles were academia (73.5%), research organizations (36.3%), and government agencies (29.1%). Industry and professional institutions (9.9%) contributed the least number of articles.
- The top three hazards identified were: *Salmonella* (37.2%), *Listeria* (30.0%) and *Escherichia coli* (28.7%).
- 15 *Salmonella*-related sanitation issues were derived, with the top three being: (a) environmental monitoring and cross-contamination control; (b) hygiene awareness and compliance, and (c) slaughterhouse/carcass process management.
- The key food sectors associated with *Salmonella* were meat and poultry (43.4%), retail and foodservice (22.9%), and produce (19.3%).

Significance: This study shows that publications such as JFP can offer a repository of credible knowledge that supports food professionals in the development of *Salmonella* control strategies and similar programs. However, there is need for: (I) a navigable method for tagging articles, (II) more industry contribution to the publication, and (III) a greater alignment of future research to technological applications essential for key food sectors.

P2-172 Novel Use of Omics to Evaluate the Hygienic Design and Operation of Equipment in a Live Factory Environment

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Introduction: Evaluating hygienic design and operation is a significant challenge as current techniques are not suitable for use during production operations.

Purpose: The study was to conduct proof-of-concept using 16SrRNA sequencing for assessing the occurrence and persistence of microorganisms in a live factory environment in a way that was intuitive and meaningful to manufacturing personnel.

Methods: A total of three projects were conducted over 11 months.

Project1 involved comparing two locations within the same care zone and three sampling points. Project2 was an operational evaluation on the impact of wet cleaning in a dry environment. Ten sampling points two locations, pre and post cleaning, over three cleaning cycles. Project3 compared equipment that had been upgraded to reduce condensation. Three sampling points on three sets equipment (one upgraded, two not) with 5 units each. .

Swabbing SOP's were used, including sterile and contaminated swabs as controls.

The DNA samples were prepared on Illumina® MiSeqTM with a v3 reagent kit (600 cycles). Significant effort was made to simplify the relevant taxonomy.

Results: Project1: found significant differences in the microbiome, with one location having higher abundance in spore formers and a greater number of dominant species. Project2: wet cleaned equipment showed more species of concern, less diversity and higher dominance. Project3: the upgraded equipment had an increase in Firmicutes, specifically *Bacilllus spp*. at the expense of species diversity without reducing the relative presence of Proteobacteria. The overall hygienic design evaluation requires further study.

This information was presented in an interactive dashboard, combining microbiome analysis and metadata. Resolving the analysis to a single score served as a key communication tool.

Significance: While further studies are recommended, this has demonstrated genomics provides a unique, timely and powerful insight into hygienic design and operation. This has been almost impossible to achieve in a live production environment here-to-date.

P2-173 Efficacies of Cleaning and Sanitizing Treatments for Blueberry Harvest Containers

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❖ ***Developing Scientist Entrant***

Introduction: Among blueberry-contact surfaces in fresh berry packing facilities, lugs are among the most heavily contaminated with hygiene indicator microorganisms. Berry grower/packers have used different approaches to clean/sanitize harvest containers.

Purpose: This study examined the efficacies of several treatments in cleaning and sanitizing blueberry harvest containers.

Methods: Each of four blueberry facilities in Georgia (n=2) or Oregon (n=2) was randomly visited twice in the summer of 2021. Swab samples (100 cm^2) of dirty berry lugs (DL), cleaned lugs (CL), dirty picking buckets (DH), and cleaned buckets (CH) were collected in Georgia (n =160). In Oregon, swab samples of dirty berry flats (DF), cleaned flats (CF) were collected along with the samples of DH and CH (n = 160). Population of total aerobes (TA), total yeast and mold (YM), and total coliforms (TC), as well as the presence of fecal coliform (FC), and enterococci (EC) in the swab samples were determined. Data collected were analyzed using the Split-Plot ANOVA of SAS.

Results: In Georgia, DL had significantly (P<0.05) higher TA and YM counts than CL, while no difference in TC counts was observed. Moreover, DH had significantly higher TA and YM counts than CH. FC and EC were detected from 1.3% or 3.8% of the Georgia samples. TA counts on DF and CF in Oregon were not significantly different, while DF had significantly higher YM and TC counts than CF. FC and EC were detected from 3.8% or 10.6% of the Oregon samples. Containers soaked in chlorinated water followed with manual washing had lower TA and YM counts than using wash machine and un-chlorinated water. Chlorination of the water in wash machine after each use was, nevertheless, more effective than manual washing.

Significance: Research confirmed that evaluated cleaning/sanitation approaches are largely working, but improvement could be made to further improve the hygiene status of harvest containers.

P2-174 Formation/Removal of Biofilms on/from Food-Grade Elastomeric Polymers vs. Plexiglass Used for the Fruit-Catching Plates of OTR Machine Harvesters

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Introduction: To reduce the physical impact and bruising potential of machine-harvested fresh market blueberries, manufacturers of over-the-row (OTR) machine harvesters are replacing the hard, plexiglass fruit-catching plates with soft, elastomeric polymers.

Purpose: This study assessed whether some selected soft, food-grade elastomeric polymers, that have the potential to be used in OTR harvesters, tend to attract more microbial cells and encourage more microbial buildups, making the cleaning and sanitation of the equipment a greater challenge.

Methods: Coupons (2 x 5 cm) of plexiglass, silicone, neoprene, and ethylene propylene diene monomer (EPDM) with a smooth texture and 1/8" thickness were exposed to five sets of single or mixed fecal coliform isolate(s) from various sources in Luria-Bertani no salt broth for 7 days at 25 °C for biofilm development. The surface coupons with developed biofilms were treated with sodium hypochlorite, peracetic acid, isopropyl alcohol-based quaternary ammonium compounds (AlpetD2), or a commercial dish soap. Biofilms, and their residuals after the sanitizer treatments were quantified using the crystal violet binding assay.

Results: Results showed that the fecal coliforms previously isolated from the surface of OTR harvesters developed significantly (P < 0.05) more biofilms than those isolated from fruits, hand gloves, and fresh blueberry packing equipment. EPDM coupons had significantly more biofilms than those on plexiglass coupons, while neoprene and silicone coupons had similar (P > 0.05) amounts of biofilms to those on plexiglass coupons. The dish soap was more effective than sodium hypochlorite and peracetic acid in removing biofilms from surface coupons. The efficacy of the treatment with AlpetD2 was not significantly different from the other three treatments. After sanitizer treatments, EPDM coupons had significantly more biofilm residues, while neoprene and certain silicon coupons had significantly less than plexiglass coupons.

Significance: The study suggests that neoprene did not attract more microbial cells and encourage more microbial buildups compared to plexiglass.

P2-175 The Effect of Different Sanitation Treatments on the Frequency of *Listeria monocytogenes* Detected on Non-Food Contact Surfaces in Apple Packinghouses

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❖ ***Developing Scientist Entrant***

Introduction: To control *Listeria monocytogenes* in fresh fruit, effective cleaning, and sanitizing procedures need to be applied in fruit packinghouses.

Purpose: We evaluated the effect of four cleaning and sanitizing treatments on the frequency of *L. monocytogenes* detected in three apple packinghouses.

Methods: Five sites underneath a packing line were sampled before and after the application of four sanitation treatments in three packinghouses using sterile 3M sponges. The first treatment (T1) included a sanitation practice used by each packinghouse before the start of the project; the second treatment (T2) consisted of a pre-rinse, a spray application of a dewaxer, a foam application of an alkaline chlorinated cleaner, and a spray application of 230 ppm peroxyacetic acid sanitizer (PAA). The third treatment (T3) followed the same routine as T2, with the addition of a sulfuric acid-based cleaner, and the fourth treatment (T4) followed the same routine as T2, but with the addition of three applications of Sterilex Ultra Disinfectant and Activator prior to the PAA application. Collected samples were analyzed to determine the aerobic plate count (APC) and detect *L. monocytogenes* using the FDA BAM enrichment and MPN protocols. APCs before and after each treatment were statistically compared using a t-test; statistical differences in the frequency of *L. monocytogenes* detected before and after each treatment were assessed using a chi-square test.

Results: APC values were significantly reduced by an average of 0.62, 1.57, 1.66, and 1.75 $\log_{10}$ CFU/sponge after application of treatments T1, T2, T3, and T4, respectively (p<0.05). However, only treatments T2 and T4 significantly (p<0.05) reduced the frequency of detected *L. monocytogenes* after the treatment.

Significance: Treatments T2 and T4 were most effective in reducing the frequency of detected *L. monocytogenes* and warrant further evaluation.

Poster

P2-176 The Residue Levels and Decontamination Ability of Disinfectants in the Sanitizing Process of Washed Eggs

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Introduction: Recently consumers have been conscious of food safety issues including eggs, which is a common ingredient in our daily diet. The washed eggs are popular to consumers because of their neat and clean appearance that is disinfected by food detergents.

Purpose: We simulated the washing process in different available chlorine concentrations (ACC) of two kinds of detergent (sodium hypochlorite and slightly acid electrolyzed water), washing temperature and duration time. We visited 10 traders including individual egg washing traders and affiliated washing factory of egg farm in Taiwan. Finally, we investigated the brushes and the washed eggs' eggshell to test *Salmonella spp.* and chlorine residues, in order to identify the feasibility of the washing process in industry.

Methods: The washed eggs after simulated washing and from supermarket were evaluated by the residue levels of chlorine and *Salmonella spp*. The washed eggs and brushes were sampled on site from 10 traders and evaluated by the residue levels of chlorine and *Salmonella spp*. The sanitizing process of washed eggs from industries were monitored for improvement.

Results: From the chlorine residues on eggshell, egg membrane and egg whites, all detergent residues were below the regulation limit (1 ppm) and prevent from the chemical hazard. If traders wash with proper detergents and protocols, it remains low risk of food safety. Regarding washing egg protocols, there were several non-conformance reports under the [Guidance of Egg Washing] by Taiwan Food and Drug Administration (TFDA). The domestic brands of egg washers have 34% positive detection rate of *Salmonella spp.* on brushes, much higher than the imported brands. Whether residues of *Salmonella spp.* contaminated on brushes will cause the second pollution of washed eggs needs to be further investigated.

Significance: This study highlights the impact of food detergents in egg washing industry on disinfection capability and the importance of elucidating potential brushes contamination in the egg washing environment.

P2-177 The Impact of Surface Conditions on Sanitizer Efficacy Against *Listeria monocytogenes* Biofilms on Food-Contact Surface

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❖ ***Developing Scientist Entrant***

Introduction: Microcracks on worn and aged food-contact surfaces could be persistent contamination sources for *Listeria monocytogenes*.

Purpose: This study aimed to test the impact of surface conditions on sanitizer efficacies against *L. monocytogenes*biofilms on different food-contact surfaces.

Methods: The 7-day-old *L. monocytogenes* biofilms were formed on different food-contact surface coupons, then treated with chlorine (200 ppm, 5 min), quaternary ammonium compound (QAC, 400 ppm, 5 min) and peroxyacetic acid (PAA, 160 ppm, 1 min). *L. monocytogenes* survived on surface coupons was enumerated after each treatment.

Results: The antimicrobial effect of chlorine was significantly ($P < 0.05$) decreased on worn polyvinyl chloride (PVC) and polyester (PET) surfaces, which reduced 2.3 – 3.4 $\log_{10}$ CFU/coupon *L. monocytogenes* on worn surfaces compared to 2.7 – 3.9 $\log_{10}$ CFU/coupon on new surfaces. QAC efficacy was significantly ($P < 0.05$) lowered on all worn surfaces except worn rubber, for 1.7 – 2.9 $\log_{10}$ CFU/coupon reductions on worn surfaces compared to 2.4 – 3.7 $\log_{10}$CFU/coupon on new surfaces. The anti-*Listeria* effect of PAA was significantly ($P < 0.05$) reduced on worn SS while was not negatively impacted on other worn surfaces, causing 3.8 – 4.5 $\log_{10}$ CFU/coupon reductions on worn surfaces compared to 3.7 – 4.6 $\log_{10}$ CFU/coupon reductions on new surfaces. Organic matter coating on worn surface significantly ($P < 0.05$) compromised the efficacies of QAC and PAA regardless of surface types.

Significance: Data highlighted the importance of food-contact surface maintenance to ensure effective sanitization.

P2-178 Efficacy of Sodium Acid Sulfate to Reduce Shiga-Toxigenic *Escherichia coli* and Their Biofilms *in Vitro* and on Water-Contact Surfaces

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Introduction: Shiga-toxigenic *Escherichia coli* (STEC) have caused numerous foodborne outbreaks, mostly linked to beef. STEC are known to persist in the cattle farm-environment, with water-troughs and incoming water supplies harboring them for extended periods due to formation of bacterial biofilms. Removal of these biofilms from water-contact surfaces could be a control strategy. Sodium acid sulfate (SAS), a food-acid, is poorly understood for its biofilm-disrupting capabilities and was evaluated in this study.

Purpose: Efficacy of SAS to prevent STEC biofilm formation *in vitro* and on water-contact surfaces.

Methods: SAS (0.1, 1, and 3%) was compared with water, chlorine (200 ppm) and peracetic-acid (PAA; 200 ppm) to disrupt STEC biofilms *in-vitro* and on Polyvinyl Chloride (PVC) and Chlorinated PVC (CPVC). A 3-strain cocktail of *E. coli* O157:H7 and 14-strain cocktail (2 strain/serotype) of non-O157 serotypes (O26, O45, O103, O111, O121, O145) was used. Biofilms were formed in 96-well-plates for 24h, followed by treatment for 0, 10 and 480min. PVC and CPVC coupons (2x5 cm^2) were placed in water containing one of the treatments and the pathogens (~6 logs CFU/cm^2) allowed to form biofilms for 7 days (25C). Biofilm disruption was determined *in vitro* by measuring absorbance (A_{595}) and on water-contact surfaces using scanning electron microscopy (SEM) and enumeration of pathogens. Data were analyzed using one-way ANOVA ($P<0.05$).

Results: SAS-treatments significantly ($P<0.05$) disrupted STEC biofilms *in-vitro,* with 1% (A_{595}=0.249) and 3% (A_{595}=0.260) being the most effective, compared to the control (A_{595}=1.392). On PVC and CPVC, compared to the control (7.4 logs CFU/cm^2), STEC populations were reduced to undetectable levels by all the treatments except water ($P<0.05$), over 7 days. The SEM revealed disrupted biofilms and inactivated cells on SAS-treated surfaces. The same was not observed with chlorine and PAA, where undamaged bacterial cells, embedded in biofilms, were observed.

Significance: SAS indicated its potential use as a biofilm-removing antimicrobial.

P2-179 Inactivation of Human Coronavirus 229E by Various Disinfectants in Suspension

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Introduction: The novel coronavirus disease (COVID-19) is caused by the SARS-CoV-2. Various transmission routes of SARS-CoV-2 have been proposed. Food and food-contact surfaces might be potential carriers for SARS-CoV-2 through coughing or touching by an infected person. Therefore, it is important to disinfect food and food-contact surfaces with sanitizers to avoid contamination with SARS-CoV-2.

Purpose: This study investigated the effects of various disinfectants including ethanol, sodium hypochlorite (NaOCl), chlorine dioxide (ClO_2), peroxyacetic acid (PAA) and hypochlorous acid water (HClO) on the inactivation of human coronavirus 229E (HCoV-229E), as a surrogate of SARS-CoV-2 in suspension to serve as a guide to effective concentration ranges of disinfectants for control of SARS-CoV-2 on food and food-contact surfaces.

Methods: Each concentration of disinfectants (ethanol at 50-70%, NaOCl at 50-200 ppm, ClO_2 at 2.5-20 ppm, PAA at 25-100 ppm, HClO at 20-80 ppm) was prepared. 0.1 mL of virus suspension (5-6 $\log_{10}$ $TCID_{50}$/mL) was mixed with 0.9 mL of the manufactured disinfectant for 1 or 5 min, then 0.1 mL of the mixture was neutralized with 0.9 mL of MEM with 1% FBS. Viral titers were calculated by $TCID_{50}$ assay. All experiments were replicated three times.

Poster

Results: The values of HCoV-229E titer were significantly reduced as the concentration of each disinfectant increased ($P < 0.05$). As a result of treatment with ethanol at 50-70%, NaOCl at 100 ppm, ClO_2 at 15-20 ppm, PAA at 100 ppm, HClO at 60-80 ppm, the virus was not detected (> 2.5 $\log_{10}$ reduction, detection limit = 0.5 $\log_{10}$ $TCID_{50}$/mL).

Significance: This study suggests that ethanol (50-70%), NaOCl (100 ppm), ClO_2 (15-20 ppm), PAA (100 ppm) and HClO (60-80 ppm) can be effective for inactivating HCoV-229E. Also, ClO_2 can be effective even at low concentrations compared to other disinfectants.

P2-180 Combination of Essential Oils with Bacteriophage Against *Listeria monocytogenes* Biofilms on Abiotic Surfaces

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Introduction: Essential oils (Eos) and bacteriophage are ecofriendly antimicrobials to reduce and inhibit foodborne pathogens biofilms and they are promising strategy to secure food safety by using it as a single or hurdle technology.

Purpose: The goal of this study was to investigate single or combination treatment of Eos and bacteriophage effect to remove *L. monocytogenes* biofilms on abiotic surfaces (polyethylene [PE], polypropylene [PP], stainless steel [SS]).

Methods: Minimal inhibitory concentration (MIC) of thymol and multiplicity-of-infection (MOI) 100 of *L. monocytogenes* specific bacteriophage cocktail, which were isolated and characterized before, were used in this study to evaluate biofilm eradication ability of its single or combination treatment. *L. monocytogenes* biofilms were formed on abiotic surfaces for 24 h and each treatment (single or combined treatment) lasted for 6 h. Field emission scanning electron microscopy (FE-SEM) and confocal laser scanning microscope (CLSM) were conducted to deep investigate of biofilm structure before and after treatment.

Results: Single treatment of MIC of thymol reduce 1.32, 0.86, 1.04 log CFU/cm^2 and single treatment of MOI 100 bacteriophage cocktail reduce 0.54, 0.62, and 0.59 log CFU/cm^2 in PE, PP, and SS, respectively. Combination of thymol and bacteriophage reduce 2.15, 1.77, 1.83 log CFU/cm^2 and synergistic effect of combination was 0.29, 0.29, 0.2 log CFU/cm^2 in PE, PP, and SS, respectively. FE-SEM and CLSM revealed that combination treatment was more effective in eradicate biofilms from abiotic surfaces and broke biofilm structure.

Significance: These results demonstrate that combination treatment of Eos and bacteriophage show synergistic effect and has the potential to be used as eco-friend antimicrobials.

P2-181 Antibiofilm Effects of Quercetin Against *Salmonella enterica* Biofilm Formation and Molecular Mechanism on Food and Food-Contact Surfaces

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Introduction: Salmonellosis is a prevalent food poisoning disease caused by *Salmonella* spp. that affects millions of people throughout the world. Contamination of chicken meat and processing equipment with *Salmonella* is a major issue in the food industry. Therefore, it is necessary to study not only food but also food contact surfaces [plastic (PLA) and rubber gloves (RG)] that can cause cross-contamination during processing of food.

Purpose: The objectives were to determine the antimicrobial efficacy of quercetin against QS-regulated phenotypes of *S. enterica*, including biofilm formation and flagellar-mediated motility, in association with its effects on virulence and QS gene expression at sub-minimum inhibitory concentrations (sub-MIC).

Methods: The Minimum Inhibitory Concentration (MIC), motility assays, biofilm inhibition on PLA, RG, and chicken skin surfaces, field emission scanning electron microscopy (FE-SEM), confocal laser scanning microscope (CLSM), and gene expression patterns by real-time PCR were performed to identify the antimicrobial mechanism of quercetin.

Results: The results established that different concentrations of quercetin sub-MIC (0-125 µg/mL) potentially reduced biofilm formation, motility assays, and downregulated the different genes. The inhibitory effect was 1.50~2.61 log CFU/cm^2 (125; 1/2, 62.5; 1/4, and 31.25 µg/mL; 1/8MIC), respectively. Furthermore, the initial biofilm formation rate of both bacteria was greater on the chicken skin surface than on the food contact surfaces. The inhibitory impact was visually observed using FE-SEM and CLSM. The swimming and swarming motility was also found to be inhibited from 1/8 MIC. As determined by qRT-PCR, quercetin downregulated the expression levels of virulence (*avrA*, and *hilA*), stress response (*rpoS*), and quorum-sensing (*luxS*) genes.

Significance: This study results suggested that plant-derived quercetin can be used to control *S. enterica* biofilms as well as other bacteria in the food industries to enhance food safety.

P2-182 Reduction of Feline Calicivirus and Tulane Virus by Aqueous Ozone in Clean and Organic Load Containing Water

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Introduction: Human noroviruses (HuNoV) are globally recognized as epidemiologically significant viruses causing acute gastroenteritis. Due to the ongoing challenges with HuNoV cultivable assays, feline calicivirus (FCV-F9) and Tulane virus (TV) are used as surrogates to evaluate inactivation methods. Aqueous and gaseous ozone are alternate control strategies to corrosive chlorine.

Purpose: The objective of this study was to determine the ability of 1 ppm aqueous ozone to inactivate FCV-F9 and TV over time.

Methods: Twenty mL of FCV-F9 (~8 log PFU/mL) or TV (~6 log PFU/mL) was added into 180 mL sterile water and treated for 0 to 5 min with ~1 ppm ozone (pH 7 to 6). Additionally, 25 mL virus was added to 200 mL sterile water containing 25 mL newborn calf serum (as organic load) for 0 to 38 min treatments. Each treatment was replicated thrice and virus infectivity was determined by plaque assays. Data were statistically analyzed using ANOVA and Tukey's adjustment (SAS. 9.4).

Results: FCV-F9 was reduced by 0.35 to 6.07 log PFU/mL after 1 to 5 min in clean water showing significant differences in reduction over time ($p<0.05$), and by 0.44 to 4.63 log PFU/mL after 4 to 32 min and was non-detectable after 36 min in organic matter-containing water using 1 ppm aqueous ozone. TV resulted in 0.34 to 4.28 log PFU/mL reduction after 1 to 4 min and was non-detectable after 4.5 min in clean water, while in organic matter decreased by 0.4 to 4.13 log PFU/mL after 2.5 to 20 min and was non-detectable after 22.5 min treatment.

Significance: Although, >6 log PFU/mL reduction of FCV-F9 occurred after 5 min treatment and 4 min for TV, longer treatment is required for ≥4 log reduction in organic matter. Thus, prior water filtration or removal of organic load is suggested before ozonation for increased inactivation efficacy and decrease treatment time.

P2-183 Evaluation of Sanitizer Efficacy Against Human Norovirus on Surfaces: The Role of Wiping

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Introduction: Fomites contribute to pathogen transmission, but many surface sanitizers perform poorly against human norovirus (hNoV). Wiping is a critical final step of surface sanitizing, but its contribution to efficacy is poorly understood.

Purpose: Characterize the combined contribution of surface sanitizer application and wiping on sanitizer efficacy against hNoV and the Tulane virus (TuV) cultivable surrogate.

Methods: Four commercial products [A (29.4% ethanol); B (0.06% dodecylbenzenesulfonic acid and 0.15% lactic acid); C (200ppm sodium hypochlorite); D (400ppm quaternary ammonium compound)]; and water (control) were evaluated against a semi-purified TuV cell culture lysate and two hNoV strains (GI.6 and GII.4 Sydney, 20% clarified fecal stock). Virus inactivation and removal were assessed on Formica™ coupons using a modified ASTM E1053-11 protocol in conjunction with the Gardner-scrub abrasion tester (Gardco, Popano Beach, FL) for wiping using paper towels. Virus was eluted from coupons and spent paper towels and quantified by plaque assay (TuV) or RNase-RT-qPCR (hNoV). Sanitizer efficacy was expressed as $\log_{10}$ reduction (LR) of virus from coupons after treatment. All experiments were repeated in triplicate.

Results: For TuV, the efficacy of combined sanitizing and wiping were [in LR; limit of detection (LOD)=6.5]: Product A, 6.5±0.1; B, 2.6±0.3; C, 3.8±0.8; D, 1.6±0.1; water, 2.4±0.1. For hNoV GI.6, the efficacy was as follows [in LR, LOD=3.9]: Product A, 3.9±0.1; B, 2.0±0.1; C, 2.5±0.4; D, 2.4±0.2; and water, 1.9±0.4. No virus could be recovered from the spent paper towels used with Product A, while most of the input virus was recovered from those used with Products B, D, and water. Similar results were obtained for hNoV GII.4 Sydney.

Significance: There was wide variability in product performance with only one (A) achieving elimination of virus. Residual virus on surfaces or spent towels presents a cross-contamination risk. These data highlight the importance of considering the impacts of sanitizer efficacy and wiping in evaluating products for anti-noroviral activity.

P2-184 Inactivation of Tulane Virus in Buffer and on Formica Coupons by 254nm and 279nm UV-C Systems

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Introduction: Increasing outbreaks of human noroviruses continue to occur annually. Novel processing approaches to inactivate these resilient viruses are researched to enhance food safety. Tulane virus (TV) is a cultivable human norovirus surrogate useful to determine inactivation by processing approaches.

Purpose: The objective of this research was to determine the UV-C dose-response curve of TV in phosphate buffered saline (PBS) and on Formica coupons using the traditional 254 nm UV-system and a novel environmentally friendly 279 nm UV-LED system.

Methods: Five-mL TV (~5 log PFU/mL) in PBS within 10 mL beakers was continuously stirred during 254 nm UV-C treatments of 0 to 34 mJ/cm^2 or 279 nm UV-C-LED treatments of 0 to 4.14 mJ/cm^2. Additionally, TV (100 µL) was surface-spread on Formica coupons (3×3 cm^2), air-dried, and treated with UV-C ranging from 0 to 28 mJ/cm^2 (N = 3). Optical properties of virus-suspensions were determined using a UV-Vis spectrophotometer. Recovered viruses were enumerated by plaque assays using confluent host cells in 6-well plates in duplicate from three replications. Statistically analysis of data was carried out using mixed model analysis of variance (adjust=tukey, SAS).

Results: TV titers in PBS decreased by 1.05±0.11 log PFU/mL using 279 nm UV-LED at the maximum dose (4.14 mJ/cm^2) with D-10 values of 3.91±1.03 mJ/cm^2 and decreased by 1.87±0.26 log PFU/mL using 254 nm UV-C at the maximum dose of 33.89 mJ/cm^2, with D-10 values of 18.76±3.16 mJ/cm^2. On Formica coupons, D-10 values were 16.34±3.6 mJ/cm^2 with 279 nm UV-C LED and D-10 values of 6.83 mJ/cm^2 with 254 nM UV-C. Thus, TV showed reduction in clear fluid suspensions with stirring using both the UV-C LED and the traditional 254 nm UV-C systems.

Significance: Results from this study are beneficial to calculate and deliver the desired UV-C doses for target TV log reductions on surfaces and in clear fluid suspensions.

P2-185 Phi6 Inactivation on Surfaces with Hypochlorous Acid Based on Surface Type, Inoculum Matrix, and Contact Time

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Introduction: Phi6 (φ6) is an enveloped bacteriophage that is used as a surrogate for SARS-CoV-2. Identification of safe, effective sanitizers/disinfectants against enveloped viruses on the various surfaces found in consumer-facing environments is needed.

Purpose: To determine φ6 inactivation with hypochlorous acid (HOCl) on common surfaces inoculated within two different matrices.

Methods: Stainless steel and vinyl (5x5cm) were inoculated with 6.87±0.04 log φ6 PFU/surface in artificial saliva or tripartite and dried for 45min. HOCl (pH 6.0, 500 or 1000ppm) was applied to inoculated surfaces with a spray bottle (3 sprays, 30 cm distance) for 0, 5, 30, and 60s. Thereafter, φ6 was eluted with 2mL D/E buffer, diluted, and plated on LC agar via the double agar overlay assay. Experiments were performed in technical duplicates with two experimental trials. Three-way ANOVA with Tukey's test was performed to determine differences in log PFU reduction based on HOCl concentration, surface type, and inoculum matrix at 60s exposure (alpha=0.05).

Results: Among all stainless steel and vinyl surfaces exposed to 1000ppm HOCl (regardless of matrix), log PFU reduction averaged 0.58±0.16, 1.18±0.32, and 2.21±1.19 at 5, 30, and 60s, respectively. At 60s exposure, log PFU reduction differed significantly based on surface type (P < 0.05) but not for inoculum matrix or HOCl concentration. For φ6 in artificial saliva on vinyl and stainless steel, the mean log PFU reduction was 1.16±0.34 and 3.33±2.04, respectively, when exposed to 1000ppm HOCl for 60s. On vinyl and stainless steel, the mean log PFU reduction was 1.62±0.53 and 2.75±2.46, respectively, for φ6 in tripartite exposed to 1000ppm HOCl for 60s.

Significance: Determining the impact of surface type, inoculum matrix, and HOCl concentration on φ6 inactivation will help guide surface disinfection measures. Additionally, realistic contact times should continue to be investigated to determine suitable virus reduction in public settings.

P2-186 Thermal Inactivation as a Function of Microbial Target and Food Matrix Composition during Superheated Steam Treatments

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❖ ***Developing Scientist Entrant***

Introduction: Sanitation in dry food processing environments is challenging due to the exclusion of water. We evaluated microbial inactivation by superheated steam (SHS). The high sensible heat of dry steam prevents condensation on surfaces.

Purpose: The goal of this study was to 1) assess thermal inactivation of vegetative and spore forming bacteria and 2) determine the effect of food matrix composition on inactivation.

Methods: Capillary tubes with vegetative cells (*Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, or *E. faecium*), or *B. cereus* spores (100 µL) were SHS treated at 135±1°C for 1 or 2 s. Additionally, peanut butter formulations (peanut powder, oil, and water) and milk powder (whole and nonfat) inoculated with *B. cereus* spores on aluminum foil coupons (2 x 3 x 0.5 cm^3) were SHS treated at 161±1°C for 15-270 s. Log-linear and Weibull models were used to fit the experimental data, and Fisher's exact and ANOVA were used to determine statistical significance.

Results: A 10.0 Log_{10} reduction for vegetative cells and 0.2 Log_{10} reduction for *B. cereus* spores was achieved after a 1 s treatment at 135±1°C. The $D_{161°C}$ values for *B. cereus* spores ranged from 6.53 s (low fat, high moisture) to 198.45 s (high fat, low moisture) for various peanut butter formulations. Whole milk powder had higher $D_{161°C}$ (58.50 s) than nonfat milk powder (46.16 s). Statistical analysis revealed that spore inactivation was significantly affected by product formulation ($p < 0.05$). Compared to the log-linear model (R^2 range 0.83-0.98), the Weibull model had better model fitness (R^2 range 0.93-0.99).

Significance: The findings from this study can inform the development of superheated steam centered sanitation conditions based on target organism and food residue.

P2-187 Inactivation of *Listeria monocytogenes* Biofilms on Stainless Steel Surfaces Using Blue Light

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Introduction: Surface disinfection is critical for controlling *Listeria monocytogenes* (*Lm*) in food processing environments. Blue light has been reported to be an emerging antimicrobial intervention technology, but research on *Lm* biofilms on inert surfaces is limited. A better understanding of antimicrobial blue light (aBL) is necessary to assess its potential for *Lm* control.

Purpose: This study was conducted to evaluate the viability reduction of *Lm* biofilms on stainless steel (SS) coupons by aBL.

Methods: Mixtures of five *Lm* strains were used to grow biofilms on SS coupons for 48 h. Biofilms with approx. 7 log CFU/cm^2 were exposed to different aBL emission doses at three wavelengths (405, 420, and 460 nm) alone or with a photosensitizer (gallic acid; GA). Coupons were processed by sonication, and counts were measured by standard plating on tryptic soy agar, followed by incubation at 37°C for 24 h. Independent experiments were conducted in triplicate. Statistical analyses were performed using ANOVA. Confocal laser scanning microscopy (CLSM) with Syto® 9 and propidium iodide staining was used to observe the biofilm structures.

Results: At 405 nm, the viable counts after the highest dose (2,672 J/cm^2) were reduced by 3.5 log CFU/cm^2 ($P<0.05$), and application of GA did not increase inactivation. The highest dose (967 J/cm^2) at 420 nm reduced viable counts by 1.9 log CFU/cm^2 ($P<0.05$). At 460 nm, after 800 J/cm^2, counts were reduced from 6.9 to 5.3 log CFU/cm^2 ($P<0.05$), and GA treatment had no effect. Biofilm CLSM micrographs displayed shifts in biofilm structure from live to dead cells after aBL treatment confirming the viable count measurements.

Significance: This study provides evidence that aBL was capable of inactivating *Lm* biofilms on SS surfaces and suggested that this can be a new tool for the improvement of microbial safety in food processing plants.

P2-188 Blue Light Efficacy Against *Listeria monocytogenes* Dried on Inert Surfaces

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Introduction: *Listeria monocytogenes* (*Lm*) survives in food processing and packing facilities. Because antimicrobial blue light in the 400–470 nm wavelength range has been applied to treat surfaces against other bacteria, it has the potential to be a valuable tool in minimizing *Lm* persistence in the food processing environment.

Purpose: This study was conducted to investigate the surface inactivation of Lm cells by antimicrobial blue light (aBL) influenced by different materials used in food processing

Methods: Five materials were investigated: stainless steel (SS), high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), and rubber (RUB) coupons. Five-strain *Lm* mixtures (6 log CFU/cm^2) were placed on individual coupons and dried on surfaces. Three wavelengths (405, 420, 460 nm) were tested against dried cells. Viability was determined after different emission doses. Cells were removed by coupon sonication, and counts were measured by standard plating on complex media, and incubation at 37°C for 24 h. Statistical analyses were performed using ANOVA.

Results: At 405 nm, the effectiveness of aBL varied depending on the surface material, causing the least reductions of *Lm* on PVC, 2.5 log CFU/cm^2 after 2,672 J/cm^2 ($P<0.05$). On PS, cells were inactivated by 4.5 log CFU/cm^2 after 2,672 J/cm^2. Exposure to 420 and 460 nm also caused significant reductions. Treatments at 420 nm (1,440 J/cm^2) resulted in 3.2 log CFU/cm^2 from PVC and 3.8 CFU/cm^2 from RUB ($P<0.05$). At 460 nm after 800 J/cm^2 on SS cell counts were reduced from 6 to 4.8 log CFU/cm^2 ($P<0.05$). Exposure at 460 nm to 1,200 J/cm^2 reduced viability by 2.2 log CFU/cm^2 from PVC and HDPE.

Significance: aBL could inactivate *Lm* on different materials, but its effectiveness was influenced by wavelength and type of material. aBL technology could be environmentally friendly, safe, and adaptable to different production systems.

P2-189 Quantifying the Electrostatic Adhesion Force of Powders on Food Contact Surfaces for Dry Cleaning and Sanitization

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✦ ***Undergraduate Student Award Entrant***

Introduction: The Food Safety and Modernization Act has no guidelines for the sanitization of processing equipment in a low-moisture environment as compared with wet cleaning. Therefore, it is necessary to understand the mechanisms of adhesion of food products to a food contact surface to develop better sanitation methods for low moisture environments.

Purpose: The study aims to analyze the relationship between electrostatic charge and particle adhesion to food contact surfaces.

Methods: Silicon quartz powders with mesh sizes of 100, 400, 1250, 3000, and 5000 were used. Initial triboelectric charge was measured using a handheld digital electrostatic field meter. Powders were agitated for 10 min to increase static charge in a rotating stainless-steel drum. Thereafter, 3 g of each powder was evenly attached across stainless steel coupons (10 mm x 10 mm) which were centrifuged at 119.5 × g for 1 min in a special holding apparatus. The amount of powder dislodged from the coupons surface was weighed. Three trials were conducted for each powder mesh size in both control and tumbled groups. The difference between the average percent loss for control and tumbled groups was calculated for each mesh size. The electrostatic charge before and after tumbling was compared with a 95% confidence paired t-test.

Results: The powders of mesh sizes 400, 1250, 3000, and 5000 were retained 2.21, 5.38, 23.81, and 5.62% more respectively after being tumbled and electrostatic charges (0.07, 0.60, 0.50, and 0.83 kV) were increased. However, the largest mesh size (100) powder was retained 18.53% less after being tumbled (0.81 kV). Electrostatic charge was significantly greater after tumbling ($P < 0.01$) for all powders.

Significance: By understanding the fundamental adhesion mechanics of powders, effective dry-cleaning methods can be developed to prevent pathogen cross-contamination.

P3-01 A Study on Growth and Toxin Production of *Clostridium botulinum* in Cold Brew Coffee during Long Term Storage

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Introduction: Unlike traditional hot brew coffee, cold brew coffee is prepared by brewing the coffee grounds at ≤25°C for approximately 8 to 36 hours. Since brewing temperature greatly affects the aqueous solubility of compounds and the chemical composition, the antimicrobial activity of cold brew coffee extracts likely differs from that of traditional hot brew coffee.

Purpose: The purpose of this study is to determine if cold brew coffee inhibits the growth and toxin production of *Clostridium botulinum* spores.

Methods: The chemical properties (pH, water activity, Brix and total dissolved solids) were measured of 5 cold brew coffee products purchased at local grocery stores. The coffee products were also diluted with sterile water to achieve approximately 0.5% coffee solids concentration, for a total of 10 coffee variations. A 10-strain cocktail of proteolytic *C. botulinum* (5 Type A and 5 Type B strains) heat shocked (80°C for 10 minutes) spores were inoculated in cold brew coffee at a concentration of 10^3 spores/ml, stored long term at 27°C under anaerobic conditions and periodically tested for toxin production. Two

additional coffee products were chosen for a challenge study with 1, 5, 10 and 20% sucrose, half & half or coffee creamer. Presence of toxin was measured using Endopep-MS assay.

Results: The 10 coffee samples ranged in pH (4.98-6.58), water activity (0.999-1.000), Brix (1.3-2.3) and total dissolved solids (0.86-1.89%). After one month incubation, all black coffee samples, and coffee with added sucrose (1, 5, 10 and 20%) were negative for toxin production. However, coffee samples with creamer (5, 10, and 20%) or half & half (20%) were positive for the presence of Type A and B toxin.

Significance: This study will determine whether cold brew coffee is inhibitory to the germination and outgrowth of proteolytic *C. botulinum* spores and will provide the foundational data for future studies identifying the chemical compounds inhibiting germination of *C. botulinum* spores.

P3-02 Culture-Dependent and Independent Approaches for Microbial Characterization of Home-Brewed Ginger Beer

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Introduction: Ginger beer is one of the carbonated beverages flavoring ginger root which is fermented by a starter culture naturally present in ginger. The presence of live microorganisms in ginger beer considered to a probiotic beverage.

Purpose: The aim of this study is to investigate microbial changes in home-brewed ginger beer and the effects of endogenous microbiota on ginger beer production.

Methods: Microbial characterization was analyzed during ginger beer production processes: raw ginger, ginger bug, and ginger beers fermented for 1, 2, and 3 weeks. Aerobic bacteria, coliform, and yeast and mold counts were evaluated using 3M™ Petrifilm™. Alcohol content of ginger beer was measured using an Anton-Parr DMA™ 4500 M (Anton Paar GmbH, Graz, Austria) and Alcolyzer ME (Anton Paar GmbH). Microbiome analysis based on 16S rRNA gene was performed and analyzed using a QIIME2 pipeline. Paired t-test was used to evaluate the significant difference among samples.

Results: Both APC and CC in ginger bug showed the highest than raw ginger and fermented ginger beers. No more than 1% v/v alcohol content was measured during ginger beer fermentation process. The ginger bug used for making ginger beer significantly influenced on the diverse taxa in the fermented ginger beers. Microbial diversity in each sample (raw ginger, ginger bug, and ginger beers) was clustered.

Significance: This study will contribute to understand the microbial changes from raw ginger to fermented ginger beers and their potential roles associated with safety and quality.

P3-03 Acid Adaptation Increases Resistance of *Escherichia coli* O157:H7 in Bok Choy (*Brassica rapa* subsp. *chinensis*) Juice to High Pressure Processing

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❖ ***Developing Scientist Entrant***

Introduction: The adaptive response of *Escherichia coli* O157:H7 to acid stress, a major stressor in juice processing environments, has been shown to confer cross resistance against pasteurization techniques such as heat, X-ray and ultrasound. However, its effect on resistance to High Pressure Processing (HPP) has yet been studied.

Purpose: This study aimed to evaluate the effect of acid adaptation on the resistance of *E. coli* O157:H7 in bok choy juice to HPP. Bok choy is a leafy green commonly consumed in Asia.

Methods: Acid adaptation of *E. coli* O157:H7 was performed in tryptone soya broth without dextrose, adjusted to pH 5.0, at 37˚C for 24 h. Unadapted cells were prepared in similar conditions, without pH adjustment (pH 7.3). Inoculated bok choy juice (~10-log CFU/mL) was subjected to HPP at 400 MPa, 5˚C. *D*-values were obtained from survival curves. Changes in viable counts were further monitored during post-HPP storage at 4˚C. Sub-lethally injured populations were measured via plating on selective media, as well as differential fluorescent staining and flow cytometry. A Student's *t*-test was employed to compare acid adapted and unadapted cells (n=3).

Results: The *D*-value at 400 MPa of acid adapted cells (1.2 ± 0.1 min) was significantly higher ($p < 0.05$) than that of unadapted cells (0.8 ± 0.1 min). Furthermore, significantly lower reductions ($p < 0.05$) in total viable counts were observed after 72 h of post-HPP storage for acid adapted cells (3.4 ± 0.2 log) as compared to control cells (5.0 ± 0.1 log). Lastly, acid adapted cells experienced significantly lower levels ($p < 0.05$) of membrane, DNA and enzyme damage than unadapted cells, suggesting that acid adaptation may protect *E. coli* O157:H7 against HPP-induced cellular damage.

Significance: The effect of acid adaptation should be considered in the selection of HPP parameters for *E. coli* O157:H7 inactivation.

P3-04 Survival of Probiotic *Lactobacillus* spp. during Kombucha Fermentation

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Introduction: Kombucha is noted for its taste, sensorial qualities, and purported health benefits. The beverage is perceived to contain probiotics, but most culture systems do not contain significant levels of these organisms.

Purpose: This study will determine if specific probiotics inoculated into unfermented sweetened tea can survive kombucha fermentation in appreciable quantities.

Methods: Growth curves for six probiotic *Lactobacillus* spp. in MRS broth or sweetened black tea incubated at species-recommended temperature (30ºC or 37ºC) or 25ºC were created. Inoculated (~9 logCFU/mL) sweet tea was also fermented with kombucha SCOBYs (n=3), until a pH of 3.0 was reached. Probiotic populations were documented throughout fermentation and storage and were compared to previously constructed growth curves. Model comparison was performed using multiway ANOVA in R studio to decipher significant differences ($p<0.05$) between growth media/temperature and between probiotics.

Results: Temperature and medium both had significant effects on the probiotic growth rates, and results showed the probiotics survived better in sweetened tea at 25ºC than in sweetened tea at recommended incubation temperatures. Differences in pH indicated the probiotics were unable to acidify the tea pre-fermentation. Fermentation lasted on average 8.3 days, and probiotic populations declined significantly with acidification around day 6. Survival was highly heterogeneous across *Lactobacillus* species, with final populations ranging from >1.0 to 3.0 logCFU/mL.

Significance: Probiotic *Lactobacillus* spp. are not well suited for a probiotic kombucha beverage. Of the tested probiotics, *L. brevis* ATCC 14869 and *L. fermentum* ATCC 9338 are the best candidates according to the growth curves. Other probiotics, such as *Bacillus coagulans*, should be further explored before and during fermentation to produce a probiotic kombucha beverage.

P3-05 Variation in Biological and Chemical Characteristics of Cabbage-Based Ferment Produced in Home and Laboratory Conditions

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Introduction: Traditionally prepared sauerkraut is typically considered safe, however introductions of additional ingredients into cabbage-based fermentations can impact the quality and safety of the final product. As home fermentations continue to increase in popularity the impact of individual and environmental variations on biological and chemical characteristics of cabbage-based fermentations remains understudied.

Purpose: To investigate chemical and biological dynamics between home cabbage fermentations compared to controlled laboratory conditions.

Methods: Purple cabbage (907 g) and carrots (454 g) with 3% salt (w/w) were fermented at five households in Davis, CA. The same ratio of ingredients with 2% and 3% salt concentrations was used to set up fermentations controlled at 22°C in the laboratory. Cabbage was fermented 21 days. Brine and vegetable samples were collected at nine timepoints during the study period. Brine pH, salinity, and temperature were measured for all samples. Lactic acid bacteria (LAB) and yeast counts were conducted for laboratory fermentations at all timepoints and at initial and final time points for home fermentations.

Results: Statistically significant differences ($p<0.001$) were observed in day 21 LAB counts between home (8.17 ± 0.49 log CFU/mL) and lab fermentations (6.61 ± 0.10 log CFU/mL and 5.80 ± 0.18 log CFU/mL for 2% and 3% NaCl, respectively). The difference in measured salinity at the end of sampling was statistically significant among fermentations ($p<0.001$). pH measurements were not significantly different among treatments and no yeast was observed at the end of fermentation.

Significance: This study highlights variation in microbial composition between laboratory validated cabbage-based fermentations and small-batch home ferments. Although variation was observed between microbial counts and salinity measurements, by day 3, pH values were below 4.6 and a pH below 4.0 was maintained for 21 days for all fermentations indicating the safety of these cabbage-based ferments.

P3-06 Inactivation of *Escherichia coli* O157:H7 in Claussen Pickle Products Stored at 4°C

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Introduction: Cucumber pickle products are a ready-to-eat food and rely on brine acidification to ensure inactivation of pathogenic *Escherichia coli*, which is a causative agent in many foodborne outbreaks related to fresh vegetables.

Purpose: This study examined the efficacy of titratable acidity (TA) and pH of three different brines on the inactivation of *E. coli* O157:H7 in two pickle products (whole or cut) stored at 4°C.

Methods: Whole and cut cucumbers dipped in brines in 80 oz jars were inoculated with a two strain cocktail of *E. coli* O157:H7 targeting 10^6 CFU/mL and stored at 4°C for 15 days, with uninoculated controls to assess for background microflora. Brines were modified by the addition of acetic acid or NaOH solution, with the following combinations : 1) pH 3.44 and % TA 0.80, 2) pH 3.44 and % TA 0.75, 3) pH 3.77 and % TA 0.83. The surviving population of *E. coli* O157:H7 was plated in triplicate and determined during storage at 4°C by plating serial dilutions onto Trypticase Soy agar with Violet Red Bile agar overlay and incubated at 35°C for 48 h.

Results: A 5-log inactivation of *E. coli* O157:H7 was observed after 3 days in whole cucumbers and 15 days in cut cucumbers. A rapid and greater reduction of *E. coli* O157:H7 was observed in brines with higher TA levels for both cucumber types. The TA concentration in brines decreased during storage by 45%, while pH increased. For example, TA dropped from 0.75 to 0.41 % and pH increased from 3.45 to 3.86 for whole cucumbers in brine 1.

Significance: This study validated that within these pickle formulations, brine pH and titratable acidity served as critical control points and achieved 5-log inactivation of *E. coli* O157:H7.

P3-07 Detection of Spoilage Organisms in Tea Beverages Using Hygiena™ Microsnap™ Total Enrichment Device and Enhanced Nutrient Broth

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Introduction: MicroSnap™ Total and Enhanced Nutrient Broth is a rapid bioluminogenic test for detection and enumeration of the total population of viable aerobic bacteria in a sample, providing results in 7 hours.

Purpose: To evaluate the matrix effects of flavored tea beverages and to detect aerobic spoilage bacteria using the MicroSnap™ Total Enrichment Device and Enhanced Nutrient Broth.

Methods: 20% dilutions of cranberry, grapefruit, sweet tea, blueberry, peach and strawberry tea were made in BPW. Serial dilutions of mixed cultures of bacteria were spiked into the diluted teas. 1 ml of each of these dilutions were pipetted into 1.2 mL enrichment devices, 9 ml enhanced nutrient broth vials and plated on TSA agar plates respectively. Enriched samples were tested using the MicroSnap™ detection device at the end of 8 hrs and colony counts were performed from the TSA plates after 24 hrs.

Results: All the beverages had acidic pH ranges from 2.52- 4.23. Diluting the teas in BPW helped to neutralize the low pH and the background signal caused by nonmicrobial ATP present in the beverages. For blueberry, sweet tea, peach and cranberry flavored teas, correlation between RLUs and CFUs was ≥0.95 when tested with 1.2 ml MicroSnap™ Total enrichment device whereas correlation values for grapefruit and strawberry flavored beverages were 0.88 and 0.79, respectively. When testing 20% dilutions of flavored teas using 9 ml Nutrient broth, a correlation between RLU and CFU of ≥0.95 was seen for all the flavored beverages.

Significance: The MicroSnap™ system works well with the flavored tea samples when samples are first diluted in BPW and can be used as a simple, rapid, accurate method to measure spoilage organisms from flavored tea beverages. Results can be obtained within 8 hrs as compared to traditional methods which takes 24-48 hrs.

P3-08 Food Allergen and Gluten Associated Recalls of FDA-Regulated Foods from October 2012 to September 2019

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Introduction: Food allergens remain a major food safety hazard responsible for a high number of recalls every year.

Purpose: To determine the trend of food allergen and gluten (FA/G) recalls over a 7-year period and study associated root causes.

Methods: Recalls related to FA/G during fiscal year 2013-2019 were queried in the FDA's recall database. Wheat related recalls were categorized as gluten recall if they involved gluten-free products. Recall information was analyzed to study recall Class, number and type of allergens involved, associated food categories based on FDA Product Codes, and root cause.

Results: 1,705 recalls related to FA/G were identified with 1,471 unique recalls (including non-major food allergen and gluten recalls) analyzed after removing 234 downstream or related events. Among 1,471 recalls, 49.3% were Class I, 47.3% were Class II and 3.4% were Class III. Over the study period, the percentage of Class I recalls generally decreased while that of Class II recalls increased. FA/G recalls involved one (N=1,171; 79.6%), two (N=193; 13.1%) or multiple (N=107; 7.3%) allergens/gluten. Milk was the leading allergen identified in 531 recalls (36.1%), followed by soy (N=319; 21.7%) and tree nuts (N=305; 20.7%). Gluten caused 34 recalls (2.3%). For recalls involving one allergen/gluten, the majority (>60%) of recalls associated with egg, Crustacean shellfish, peanut and milk resulted in Class I recalls, whereas those associated with soy, wheat, gluten, fish and non-major food allergens resulted in Class II recalls. Among FA/G recalls that involved one product category (N=1,427), Bakery Products/Dough/Bakery Mixes/Icings (N=370; 25.9%) ranked first, followed by Chocolate/Cocoa Products (N=123; 8.6%) and Multiple Food Dinners/Gravies/Sauces/Specialties (N=117; 8.2%). Labeling associated errors were the leading cause of FA/G recalls.

Significance: Recall trend analysis and root cause evaluation can identify major areas of concern and potential corrective actions that can be implemented by the industry to reduce future FA/G recalls.

P3-09 Development of a Targeted Mass Spectrometry Method for the Detection of Egg in Multiple Processed Food Matrices

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❖ ***Developing Scientist Entrant***

Introduction: Egg is one of the most commonly allergenic foods worldwide, and immunoassay methods are routinely used to detect egg in foods. Processed foods and complex matrices often hinder such detection, but mass spectrometry (MS) methods have advantages over traditional immunoassays due to their specificity, sensitivity, and robust protein extraction.

Purpose: The objective of this study was to develop an egg-specific, targeted MS method for four common, processed food matrices.

Methods: Model food matrices (cookie, pie crust, pasta, and ice cream) were incurred with 0 or 10,000 ppm whole egg powder (WEP). Proteins from unprocessed and processed food matrices and from WEP alone were extracted, trypsin-digested, and desalted prior to MS analysis. The peptides obtained from the 15 sample types were evaluated using an untargeted proteomics approach, with label-free quantification. Candidate target peptides were selected for each matrix using a series of criteria, including specificity, sensitivity, and robustness (performance independent of matrix and processing).

Results: Peptide performance was evaluated across three sample categories: (1) unprocessed doughs for cookies, pie crust, and pasta, (2) processed finished foods for all matrices, and (3) WEP alone. Peptides with the highest observed intensities and with relative recoveries (vs. WEP) greater than 10 % were selected as candidate target peptides. Ice-cream showed the greatest number of potential target peptides (208). The wheat-based matrices delivered many fewer candidate peptides, with 49 identified in cookie, 43 in pie crust, and 56 in pasta. Across all four matrix types, 25 candidate target peptides were found in common.

Significance: These results indicate that while the performance of many egg peptides is matrix- and processing-dependent, candidate targets are available to develop an MS method capable of detecting and quantifying egg in processed food products.

P3-10 Identification of Wheat-Specific Peptides for Use in the Development of Immunoassay and Proteomic-Based Detection Methods

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❖ ***Developing Scientist Entrant***

Introduction: The food industry relies on food allergen detection methods, such as ELISA, to ensure foods are free from the unintended presence of allergen residues. Current commercial ELISA kits, however, lack the ability to differentiate the gluten-containing grains.

Purpose: A combined experimental and bioinformatics approach was utilized to identify wheat-specific peptides for use in the development of a wheat-specific immunoassay and a proteomic-based detection method.

Methods: Wheat, durum wheat, rye, barley, oat, and commercially available whole wheat and all-purpose flours were extracted using two water-soluble buffers. Phosphate buffered saline (PBS) and PBS with 0.1 M sodium sulfite (PBS-S) were used to preferentially extract albumin and globulin proteins and exclude the water-insoluble gluten fraction known to have a high degree of sequence identity among the gluten-containing grains. Sample extracts were reduced, alkylated, and digested for mass spectrometry (MS) analysis. Untargeted MS was used to yield potential peptide targets which were then systematically screened using experimental MS data and bioinformatics to identify wheat-specific peptides.

Results: Extracted samples yielded 15-30% protein which was expected as the water-soluble proteins contribute to ~20% of the total protein content of gluten-containing grains. SDS-PAGE analysis indicated the predominant presence of LMW proteins and the absence of HMW proteins, suggesting that the albumin and globulin proteins were preferentially extracted, which are more likely to be unique to wheat. Using Peaks v8.5 and proteome databases (UniProt), a total of 12,950 peptides were identified using label-free quantification. Subsequent systematic filtration steps resulted in the identification of 13 wheat-specific peptides originating from proteins such as gamma gliadin, defensin-like and Bowman-Birk-like, which may be potential targets in the development of wheat-specific detection methods.

Significance: With the ability to detect and quantify the specific presence of wheat in food products, the safety of food for wheat-allergic consumers will be improved.

P3-11 Variance Estimations When Measuring Peanut and Soy Protein in Discrete Wheat Flour Samples

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Introduction: Wheat kernels may be contaminated with soybeans and peanuts due to agricultural commingling. The milling process can alter their distribution in wheat flour, which can impact allergen quantitation.

Purpose: To determine variance associated with analyses of peanut protein (P) and soy protein (S) in wheat flour samples obtained by discrete sampling and predict total variance (Vt) at P or S concentrations (ppm) depending on test portion size (Ns; grams), and the number of aliquots analyzed (Na).

Methods: Ten wheat kernel lots (45kg each) were mixed with varying amounts of crushed, raw peanut, and dried soybean, followed by milling using a hammer mill with 0.6 mm outlet screen. From each lot, 32 flour samples (200g each) were collected during milling and each randomly split (two 100g samples) to be used in discrete and composite sampling. Test portions from each discrete sample (32, 100g samples/lot) were analyzed for S and P content in duplicate aliquots using the Morinaga Soya ELISA Kit II and Neogen Veratox for Peanut assay, respectively. The Vt was partitioned into variance between test samples (Vs) and aliquot tested (Va). Regression analysis was conducted to establish the relationship between variance and P or S concentrations.

Results: The Vt, Vs, and Va increased with an increase in P and S concentration, and their regression analyses showed a linear best fit on a log-log scale. For both peanut and soy, Vs>Va and over 95% of Vt resulted from Vs. The Vt=Vs+Va can be estimated from the equations:

Peanut: $Vs=(5/Ns)2.4235\ P^{1.5926}$ and $Va=(1/Na)0.0653\ P^{1.6033}$

Soy: $Vs=(1/Ns)1.1596\ S^{1.6204}$ and $Va=(1/Na)0.0046\ S^{1.8594}$

Significance: Variance equations can be used to predict sampling-dependent variability in peanut and soy test results. Peanut measurement variability was nearly 10 times greater than that of soy, possibly due to the differences in their composition and physical properties.

Poster

P3-12 Selection of Target Peptides for a Mass Spectrometry Method to Detect Peanut Protein in Processed Food Matrices

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❖ ***Developing Scientist Entrant***

Introduction: The ability to detect peanut protein is imperative for the food industry to protect allergic consumers. Current immunoassay allergen detection methods demonstrate inadequacies in detecting and quantifying food allergens in processed food matrices, but mass spectrometry (MS) methods may overcome both decreased recovery and cross-reactivity issues.
Purpose: The objective of this study was to identify sensitive and robust (minimally affected by matrix and processing) target peanut peptides for inclusion in a mass spectrometry-based peanut detection method.
Methods: Cookie and dark chocolate matrices incurred with peanut flour (0 and 5,000 ppm peanut protein) were extracted and digested with trypsin for untargeted MS analysis, including label-free quantification. Candidate target peptides were selected based on signal intensity, specificity, and recovery in matrices. Further filtration was performed using iterative rounds of targeted mass spectrometry to select the best-performing target peptides for each matrix.
Results: Using selection criteria applied to label-free quantification data, 32 and 67 peptides were identified as candidate target peptides for the cookie and dark chocolate matrices, respectively. After filtration by targeted mass spectrometry, nine peptides were determined to be sensitive and robust targets. Detectable levels in diluted digests of incurred food matrices ranged from 2.5–50 ppm peanut protein. All final targets came from major peanut allergens; seven from Ara h 3, one from Ara h 1, and one from Ara h 2.
Significance: This study identified nine peanut peptides that would be robust targets in an MS-based peanut detection method. The implementation of such an orthogonal, confirmatory method for processed food matrices would increase confidence in analytical results obtained by industry and regulatory stakeholders.

P3-13 Detection of Edible Insects Using Three Crustacean Allergen Detection Methods: ELISA, xMAP Food Allergen Detection Assay, and Real-Time PCR

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Introduction: Increased consumption of insects in recent years, especially in Western countries, has raised concern about their potential to cause allergic reactions. Insects are known to cross-react with crustacean shellfish such as shrimp, crab, and lobster, which are regulated as food allergens by the United States Food and Drug Administration (FDA).
Purpose: The purpose of this work was to investigate the performance of crustacean allergen detection methods currently employed by FDA with edible insect samples.
Methods: Detection methods used for this work included ELISA and xMAP-based Food Allergen Detection Assay (xMAP FADA), which detect proteins, and real time PCR, which detects DNA. A total of 10 edible insect samples were analyzed using three different commercial crustacean ELISAs, the xMAP FADA, and real time PCR assays for shrimp, crab, lobster, and krill. Western blot, using the detector antibodies of the ELISA kits, was carried out to further investigate specific proteins involved in cross-reactivity.
Results: Nine of the ten insect samples showed cross-reactivity with both ELISA and xMAP FADA. Response ranges for the three ELISA assays were 4.5-154 µg/g crustacea, 1.7 – 59 µg/g crustacea, and 0.03-0.09 µg/g tropomyosin with CVs of 1-13%. For the xMAP FADA, insect reactivity with the crustacean antibody yielded mean fluorescent intensities (MFI) of 386-8522 MFI with CVs of 0.5-15%. Western blot identified the major protein involved in cross-reactivity to be a 37 kDa band, which is tropomyosin based on the approximate molecular weight. Insect samples did not yield cross-reactive signals in real time PCR assays.
Significance: This work demonstrated that the protein-based crustacean allergen detection methods ELISA and xMAP FADA cross-react with potentially allergenic insect samples. However, real time PCR assays did not cross-react. PCR confirmation of positive signals from ELISA and xMAP FADA is important for differentiation of crustaceans and insects in food products.

P3-14 Withdrawn

P3-15 Performance Verification of an ELISA-Based Gluten Assay on Plant-Based Meat and Food

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Introduction: Eating plant-based food has grown tremendously in popularity due to its taste factor and mass appeal. Buying beef, chicken, and other popular meats via plant-based provides consumers with a healthier option. Plant-based companies have mastered the art of marketing, brand influence, and recipe.
Purpose: Gluten is a protein found in wheat, barley, rye and sometimes oats. People with celiac disease must stay away from all grains with gluten. People with wheat allergy often only need to stay away from wheat and can eat the other grains. So, following a gluten-free diet may be limiting. But gluten-free foods should be safe for people with wheat allergy. Are plant based meat and food available for people with wheat allergy or celiac disease? Such consumers need to check and trust on the declaration made by the supplier.
Methods: The following are bought from a local supermarket to be tested with ELISA-based Gluten Assay. Plant based sausage that contains wheat and a plant based meat that is declared "Gluten free," and also meat balls that do not list wheat gluten in the ingredients but stated "may contain gluten."
Results: The plant based sausage that contains wheat has a high amount of gluten detected, the meat balls (that may contain gluten) and plant based meat that is declared gluten free are analyzed free of gluten. The non-detectable meat and meat balls are fortified with gluten spike at at LOQ level of 4ppm and 3x LOQ at 12ppm level to determine the recovery. The recovery of gluten spike fortification is between 80-100% for the . The RSD is <10%.
Significance: The AgraQuant® Gluten G12 offers a rapid and reliable tool for testing gluten presence in plant-based food.

P3-16 Performance Verification of an ELISA-Based Assay Sesame in Pastries Found in Bakeries

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Introduction:. Sesame is found in various foods, especially Asian cuisine. Sesame seeds are a source of nutrients with significant health benefits. Despite the nutritional advantages, many people are allergic to sesame. Sesame is now the ninth major food allergen to require food labeling in USA, which could pose tremendous challenges for the food industry.
Purpose: In bakery shops in Singapore and round the region, the Chinese pastries are getting very popular, we check whether sesame allergy consumers are able to consume these items. These include Chinese pastries, such as buns, tarts and bread. Other than ones with sesame filling or sesame seeds that are decorated on the buns, we do not exactly know whether the pastry items contain sesame as there is no need for declaration of allergen in bakeries in Singapore.
Methods: We scouted a Chinese bakery to check on the popular Chinese pastries in Asia to purchase pastries with sesame ingredients and without. We tested both the the crust layer and the fillings of each sample using ELISA-based Assay Sesame kit.

Results: In the 20 types of Chinese pastries analyzed, sesame were detected in all the samples' filling. No sesame was detected on the crust layer that do not have sesame ingredients. The non-detectable samples are fortified with sesame spike at LOQ level of 2ppm and 3x LOQ at 6ppm level to determine the recovery. The recovery of sesame spike fortification is between 80-100%. The RSD is <10%. There seems to be cross contamination of sesame on the pastries that are sold in the bakery. There is a need of caution if sesame allergy users are to purchase from bakeries.
Significance: The AgraQuant® sesame Allergen offers a rapid and reliable tool for testing sesame in pastries.

P3-17 Ergot Occurrence in Asian Grains

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Introduction: Ergot alkaloids are potent toxins that occur in rye, wheat and triticale. They are produced by fungi of the Claviceps species. These fungi are able to produce a wintering body, also known as sclerotium. Sclerotia contain different classes of alkaloids, the most prominent being ergometrine, ergotamine, α-ergosine, ergocristine, α-ergocryptine and ergocornine.Most countries have established regulations to protect consumers from the harmful effects of ergot alkaloid intake. New regulations for ergot alkaloids go into effect in the European Union on January 1, 2022.
Purpose: Many countries in Asia and Australia and are exporting countries of food to European countries, thus there is an interest to understand the occurrence of ergot in Asia grains.
Methods: Samples from Thailand and Australia are sent to Romer Labs Analytical Service lab which is ISO17025 certified for Ergot analysis using LCMS/MS, which is based in Singapore. The samples include rye, barley and wheat and feed. The 12 ergot alkaloids analyzed include Ergocornine,Ergocorninine Ergocristine, Ergocristinine, Ergocryptine,Ergocryptinine, Ergometrine, Ergometrinine, Ergosine, Ergosinine,Ergotamine and Ergotaminine.
Results: The results for 100 samples sent between 2016 – 2021 indicate 10 % of the samples received contained ergot presence and are mostly ergometrine, and ergotamine. This gives a good indicator that is presence of ergot in Asia wheat grains.
Significance: It is recommended be tested for Ergots in wheat grains, to protect the consumers and to adhere to the European regulation.

P3-18 Controlled Fermentation with Selected *Lactobacilli* and Yeast Probiotics Decontaminates Aflatoxins in Maize Gruel and Changes the Amino Acid Profile Differentially

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Introduction: Fermented maize gruel (*ogi*) is commonly consumed as a nutritious food by breastfeeding mothers, children, and the convalescent in West Africa. However, when aflatoxin contamination is present, such food becomes unsafe. Chronic exposure to aflatoxin results in liver cancer, is associated with growth retardation in children, and other maladies.
Purpose: Consequently, aflatoxin decontamination was explored as an important management strategy for food safety.
Methods: Our study investigated o*gi* (n =27) processed in the laboratory under controlled conditions with maize containing toxigenic and non-toxigenic *A. flavus* genotypes (La3228 and La3279 respectively). Controlled fermentation was done with probiotics *Limosilactobacillus fermentum* W310, *Lactiplantibacillus plantarum* M26, *Candida tropicalis* MY115, and *C. tropicalis* YY25. Experimentation was done in triplicates and samples were completely randomized.
Results: These probiotics decontaminated aflatoxin B1 present by 86%, 62%, 60% and 60% respectively. Fermentation with these organisms also caused changes to amino acid concentrations. Generally, there were higher levels of amino acids ($p<0.05$) when toxigenic La3228 was present than in the presence of atoxigenic La3279. This is likely due to the secondary metabolism towards aflatoxin production by La3228, in contrast to atoxigenic La3279 that is not an aflatoxin-producer. There were differences ($p<0.05$) to the amino acid profile during fermentation by the probiotic strains. These differences were observed both intra- and inter-species. Among *lactobacilli,* there were differences in concentrations observed with serine, threonine, tyrosine, lysine. Among yeasts, differences were observed with aspartic acid, serine, glutamine, threonine, arginine, alanine, proline, cysteine, tyrosine, valine, lysine, methionine, leucine, iso-leucine, and phenylalanine.
Significance: Decontamination by selected probiotics is useful for aflatoxin management.

P3-19 Use of *Aspergillus oryzae* Koji to Inhibit Aflatoxin Production of *Aspergillus flavus* in the Production of Korean Soybean Paste

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Introduction: *Meju* is a Korean fermented soybean starter used in the production of soybean paste, *Doenjang*. *Meju* is susceptible to contamination with toxigenic fungi to produce aflatoxins (AFs), since it is generally manufactured under spontaneous fermentation.
Purpose: In this study, we evaluated the inhibition of AFs production of *Aspergillus flavus* by using koji made by inoculating *A. oryzae* into raw starch materials and investigated the physicochemical and nutritional properties in *Meju* and *Doenjang*.
Methods: Determination of AFs was carried out by high performance liquid chromatography coupled with fluorescence detection. The inhibition of AF production was evaluated by co-culturing koji with aflatoxigenic *A. flavus* when making *Meju*. Color difference, pH, salinity, amino type-nitrogen, free-amino acid contents and isoflavones (daidzein and genistein) were further analyzed.
Results: The contents of AFs produced in fermented *Meju* and *Doenjang* were 1684.5 μg/kg (894.5 to 2405.0 μg/kg) and 1266.3 μg/kg (419.1 to 1132.2 μg/kg) in the untreated group, and 1.4 μg/kg (no detection to 8.7 μg/kg) and 0.2 μg/kg (no detection to 0.4 μg/kg) in the koji-treated group. The AF production inhibition rates by adding koji into *Meju* and *Doenjang* were over 99%, which significantly lowered AFs production (Mann-Whitney U test, $p <0.001$). The color difference, amino-type nitrogen and isoflavone contents were significantly higher in the koji-treated group. Total free amino acids concentration was also higher in the koji-treated (4046.97 mg/100 g) than the untreated group (3291.96 mg/ 100 g).
Significance: The findings could be applied to inhibit AFs contamination in the production of Korean soybean paste.

P3-20 Mycotoxin Reduction after the Production of *Dawadawa* (An African Fermented Condiment) from Bambara Groundnut

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Introduction: Mycotoxins are known to be among the most hazardous naturally occurring substances that usually contaminate legumes and other food commodities. The occurrence of these contaminants in food crops extends to derived food products, which when consumed, can result in marked negative health effects on consumers.
Purpose: To necessitate safe and practicable alternatives for addressing this menace, this study was aimed investigating the extent of mycotoxins reduction in an African fermented condiment produced from Bambara groundnut.
Methods: Doehlert design of response surface methodology was used as optimization tool, different fermentation time (48–120 h) and fermentation temperature (25–45 °C) conditions were optimized to produce *dawadawa* from Bambara groundnut. Mycotoxins in the Bambara groundnut and derived *dawadawa* samples were extracted using a quick easy cheap effective rugged and safe (QuEChERS) method and a total of 14 mycotoxins were quantified using Liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: The LC-MS/MS result showed a significant ($p \leq 0.05$) reduction of mycotoxins, at varying levels after fermentation. The high percentage reduction of mycotoxins with increased fermentation time and temperature, was probably due to increased action of fermenting microorganisms (especially

Bacillus and *Lactobacillus* species) in degrading the tested mycotoxins. A 100% reduction in aflatoxin B_1, aflatoxin G_2, fumonisin B_1, fumonisin B_3, ochratoxin B, T2-toxin, alpha-zearalenone and beta-zearalenone were observed. An increase in fermentation time and temperature was also observed to have led to greater mycotoxin reduction while the selected optimal fermentation conditions (84 h 35 °C) for *dawadawa* production were obtained from higher values for pH and TTA.

Significance: This study signifies that fermentation can contribute to the safety of *dawadawa* and reduce the mycotoxin content. An insight into the reduction of mycotoxins after *dawadawa* production, is vital considering the vast use of this condiment during food preparations in developing countries.

P3-21 Impact of Fermentation on Mycotoxins Detoxification

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Introduction: Fermentation technique is brought about through the actions of microorganisms leading to substrate modification and subsequent improvement in food composition. Mycotoxins on the other hand are secondary metabolites produced by filamentous fungi, leading to negative health and economic effects. The need to control mycotoxins cannot be over emphasized and fermentation is a notable inexpensive mycotoxin decontamination strategy that can be explored not only to improve the constituents in food, but equally reduce and at best eliminate mycotoxins. This study was aimed at providing an insight into fermentation as a sustainable decontamination technique in detoxifying mycotoxin.

Purpose: Considering an array of different fermented foods in Africa and the increasing reports of mycotoxin contamination in African and non- African food crops, there is need to explore a cheap, readily available and simple technique that would not only ensure the safety of food, but also contribute to its sensorial, nutritional and health beneficial composition.

Methods: This study appraised existing literature on elucidating the mechanisms of mycotoxin detoxification/reduction processes in fermented foods.

Results: The results showed that fermentation is indeed a processing technique that generally favors mycotoxin reduction. Such reduction is through various mechanisms including binding with yeast or LAB cells and/or degradation/dissociation/bioconversion of the mycotoxin molecule. Effective handling and storage practices before fermentation may ensure complete prevention of the heinous effect of these toxins on human health. The extent of mycotoxin reduction also largely depends on the initial mycotoxin concentration. While this may be desirable, studies should also explore the toxicity of degraded mycotoxins to effectively ensure the safety of the fermented foods.

Significance: The study shows that in the absence of sophisticated monitoring and prevention mechanisms in Africa, exploiting fermentation would be vital in improving nutrition and ensuring food safety.

P3-22 Fermentation of Sorghum into Sourdough Reduces Mycotoxins Contents

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Introduction: Mycotoxins are deleterious and of global public health concern, with numerous reported adverse health and economic effects associated with its exposure. This thus necessitates measure to reduce and or eliminate them in foods.

Purpose: This study explored the reduction of mycotoxins through natural and lactic acid bacteria (LAB) fermentation during sourdough production from whole grain (WG) sorghum varieties.

Methods: Two whole grain (WG) sorghum varieties (with different tannin contents) were milled naturally (spontaneously) fermented and with Lactobacillus fermentum strains (FUA 3165 and FUA 3321), to obtain sourdoughs. Mycotoxins were extracted and quantified in WG sorghum and derived sourdoughs.

Results: Among the mycotoxins investigated, fumonisin B_1 (FB_1), B_2 (FB_2), B_3 (FB_3), T-2 toxin (T-2), zearalenone (ZEA), alpha-zearalenol (α-ZOL) and beta-zearalenol (β-ZOL) were detected in sorghum and all their concentrations significantly ($p \leq 0.05$) reduced after fermentation. In particular, *L. fermentum* FUA 3321 showed the capability to significantly ($p \leq 0.05$) reduce all the mycotoxins by 98% for FB_1, 84% for T-2 and up to 82% for α-ZOL, compared to WG sorghum flour.

Significance: Fermenting with the *L. fermentum* strains showed potential to effectively reduce mycotoxin contamination in whole grain sourdough and could be considered as suitable starter cultures for dietary detoxification of mycotoxins.

P3-23 *In Vitro* Testing of Lytic Bacteriophages Isolated from Virulent Strains of *Vibrio parahaemolyticus* for Biocontrol Applications

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Introduction: Foodborne gastrointestinal infections with *Vibrio parahaemolyticus* are primarily associated with the consumption of raw or undercooked oysters exposed to contaminated water. Bacteriophages (phages), viruses that infect bacteria, offer promising potential as an alternative to antibiotics against bacterial pathogens.

Purpose: To conduct *in vitro* testing of wild-type *V. parahaemolyticus* bacteriophages for biocontrol applications.

Methods: Bacteriophages were isolated from Florida oysters using ten environmental and clinical virulent isolates of *V. parahaemolyticus*. Host specificity determination using spot plate assay identified two lytic bacteriophages with the broadest host ranges by infecting 6 and 8 out of 10 host strains. Though not tested as a cocktail, both isolates collectively lyse all ten host strains. These two bacteriophages were further characterized based on their morphology using scanning electron microscopy, whole-genome sequencing, and a one-step growth curve.

Results: Morphological assessments and WGS analyses suggest that the isolates belong to *Siphoviridae* and *Podoviridae* and were assigned as vB_VpS_P9 and vB_VpP_P10, respectively. Sequence analyses showed that the viral genomes of vB_VpS_P9 (58.6 kb, 46.0% GC) and vB_VpP_P10 (58.6 kb, 46.0% GC) are composed of dsDNA that do not carry any virulence factor, antimicrobial resistance, or integrase genes. Specific hosts yielding the highest progenies were utilized for one-step growth curve experiments. The vB_VpS_P9 and vB_VpP_P10 were highly lytic and yielded respective 10 and 9 $\log_{10}$ PFU progenies per mL at a 0.1 multiplicity of infection. One-step growth curves for vB_VpS_P9 and vB_VpP_P10 showed a latent period of 15 min, then plateaued after 180 min with approximate burst sizes of 260 and 70 PFU per infected cell, respectively.

Significance: The outcome of this study help develop effective biocontrol strategies for mitigating risks associated with foodborne pathogens. Further research efforts focus on the environmental persistence and application of bacteriophages to control *V. parahaemolyticus* in seafood.

P3-24 Mild Lactic Acid Stress Causes Strain-Dependent Reduction in SEC Protein Levels

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Introduction: Staphylococcal enterotoxin C (SEC) is a major cause of staphylococcal food poisoning in humans and plays a role in bovine mastitis. *Staphylococcus aureus* benefits from a competitive growth advantage under stress conditions encountered in foods such as a low pH. Therefore, understanding the role of stressors such as lactic acid on SEC production is of pivotal relevance to food safety. However, stress-dependent cues and their effects on enterotoxin expression are still poorly understood.

Purpose: In this study, we aimed to determine the effect of mild acid stress on *sec* expression on both transcriptional and translational level.

Methods: The effect of lactic acid stress (pH 6.0) on *sec* mRNA and SEC protein levels was assessed in seven *S. aureus* strains using qPCR and ELISA assays. The strains represent four different SEC variants and originated from staphylococcal food poisoning and infections in humans and animals.

Results: While only a modest decrease in *sec* mRNA levels was observed under lactic acid stress, protein levels showed a significant decrease in SEC levels for some strains. These findings indicate that post-transcriptional modifications can act in SEC expression under lactic acid stress. The effect of lactic acid stress on *sec* expression could not be linked to SEC variants.

Significance: Our findings indicate that mild lactic acid stress can be used to decrease *sec* expression in *S. aureus.*

P3-25 Mild NaCl Stress Reduces the Synthesis of Staphylococcal Enterotoxin C

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Introduction: Enterotoxins produced by *Staphylococcus aureus* such as SEC are the cause of serious foodborne intoxications. *S. aureus* possesses a competitive growth advantage over accompanying bacterial flora under stress conditions encountered in foods, such as high NaCl concentrations.

Purpose: In this study, we investigate the effect of NaCl stress encountered in foods on *sec* expression.

Methods: Using qRT-PCR and ELISA assays, we investigated the influence of 4.5% NaCl on *sec* mRNA and SEC protein levels after 4 h, 10 h and 24 h, respectively. All experiments were conducted in three independent biological replicates in seven *S. aureus* strains representing four different SEC variants. The strains originated from foodborne intoxications, cases of infections in humans, cattle, and sheep. For statistical analysis, a mixed effect linear model was fitted on the $\log_{10}$-transformed fold change, with a full three-way interaction between reference gene, strain, and time effects. To determine whether individual mRNA levels were increased, we used lsmeans to perform a one-sided effect test, with Holm-Bonferroni-corrected *p*-values. Protein data was analyzed via two-way ANOVA and post-hoc Tukey's multiple comparisons.

Results: The effect of NaCl stress on *sec* transcription was time and strain dependent. In contrast, SEC protein levels were generally decreased under NaCl stress. These findings indicate that SEC expression under NaCl stress is regulated at mRNA level and that NaCl can contribute to lower SE concentration in foods. All tested strains carrying gene variant SEC2/promoter v1 (n = 3) significantly downregulated *sec* transcription in exponential or early stationary phase. Strains harboring SECbovine/promoter v2 (SAR1, SAR38) significantly upregulate *sec* transcription after 24 h. No influence of strain origin or clonal complex was apparent.

Significance: This study provides new insights into the regulation of *S. aureus* enterotoxin C expression under stress conditions. It represents a first step towards improving production parameters to minimize SEC production and increase food safety.

P3-26 Glucose Stress Lowers Staphylococcal Enterotoxin C Production

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Introduction: Staphylococcal enterotoxin C is produced by *Staphylococcus aureus* during growth in the food matrix and leads to staphylococcal food poisoning, one of the most prevalent foodborne intoxications worldwide. While the surrounding bacteria in food matrices usually repress growth of *S. aureus*, the organism possesses a remarkable growth advantage under stress conditions encountered in many foods. Examples for such food matrices are pastry and bakery products with their high sugar content that lowers water availability. While *S. aureus* can still grow in these challenging environments, it remains unclear how these conditions affect SEC expression.

Purpose: We aimed to investigate the influence of 30% glucose on *sec* mRNA and SEC protein expression.

Methods: To this end, quantitative Real-Time PCR experiments and ELISA assays were conducted in seven *S. aureus* strains. The levels of *sec* mRNA and SEC protein between 30% glucose stress and non-stressed growth conditions were compared after 4 h, 10 h and 24 h.

Results: In five out of seven strains, glucose stress led to a pronounced decrease in *sec* mRNA transcription in at least some growth phases. SEC protein levels were substantially lower under glucose stress for all strains in late exponential and stationary phase. Only two strains showed elevated SEC levels at 4h, before SEC levels were decreased in later growth phases.

Significance: Based on our findings, glucose seems a suitable food ingredient to effectively lower SEC synthesis in the food matrix.

P3-27 Whole Genome Sequencing Reveals Biopesticidal Origin of *Bacillus thuringiensis* in Foods

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Introduction: *Bacillus thuringiensis* is a microbial insecticide widely used to control agricultural pests. Although generally regarded as safe, *B. thuringiensis* is phylogenetically intermingled with the foodborne pathogen *B. cereus sensu stricto* and has been linked to foodborne outbreaks.

Purpose: The aim of this study was to extent the currently very limited data on the pathogenicity potential of *B. thuringiensis* and the occurrence of biopesticide residues in food.

Methods: We analyzed and matched whole-genome sequences of 33 *B. thuringiensis* isolates from biopesticides, food, and human fecal samples linked to outbreaks.

Results: All food and outbreak-associated isolates genomically matched (≤ 6 wgSNPs) with six biopesticide strains, indicating biopesticide products as their source. Long-read sequencing revealed a more diverse virulence gene profile than previously assumed, including a transposase-mediated disruption of the promoter region of the non-hemolytic enterotoxin gene *nhe* and a bacteriophage-mediated disruption of the sphingomyelinase gene *sph* in some biopesticide strains. Furthermore, we provide high-quality genome assemblies of seven widely used *B. thuringiensis* biopesticide strains, which will facilitate improved microbial source tracking and risk assessment of *B. thuringiensis*-based biopesticides in the future.

Significance: Our findings reveal the biopesticidal origin of *B. thuringiensis* from foods and fecal samples related to outbreaks and provide crucial data for improved risk assessment.

P3-28 Screening *Bacillus cereus* Psychrotrophic Cereulide Producers in Food Products

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Introduction: Cereulide is heat- and acid-stable dodecadepsipeptid (1.2 kDa) encoded on the *Bacillus cereus ces* gene locus. It induces food intoxication 0.5-5 h after consumption of the pre-formed toxin in food product. Rarely, this toxin can enter systemic circulation and cause fulminant liver failure, damage of other organs and death. Several fatal cases were reported as consequence of cereulide intoxication. Sensitive individuals, especially children, can be the most affected. The main control tool for the *B. cereus* growth and cereulide production is storage of food products at refrigerator temperatures.

Purpose: The purpose of this study was to isolate psychrotrophic *B. cereus* cereulide producers from food products.

Methods: Presumptive *B. cereus* isolated from food products (n=94) were subjected to refrigerator temperatures (4 °C) for one month. After visible colonies appeared, *ces* encoding gene detection by real-time PCR was used to isolate potential cereulide producers. Ability to produce cereulude was futher assessed by computer aided analysis of the boar semen motility.

Results: After exposure of presumptive *B. cereus* at 4 °C, it was concluded that 11/94 were *ces* gene carriers. Emetic psychrotrophic *B. cereus* were found in buckwheat, chantilly cream, coffee creamer, couscous, milk powder, muesli, polenta, instant vermicelli soup, and instant meat soup. Motility assay showed that 5 out of 11 emetic *B. cereus* were able to produce cereulide. Cereulide producers that are able to grow at refrigerator temperatures represent a matter of concern in food safety. Improper food storage can trigger cereulide production, which can lead to foodborne poisoning.

Significance: This study provides information about the presence and psychrotophic capacity of cereulide producers in food products.

P3-29 Persistence of Silver Nanoparticles in Fresh-Cut Romaine Lettuce during Simulated Commercial Processing

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❖ ***Developing Scientist Entrant***

Introduction: Silver nanoparticles (AgNPs) are used in agriculture mainly in the production of nanopesticides. Increased presence of AgNPs in farm environments is raising new food safety concerns for fresh-cut produce.

Purpose: This study evaluated the ability of flume washing in a chlorine-based sanitizer and centrifugal drying to remove AgNPs from contaminated fresh-cut romaine lettuce during pilot-scale processing.

Methods: Shredded romaine lettuce (1 kg) was immersed in a 85 mg/L AgNP suspension for 1 h, air-dried an additional hour, combined with 4.4 kg of Ag-free shredded lettuce, flume-washed for 90 sec in a 3.3.m-long flume tank containing 600 L of tap water with or without 100 mg/L chlorine (pH 6.5; XY-12, Ecolab), and finally dewatered in a 22.4 kg capacity centrifugal drier for 90 sec. Water samples collected from three independent trials at 30-sec intervals during flume washing and after centrifugal drying were analyzed in duplicate for Ag using inductively coupled plasma mass spectrometry. A mixed effect model was used to identify significant differences.

Results: The immersed lettuce contained 44.4 mg of Ag/kg before processing. Based on Ag concentrations in the water, the Ag content of the lettuce decreased only 2.0 and 0.8% after 90 sec of flume washing in water with and without chlorine, respectively. After centrifugal drying, Ag decreased an additional 0.9% and 0.5 % for lettuce previously flume washed in water with and without chlorine, respectively. Thus, lettuce flume-washed in water with and without chlorine still contained 43.1 Ag/kg (97.1%) and 43.8 Ag/kg (98.7%) after centrifugal drying, respectively, with persistence of these reductions in Ag not significant ($P > 0.05$).

Significance: Therefore, current commercial produce processing conditions are most likely ineffective in removing AgNPs from fresh-cut leafy greens.

P3-30 Bilious Biofilms Formation by *Salmonella* and Shiga-Toxin Producing *E. coli*

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Introduction: Biofilms are important for the environmental survival of human pathogens. Biofilm formation could occur in response to the presence of bile salts and acids

Purpose: In this study, biofilm formation in Shiga-toxin producing *E. coli* O157: H7 H1730, a non-pathogenic *E. coli*, *Salmonella* Newport and *Salmonella* Typhimurium was assessed after exposure to 5 % and 0.5 % Ox-bile and Deoxycholic acid.

Methods: Cultures of bacterial strains (6 log CFU/ml) were transferred into microtiter plates containing TSB with Ox-bile (0.5 and 5%) or Deoxycholic acid (0.5 and 5%). The formation of biofilms was determined over a duration of 24 h at 37 C. Planktonic cells were washed away by a and biofilms quantified using the microplate colorimetric crystal Violet assay. The optical density was read at 570nm using a microtiter plate reader. Experiment was conducted in triplicates and differences were analyzed by one-way ANOVA using JMP statistical software.

Results: *S.*Newport and *E. coli* O157: H7 formed the strongest biofilms with OD_{570} of 0.55 ± 0.07 and 0.54 ± 0.07 respectively in the presence of 0.5 % Ox-bile and were significantly different from the other bacterial strains evaluated ($P < 0.05$). In 5 % Ox-bile, *E. coli* O157: H7 formed the strongest biofilm of 0.49 ± 0.06 ($P < 0.05$). *Salmonella* Newport formed the strongest biofilm 0.60 ± 0.03 in 0.5 % Deoxycholic acid ($P < 0.05$). There was no significant difference ($P > 0.05$) between biofilm formation for all the bacterial strains evaluated in 5 % Deoxycholic acid. The weakest biofilm formation was observed in non-pathogenic *E. coli* and there was no significant difference between biofilm formation in Ox-bile and Deoxycholic acid at both concentrations for all bacterial strains ($P < 0.05$).

Significance: The presence of bile acids may impact biofilm formation in Shiga-toxin producing *E. coli* and *Salmonella* Newport.

P3-31 Antibacterial and Antibiofilm Performance of Far-UVC 222 Nm Light Inactivation Against Gram-Positive and Gram-Negative Foodborne Pathogenic Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: Far-UVC 222 nm light was reported to have antimicrobial effects, but there is limited information about its effectiveness on various substrates or bacterial species relevant to food processing.

Purpose: This study evaluated the effect of 222 nm light on several foodborne pathogens in different substrates, and on biofilm formation.

Methods: Early stationary phase (18h) colonies of *E. coli* O157:H7 ATCC 43895, *L. monocytogenes* 10493s, *P. aeruginosa* ATCC 15442, *S. Typhimurium* FSL S90123, and *S. aureus* ATCC 9144 were spot-inoculated on stainless steel (SS), followed by 3h air drying. *E. coli* and *L. monocytogenes* were also inoculated in 1.2mm thin liquid films. Substrates were exposed to 222nm using a FirstUVC® DF28B-20W unit, for 30s to 1000s, at 21 °C. To evaluate the effect on biofilm formation, SS coupons submerged in tryptic soy broth containing *S. aureus* (10^7 CFU/mL) were subjected to 222 nm at 37 ℃ for 24h to 48h. Untreated controls were prepared under similar conditions. Survivors were recovered, plated, and enumerated. Confocal microscopy was used to visualize biofilm structure and quantify biomass accumulation. Experiments were performed in triplicate with independently grown cultures. Data was statistically analyzed using ANOVA.

Results: Exposure to 222 nm resulted in >3-log reduction for *E. coli* and *L. monocytogenes* in liquids after 1000s. Highest inactivation was observed for *L. monocytogenes* and *S. aureus* on SS, with 5-log reduction after 1000s. Inactivation curves were non-linear, and successfully modeled with the Weibull model ($0.95 \geq R^2 \geq 0.82$). Inactivation kinetics was significantly ($p<0.05$) affected by substrate and species. The treatment reduced biomass accumulation by *S. aureus* on SS to 17 - 34% compared to untreated controls.

Significance: This is the first study to demonstrate the effectiveness of 222 nm on bacterial biofilms. These results also demonstrate that 222 nm light has good potential in the food industry, but disinfection success depends on bacterial species and substrate.

Poster

P3-32 UV-C and Heat Resistance of *Bacillus thuringiensis* and *Bacillus cereus*

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❖ ***Developing Scientist Entrant***

Introduction: Endospores of *Bacillus cereus sensu lato* are exposed to a broad variety of sublethal and lethal stresses in the food production chain, such as UV-C and heat treatment.

Purpose: This study aims to compare the resistance between *B. cereus* and *B. thuringiensis* (Bt) spores, including the commercial Bt biopesticide products, exposed to UV-C and heat treatment.

Methods: Spore solutions of lab harvested spores and commercial Bt products (granules or powders with dry spores and insecticidal crystals) were prepared with the initial concentration of 10^8 cfu/ml. UV-C treatment was performed in a closed inox box with a UVpro K17-2 lamp. The spores were subjected in liquid (under agitation) to UV-C treatment from 1 ($3.9x10^{-1}$ J/cm²) to 7 minutes ($6.2x10^{-1}$ J/cm²). A spore suspension was transferred to sterile ultra-thin walled PCR tubes and heat treated in a thermocycler with the lid temperature at 105°C. Heat treatments at 90°C for 10, 30, 60, 90, 120 min were performed for the determination of D-value. Surviving spores were serially diluted, plated on TSA agar and incubated at 30°C for 24h.

Results: Inactivation curves were fitted using GInaFit. For UV-C treatment, 3 commercial Bt products fit the log shoulder + linear regression model, all lab harvested spores fit the linear + tail regression model. Within 7 min, only 1 log reduction of spores in 3 commercial Bt products were observed, but 4 log reductions of lab harvested spores were detected. For heat treatment, the log-linear regression model were used for the calculation of D values at 90°C. Significant differences of heat resistance between commercial Bt products and lab harvested spores were found.

Significance: Bt spores with the formulation of commercial products (granules or powders) show higher UV-C and heat resistance than the spores harvested from lab.

P3-33 Correlation between Dipicolinic Acid (DPA) Release and Heat Resistance of *C. botulinum* Type A and *C. sporogenes* Spores during Thermal Processing

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Introduction: Dipicolinic acid (DPA) is a major constituent of bacterial spores and may provide protection against spore inactivation by various processes. Thermal processing induces DPA release from spores, followed by loss of heat resistance and inactivation of DPA-free spores. This suggests that the resistance of spores to thermal processes may rely on their ability to retain DPA.

Purpose: To determine if any correlation exists between DPA release and thermal inactivation of *C. botulinum* and *C. sporogenes* spores, and whether DPA release may be a quantifiable indicator of heat resistance.

Methods: Spores of *C. botulinum* (Giorgio-A) and *C. sporogenes* (PA3679) were diluted in ACES buffer (0.05 M, pH 7.0) to 7 log spores/mL, aliquoted into NMR tubes, and heat-sealed. Spores were thermally treated at 101°C, 105°C, and 108°C for predetermined treatment times in an oil bath. Surviving spores were enumerated by 5-tube MPN using Trypticase-Peptone-Glucose-Yeast extract broth with 10-12 weeks incubation. Spore suspensions were filtered through a 0.22 µm membrane, heat-treated (85°C, 10 minutes) to denature any toxin, and analyzed for DPA by UPLC-MS/MS. Experiments were repeated in triplicate. Correlation between MPN log reductions versus % DPA release was performed using Pearson's correlation coefficient.

Results: Released DPA content from spores and log reductions increased with increase of treatment time and process temperature from 101 to 108°C for strains Giorgio-A and PA3679. DPA released from Giorgio-A spores at 105°C after 15 minutes was 56.20±7.71% of the total DPA content with 3.89±0.39-log reduction. DPA content in unprocessed controls was below limit of quantification. A strong, positive correlation was observed between % DPA release and log reduction of spores from Giorgio-A and PA3679 at all thermal treatments (r = 0.8033-0.9120).

Significance: These results suggest that a correlation exists between DPA release and heat resistance of spores of Giorgio-A and PA3679. Further studies are underway with other *C. botulinum* strains.

P3-34 Phenotypic and Genotypic Comparisons of *Campylobacter jejuni* Strains with Different Clinical Manifestations

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Introduction: Infection with *Campylobacter jejuni* produces two different diarrheal outcomes: a bloody, inflammatory diarrhea or a watery diarrhea. However, little is known about the underlying genetics, pathogenesis, or host factors involved in the clinical manifestation differences. The neonatal piglet model differentiates between these two diarrheal outcomes.

Purpose: In this study, we compared the genotypes and phenotypes of ten *C. jejuni* strains with defined and consistent diarrheal outcomes in the neonatal piglet model to better understand the factors associated with different *C. jejuni* diarrheal outcomes.

Methods: Core and accessory gene presence were explored in five strains associated with watery diarrhea, and five with bloody, inflammatory diarrhea, but no clinical manifestation specific genes were identified. SNP analysis identified 18 SNPs, in 12 genes, unique to a particular diarrheal outcome, of which seven SNPs were non-synonymous (from four genes). SNPs associated with bloody diarrhea were also identified in additional clinical isolates that clinically presented with bloody diarrhea, including a distinctive SNP combination in the RacRS system. Phenotypic assays including motility, cytolethal distending toxin (CDT) production, biofilm, growth curves, invasion/attachment to INT-407 and CaCo-2 BBE tissue culture cells at both 5% CO_2 and 10% CO_2 and macrophage survivability were investigated between the ten strains to determine if virulence differences existed between strains associated with a particular clinical manifestation. *Salmonella* Typhimurium and *Escherichia coli* str. K12 were used as controls where appropriate.

Results: Using a Student's t-test to compare between the two groups found statistically significant differences with invasion in both INT-407 cells (p value = 0.02387) and CaCo-2 BBE cells (p value = 0.004433), CDT production (p value = 0.02035), macrophage survivability after 24 hours (p value = 0.004971) and 72 hours (p value = 0.004887).

Significance: Differences in *C. jejuni* diarrheal outcome cannot be fully explained by differences in gene presence but appear to be a complicated system that could involve epistasis between linked SNPs, gene expression and the host immune system.

P3-35 Evaluation of Impact of Emulsion Matrix on Survival of *Salmonella* during Simulated Gastric Digestion

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❖ ***Developing Scientist Entrant***

Introduction: While *Salmonella enterica subsp. enterica* serovar Typhimurium's resistance to acid, osmotic stresses, and pathogenicity have been investigated over the years, there is the necessity to systematically study how food matrices impact *Salmonella*'s survivability in the digestive tract.
Purpose: The purpose of this study was to investigate the potential effect of emulsion structure on *Salmonella* survivability during gastric digestion.
Methods: Water-in-oil (W-O) emulsion and oil-in-water (O-W) emulsion were prepared with peanut oil and water with 10% dispersed phase and stabilized with 2% (w/v) soy lecithin for W-O emulsion or 3% (w/v) sunflower lecithin for O-W emulsion. Samples were inoculated with *Salmonella* in oil or water phase and equilibrated at room temperature (21 ± 2°C) overnight. The samples were challenged with simulated gastric fluid (SGF) (pH 2, 3g/L pepsin) facilitated with stomacher mixing at 37°C, and samples were taken periodically, serial diluted and plated on tryptic soy agar (TSA) to measure bacterial inactivation.
Results: With *Salmonella* in the dispersed phase (water) of the W-O emulsion, 2.4±0.41 log CFU/ml reduction was achieved after 120 minutes of SGF exposure. In contrast, a significantly ($P<0.05$) higher 5.08±0.44 log CFU/ml reduction was achieved when it was inoculated in the dispersed phase (oil) of the O-W emulsion. A similar trend persisted when *Salmonella* was inoculated in the continuous phases, in that higher reduction was observed in O-W emulsion than in W-O emulsion ($P<0.05$), indicating that protection offered by W-O emulsion to *Salmonella* inactivation was independent of its phase of inoculation.
Significance: W-O emulsion offers better protection to *Salmonella* against inactivation than O-W emulsion during simulated gastric digestion indicating food matrix can affect *Salmonella* survivability during digestion.

P3-36 Microbiology Studies in a Variety of Plant-Based Alternative Foods

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Introduction: Little is known about the microbiological impact and safety of plant-based foods and beverages.
Purpose: We review multiple plant-based product studies utilizing pathogen screening as well as enumeration and growth of spoilage bacteria in different product types.
Methods: Pathogen verification studies performed on a plant burger included 7 spiked samples (~10 CFU) and 1 uninoculated sample. Twenty-five gram samples were dual spiked with *Salmonella* Typhimurium and *E. coli* O157:H7, incubated 18 hours at 42°C and evaluated on GENE-UP SLM, ECO, EHEC, and PEC assays. Additional samples were spiked with *Listeria monocytogenes*, incubated for 20 hours at 35°C and evaluated on GENE-UP LMO. Additional *Salmonella* (375g) and *Listeria (*125g*)* pathogen studies were performed on Tofu and Soy Milk products. Studies included 20 low (~0.0-2.0 cfu), 5 high (~5.0 cfu), and 5 uninoculated samples.
Enumeration studies were performed comparing an automated enumeration method (TEMPO) and traditional plating for natural contamination of aerobic and lactic acid bacteria on plant-based meat finished and raw ingredients.
Challenge studies were performed on Oat, Almond, Coconut, Soy and Hazelnut drinks by spiking each container (46oz) with spoilage bacteria and monitoring growth at 32-35°C over a 5 or 7 day period.
Results: Pathogen studies on a plant burger indicated 100% alignment with culture confirmation. Tofu and soymilk studies showed no significant differences with CI of > 95% and POD values of 0.0 to - 0.05.
No statistically significant bias was observed between the automated enumeration method and traditional plating.
Spiking studies on plant-based beverages demonstrated *Bacillus, Staphylococcus and E.coli* growth within 2 days. However, Lactics, Yeast and a *Bacillus coagulans* strain required 3 days. Hazelnut displayed natural antimicrobial properties.
Significance: As the landscape of plant-based foods grows in need and complexity, we look to understand their microbial challenges to verify proper microbial control during production.

P3-37 Monitoring Real-Time Resuscitation of Sub-Lethally Injured or Dormant Cells of *Listeria monocytogenes* with Direct Time-Lapse Cell Imaging

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Introduction: Within genetically similar populations, sub-populations in different physiological states co-exist and may have enhanced survival capacity upon transition to a new environment. Sub-lethally injured or dormant cells of *Listeria monocytogenes* remain viable, with varying resuscitation capacity at single-cell level.
Purpose: (i) To monitor real-time resuscitation capacity of stressed *L. monocytogenes* single-cells with direct time-lapse cell imaging; (ii) to examine the variability in the division time of individual cells after exposure to different sub-lethal stresses related with food-processing; and (iii) to detect any non-growing fractions
Methods: Acetic acid (AA) and hydrochloric acid (HCl) (adjusted to pH2.5-3.0; 20°C for 5h) were used to induce sublethal injury of *L. monocytogenes*. Peroxy-acetic acid (PAA) (10, 20, 30 and 40 ppm; 20°C for 3 h) and sodium hypochlorite (400, 500, 600ppm; 20°C for 3 h) were used for the induction of the VBNC state. After stress exposure, resuscitation was monitored for 6 hours, on Tryptic Soy Agar supplemented with 0.6% Yeast Extract (TSAYE) with time-lapse microscopy at 37°C. Temperature changes were monitored by placing a K-type thermocouple on the surface of the microscope slide. Time-lapse images were analysed by a customized software for cell quantification and tracking along time.
Results: AA treated cells (pH3.0 and 2.7) formed colonies and "recovered" even with larger lag phases than the control group. Time-lapse observation on TSAYE showed no resuscitation after 3 hours of exposure to 40ppm, PAA. Snapshots after 18 hours on TSAYE showed variable resuscitation potential with micro-colonies and single cells in between with no growth. No resuscitation was evident after treatment with 600 ppm, SH.
Significance: This study provides new insights into the recovery of dormancy states of *L. monocytogenes* and offers a new approach for investigating single-cell resuscitation capacity of VBNC foodborne pathogens in food safety.
Acknowledgment: *This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement [No1788].*

P3-38 Thermal Inactivation Kinetics of *Escherichia coli* K12 in Watermelon, Cantaloupe, Blueberry, and Grapefruit Juices Determined by Aluminum Thermal-Death-Time Disks

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Introduction: Microbial contamination, and outbreaks of foodborne pathogens are still a concern in the fruit juice industry.

Poster

Purpose: This study was conducted to evaluate thermal inactivation kinetics of *Escherichia coli* K12 in watermelon, cantaloupe, blueberry, and grapefruit juices, using thermal-death-time (TDT) disks over the temperature range from 52 to 62 ℃.

Methods: *E. coli* (ATCC 25404), a nonpathogenic wild-type K12 strain, was used as a surrogate of *E. coli* O157:H7 for safety concerns. The washed pellet of 3, 9-mL stationary phase *E. coli* grown in TSB, were inoculated to 30 mL juice, and stored at 4 ℃ for 24 h to allow acid habituation to obtain cell population of ca. 7-8 log CFU/mL. Heat treatment conducted in water bath. At designated intervals, duplicate TDT disks were taken out and cooled in iced water bath. Appropriate dilutions were spread-plated in duplicate on tryptic soy agar containing 0.6% yeast extract and incubated at 37 ℃ for 24 h. Bacterial population reductions were calculated by subtracting the survivors (N) from the initial population (N_0). The mean D-values were obtained by three independent replicates.

Results: The D_{56}, D_{58}, D_{60}, and D_{62} values of *E. coli* K12 in watermelon juice were 6.73, 2.56, 1.21 and 0.64 min, and in cantaloupe juice were 4.47, 1.96, 0.88 and 0.38 min. In grapefruit juice, D_{52}, D_{54}, D_{56} and D_{58} values were 4.72, 1.71, 0.58 and 0.21 min, and in blueberry juice were 5.28, 2.43, 0.80 and 0.37 min. The z -values in watermelon, cantaloupe, blueberry, and grapefruit juices were 5.90, 5.67, 5.08, and 4.42 ℃, respectively.

Significance: The first-order inactivation kinetic model represented by D- and z-values are crucial in designing thermal pasteurization of fruit juices to achieve 5-log microbial population reduction of the target microorganism. This work is first to investigate the inactivation kinetics of *E. coli* K12 in mentioned fruit juices.

P3-39 Validation of 13 Enrichment Procedures for the Detection of *Listeria* Species from Environmental Samples Using the Hygiena™ BAX® System

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Introduction: Food processors collect four-times more samples throughout the production facility compared to product samples to ensure that the environment and equipment are not contributing to the risk of *Listeria* contamination in final products. The use of selective and differential enrichment media for the recovery of *Listeria* species from environmental surfaces combined with the use of molecular methods for detection can be costly for laboratories and food processors.

Purpose: The purpose of these studies were to validate multiple enrichment procedures for the detection of *Listeria* species from environmental surfaces using a PCR-based method.

Methods: Following the technical guidelines described in Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, a variety of environmental surfaces including plastic, stainless steel, epoxy and ceramic were inoculated with different *Listeria* species in 30 sample sub-sets. Thirteen separate enrichment protocols using 6 different medias, ranging from 40 mL – 225 mL, were evaluated in a paired or unpaired methodology against either the USDA FSIS MLG or FDA BAM reference methods. All samples were tested using BAX® System PCR methods and confirmed with the appropriate reference culture method.

Results: Each enrichment method evaluated returned consistent results between PCR and culture demonstrating 100% sensitivity and 100% specificity. When compared to the USDA FSIS and FDA BAM reference methods, the probability of detection (POD) indicated no significant differences.

Significance: The results from these studies provide 13 validated enrichment protocol options, coupled with the BAX® System Q7 PCR assays, to screen environmental samples for *Listeria* that support laboratory workflow efficiencies while being cost-effective.

P3-40 Compatibility of the Hygiena™ BAX® System and the 3M™ Environmental Scrub Sampler for the Detection of *Salmonella* and *Listeria* from Stainless Steel and Plastic Surfaces

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Introduction: There are many sample collection devices and transport broths available for environmental monitoring of foodborne pathogens. Choosing one that will neutralize a wide range of biocides and disinfectants used during sanitization without affecting pathogen growth and subsequent downstream detection methods must be considered for an effective environmental monitoring program.

Purpose: This study was designed to evaluate the compatibility of the BAX® System Real-Time and Standard PCR assays and the 3M™ Environmental Scrub Sampler with a Wide Spectrum Neutralizer for the detection of *Salmonella* and *Listeria* from environmental surfaces.

Methods: In two separate studies, stainless steel and plastic surfaces were inoculated with *Salmonella* Typhimurium or *Listeria monocytogenes,* respectively and a competitor organism. Each method under evaluation consisted of 20 low-level samples, 5 high-level samples, and 5 uninoculated controls. The inoculum was desiccated for 24 hours, collected by swabbing and held at room temperature for 2 hours. For each target analyte, half of the sponges were enriched according to the test method and analyzed using real-time and standard PCR, while the second half were enriched and confirmed according to the appropriate FDA BAM reference methods.

Results: All PCR results from the test method displayed no false positives or false negatives as results were in complete agreement with culture for each organism. Using the probability of detection (POD) to compare the test and reference methods, no significant difference was observed between low-spiked *Salmonella* or *Listeria*, or high-spiked Listeria. For high-spiked *Salmonella*, a significant difference was observed with the test method achieving a higher proportion of positives.

Significance: Results from both studies demonstrated statistically equivalent or superior performance of PCR compared to the appropriate reference method, validating the suitability of the BAX® System to be used with the scrub sampler hydrated with a wide spectrum neutralizer to accurately detect *Salmonella* and *Listeria.*

P3-41 A New Approach to Sampling Biofilms Using the 3M™ Environmental Scrub Sampler with 10mL Wide Spectrum Neutralizer (ESSWSN)

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Introduction: Formation and buildup of biofilms on surfaces within the food processing environment can lead to cross contamination and impact food safety and quality. Precise and accurate sampling of biofilms requires bacteria to be efficiently collected for the chosen detection method.

Purpose: Demonstrate the efficacy of ESSWSN of sampling three types of biofilm (wet, dry, and low density) generated under high shear on stainless steel coupons.

Methods: A high-shear biofilm was generated on stainless steel coupons in a CDC Biofilm Reactor® containing 300mg TSB/L inoculated with 1mL of *P. aeruginosa* (ATCC15442) (*P.a.*). Biofilms were grown at 21±7°C in batch conditions for 24h, followed by a continuous flow of 100mg TSB/L for an additional 24h. Two coupons were sampled using the ESSWSN directly after biofilm generation to simulate wet biofilm (WB). Dry biofilms (DB) were generated by drying the coupons for 2h; two coupons were sampled using the ESSWSN and two by scraping. Low-density biofilms (LDB) were generated using 10mg TSB/L inoculated with 1mL *P.a.*. Three coupons were sampled using the ESSWSN and two were sampled by scraping. All biofilm types were observed microscopically using confocal laser scanning microscopy while the LDB was also evaluated by enumeration.

Results: Collected images show the before and after appearance of WB, DB and LDB on stainless steel coupons following the incubation conditions and sampling with ESSWSN or by reference scraping method. Quantitative recovery enumeration of the LDB with ESSWSN (4.861 Log cfu/cm^2) showed no statistical differences when compared with the reference scraping method (4.350 Log cfu/cm^2) (P=0.644).

Significance: These data demonstrated that the scouring feature on the ESSWSN works as effectively as reference methods in the disruption and sampling of the biofilm buildup on stainless steel.

Poster

P3-42 Detection of *Salmonella* Typhimurium from Environmental Cellulose and Polyurethane Sponge Swab Rinsates as Compared to Direct Sponge Enrichment

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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 which could allow the detection of multiple pathogens from one sponge.
Purpose: To assess whether detection of *Salmonella* from cellulose and polyurethane sponge swab rinsates is equivalent to detection from direct sponge enrichments.
Methods: Cellulose sponges with DE neutralizing broth and polyurethane sponges with HiCap™ broth were inoculated with *S.* Typhimurium at 0.25CFU (n=10), 2.5CFU (n=10), 25CFU (n=5) and uninoculated (n=3); coinoculated with *Citrobacter freundii* (competitor) at 10X the pathogen inoculation level. Sponges were refrigerated 40h to simulate shipping conditions prior to enrichment.
Using an FDA BAM sponge procedure, lactose broth was added to one set of reference sponges (direct enrichment) and incubated. Fifty mL BPW was added to another sponge set and homogenized for 5 or 30 seconds manually or by stomacher. The rinsate from each was transferred to lactose broth and incubated. All incubated enrichments were streaked to XLD and chromogenic agars, and the plates examined for typical colonies.
Results: An unpaired statistical analysis compared rinsates to reference method sponges (Mantel-Haenszel Chi Square and relative sensitivity rate). A statistically significant difference was determined by Chi Square values of >3.84 between the reference method and the rinsate methods regardless of sponge type, homogenization time or procedure. At a 2.5CFU contamination level, a statistically lower recovery rate for rinsates was observed for 75% of the treatments.
Significance: Sponge rinsates result in a lower *Salmonella* recovery rate and are not comparable to a standard direct enrichment (FDA BAM) for the detection of *Salmonella* from environmental sponges.

P3-43 Detection of *Salmonella enterica* in Environmental Surface Samples Using the *Salmonella* Canary® Zephyr Assay

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Introduction: *Salmonella* can cause salmonellosis which is the second leading cause of foodborne illness in the U.S. The CANARY® Zephyr is an immuno-based biosensor platform that allows for rapid and sensitive detection of *Salmonella enterica* from environmental surface samples.
Purpose: The purpose of this study was to evaluate the performance of the new *Salmonella* assay for its inclusivity, exclusivity, and sensitivity.
Methods: Overnight cultures of *Salmonella enterica* strains (n=102, including six subspecies) were diluted to 100 times of the limit of detection (LOD) for testing (10^4 CFU/mL). Thirty-three non-target strains were cultured and tested without dilution. Matrix studies were conducted in various environmental surfaces including stainless steel, silicone rubber, HDPE, and glazed ceramic (1″ × 1″ test area). Each matrix was inoculated with diluted *Salmonella* cultures at a low-level expected to yield fractional positive results and a high-level expected to yield all positive results. Non-inoculated areas were included as negative controls. Environmental surfaces were sampled with swabs and enriched in 10 mL *Salmonella* Enrichment Media at 37°C for 24±2 hours. After enrichment, samples were prepared and tested following the assay user guide. Assay performance were compared to the FDA reference method and all presumptive results were culture confirmed following FDA guideline.
Results: All 102 *Salmonella enterica* isolates were correctly identified, and all 33 non-target strains were tested negative. All presumptive results were confirmed by culture. No statistically significant differences were observed between the candidate and the reference method for environmental surfaces by probability of detection (POD) analysis.
Significance: The new assay demonstrated equivalent performance to the FDA BAM reference method. It can be used as an alternative method for rapid and reliable detection of *Salmonella enterica* in environmental surface samples with a 5-minute sample preparation and instrument-reading time.

P3-44 Targeted Sequencing Improves Detection of *Salmonella* in Environmental Samples

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Introduction: Current methods of detecting *Salmonella* from environmental and food samples have limited sensitivity due to the low relative abundance of the target to background organisms.
Purpose: The purpose of this study was to evaluate targeted enrichment with RNA baits prior to sequencing as a means of increasing sensitivity of detection of *Salmonella* in environmental samples.
Methods: *Salmonella*-specific RNA baits (120nt) were designed against the 373 O and H antigen sequences in the SeqSero2 database and an additional four virulence genes (*inv*A, *mgtC*, *sopB*/*sigD*, and *spiC*/*ssaB*). Samples were prepared with 1%, 10%, or 50% of *Salmonella* serovars Mississippi ($10.3x10^8$ CFU/mL) or Midway ($5.3x10^8$CFU/mL) with and without a competitor, *Citrobacter braaki* ($5.5x10^8$ CFU/mL), spiked into a manure background. Pre- and post- hybridization DNA libraries were sequenced on an Illumina MiSeq. Data were analyzed with Kraken2 and Bracken to estimate abundances and a custom reference alignment-based bioinformatics pipeline for serovar identification.
Results: Unhybridized samples without a competitor (n=4) resulted in 32-80% of reads being assigned to *S. enterica* whereas the hybridized libraries of the same samples resulted in 98-99% of reads assigned to *S. enterica* with Kraken2 and Bracken. In the presence of a competitor (n=4), the percentage of reads assigned to *S. enterica* ranged from 5-24% for unhybridized and 90-94% for hybridized runs. SeqSero2 did not always correctly return the expected serotype for samples in the presence of a competitor but did correctly identify *S. enterica* Mississippi at 10% and 50% in hybridized samples. The custom pipeline accurately aligned reads from hybridized samples to expected antigen targets and serovars could be correctly predicted from all hybridized samples using the custom pipeline metrics.
Significance: This study shows proof of concept that targeted sequencing can increase the ability to accurately detect *Salmonella* in complex backgrounds.

P3-45 Comparison of Real-Time PCR, VIDAS LIS, and the FDA BAM Culture Method for Detecting *Listeria monocytogenes* on Stainless Steel Surfaces Co-Inoculated with *Enterococcus faecalis*

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (*Lm*) is frequently isolated from food processing environments including stainless steel surfaces that have other background flora. Establishing a rapid detection method is critical for *Lm* surveillance strategy in food safety.

Poster

Purpose: This study was designed to compare real-time PCR (qPCR), VIDAS LIS and the culture method in the FDA Bacteriological Analytical Manual (BAM) for the rapid detection of *Lm* in the presence of competitive microflora on stainless steel surfaces. Automatic and manual DNA extraction were compared for qPCR.

Methods: A low level (i.e., 1.7 log CFU/square) of *Lm* was artificially inoculated on stainless steel surfaces with a competing species, *Enterococcus faecalis*, at a level of 2.7 log CFU/square. The uninoculated surfaces and the surfaces inoculated with a high level of *Lm* and *E. faecalis* (i.e., 2.7 and 3.7 log CFU/square) were included as negative and positive controls, respectively. After 18-h of desiccation stress of the inocula, each surface was swabbed using a premoistened sponge, resulting a test portion. The test portions were then enriched following the FDA BAM enrichment scheme. For qPCR, the 48-h enriched cultures were subjected to two DNA extraction methods, MagMax/Kingfisher apparatus for automated extraction and QIAGEN Blood & Tissue kit for manual extraction. The qPCR was performed on the AB7500 FAST instrument. VIDAS LIS that detects *Listeria* antigen was performed on each enriched test portion.

Results: qPCR, VIDAS LIS and the FDA BAM cultural method all generated 14 positive results in 20 test portions, which met the fractional positive requirement in the validation guideline by FDA and AOAC International. There were no false positive and false negative results. No difference was observed between automated and manual DNA extraction methods.

Significance: Two rapid methods were compared and shown to be equivalent to the FDA BAM cultural method for detecting *Lm* on stainless steel surfaces co-inoculated with competitive microflora.

P3-46 The Impact of Organic Matter Type on Recovery of *Listeria monocytogenes* during Environmental Monitoring

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Introduction: Environmental monitoring (EM) programs are designed to prevent the transfer of pathogens between surfaces and food. However, limited knowledge exists regarding the impact of organic matter on microbial recovery during EM.

Purpose: To determine the impact of various organic matter types on recovery of *Listeria monocytogenes* during EM.

Methods: *Listeria monocytogenes* (10^9 CFU/mL) and a liquid organic matter (skim milk, whole milk, blended lettuce, or lettuce rinsate) were mixed. One milliliter of the *L. monocytogenes* and organic matter suspension was dispersed as droplets onto sterile stainless steel surfaces. Surfaces were held in an environmental chamber for 1 h at 30°C/85% relative humidity. After the exposure period, the surfaces were swabbed with a polyurethane foam sponge pre-moistened with 10 mL of 1×PBS. The sponge was then returned to the sample bag and processed in the stomacher for one minute at 230 rpm. Samples were serially diluted and plated onto selective agar. After incubation for 48 h at 35°C, CFUs were counted. Experiments were replicated and analyzed in duplicate.

Results: When comparing the types of organic matter, data (n=16) indicate that there is no significant difference between each organic matter type when analyzing using Tukey's Honest Significant Difference test. Under similar conditions, the log loss CFU/mL of *L. monocytogenes* during recovery was similar across each organic matter type with a range of 0.51 to 0.59 log loss CFU/mL

Significance: Our results suggest that the recovery of *L. monocytogenes* from environmental surfaces does not significantly differ between the organic matter type present on the surface. This is critical information needed to help understand and inform how to improve EM programs and the EM tools utilized in the food manufacturing industry.

P3-47 Direct BAX® Q7 PCR Confirmation of Presumptive Positive Results after Rapid Screening for *Listeria* with HygienaTM MicrosnapTM Surface Express *Listeria L. Mono* Glo Devices

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Introduction: MSX *Listeria L. mono* GLO is a rapid screening test for *Listeria* species differentiating *Listeria monocytogenes* with green fluorescence. Once the bioluminescence reaches a predetermined threshold, it is considered presumptive positive for *Listeria* species (between 12 and 24 hours) and the green fluorescence indicates *L. monocytogenes*. This non-destructive assay allows further confirmation testing to be conducted directly from the incubated devices.

Purpose: Determine if presumptive positive swab devices can be directly confirmed using the BAX® System Genus *Listeria* and *L. mono* kits?

Methods: 8 *Listeria* strains were cultured overnight in TSB at 37°C, then diluted in MRD to -4, -5 and -6. 10 µL of each dilution was added to the assay swab (five replicates), activated, and incubated at 37°C. Spike levels were enumerated using TSA spread plates. RLU readings were taken in an EnSURE™ Touch luminometer at 12 14, 16 and 22 hours. After each reading, an aliquot was taken for confirmation testing by PCR assays for Genus *Listeria* and *L. mono*.

Cultures used: *Listeria grayii*, *L. innocua, L. ivanovii, L. welshmeri, L. seeligeri, L. mono.*

Results: All Listeria presumptive positive devices were correctly confirmed by PCR testing regardless of the incubation timepoint. Broth in the testing devices does not appear to inhibit the PCR chemistry. Most *Listeria* were detected at 16 hours incubation; however, the CFU varies, ranging from 20-600 CFU. *L. seeligeri* was detected at 12, 14 and 16 hours (160, 16 and 2 CFU, respectively) in the swab assay. *L. innocua* was detected at 14 and 16 hours (790 and 79 CFU).

Significance: The *Listeria* PCR kits can be used to confirm presumptive positive swab devices in less than 20 hours. Detection occurs at 14 hours with high bacterial load (~10^3CFU). Lower load (~10^2 CFU) turns positive after 16 hours of incubation.

P3-48 Swabbing Efficiency of HygienaTM Listeria Swab (MSX *L.mono* GLO)

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Introduction: Swabbing is often used as a method to determine how many microbes are present on a surface as part of an environmental monitoring plan.

Purpose: To test the efficacy of swabs at removing bacteria dried onto food testing surfaces.

Methods: The following cultures were grown overnight, diluted in MRD. To each 4" x 4" surface, 100 µL was applied by dotting 10 x 10 µL volumes. Surfaces were allowed to dry overnight in a laminar flow hood.

The dry surfaces were swabbed, and from each swab bud 20 µL was removed and plated to estimate the total bacteria present.

The swabbed area was then overlaid with cooled non-selective agar and counted.

The original culture was counted using TSA to plate all dilutions.

Cultures used – *Listeria monocytogenes* (ATCC 7655, 51780, 19111), *E. faecalis* (ATCC 51299, 49533, 51742), *L. innocua* (ATCC 51742, 43547, 11288) and *L. ivanovii* (BAA 753, ATCC 49953)

Results: Survival from surface drying was estimated using the CFUs from the swab and adding the counts from the overlaid agar. This was divided by the overnight counts from each culture. The survivability was as follows: *L. mono* (0.74%, 0.93% and 0.21%) for *E. faecalis* (0.24%, 0.25% and 0.02%), for *L. innocua* (0.0006%, 0.01% and 0.04%) and *L. ivanovii* (0.03% and 0.006%)

The swabbing efficiency was estimated by dividing the CFU count from the swab with the sum of the overlaid agar count and the swab CFU.

Swabbing efficiency was as follows: *L. mono* (96.7%, 97.9% and 89.6%) for *E. faecalis* (95.9%, 95.1% and 91.8%), for *L. innocua* (97.2%, 90.9% and 85.7%) and *L. ivanovii* (94.6% and 94.4%)

Significance: The perception in the field is that a larger swab is a superior swab. However, for smaller, distinct or irregular surfaces smaller swabs can be very efficient if swabbing is done correctly following proper procedures.

P3-49 Relationship between HygienaTM Innovate RLU Detection and pH for All Data Using *Staphylococcus aureus* as an Example

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Introduction: *Staphylococcus aureus* and similar organisms are used in routine challenge testing of UHT and ESL products. The growth of these organisms is rapid but the production of acids during growth can lag behind the production of ATP.

Purpose: To demonstrate that ATP detection can be beneficial in detecting this organism both during challenge testing and routine testing versus the use of pH measurements.

Methods: 87 products over the last 12 months were spiked with *Staphylococcus aureus* at 2 different levels; the highest level at 100 CFU/mL and the lowest level at 0.1 CFU/mL. The incubations were all in-pack with product types broken down into the following groupings – dairy (42%), non-dairy alternatives (25%), soups (15%), sports drinks (15%) and high protein shakes (3%).

Packs were incubated at 32°C ±1°C and assayed every 24 hours for extractable ATP RLUs and pH measurements.

Results: Resultant RLU (Relative Light Units) measurements and pH measurements can be used to calculate the PoD% (probability of detection) when compared to standard plating confirmations run from each aliquot.

The PoD% for the ATP system and pH was as follows, at 24 hours 65% and 32%, at 48 hours 100% and 84% and for all other further incubation periods, results were 100% for both methods.

The mean RLU and pH measurements were as follows, at 24 hours 14,486 RLU and pH 7.2; at 48 hours, 37,415 RLUs and pH 6.66. The RLU level remained consistent through Day 7, with a RLU value of 49,107. The pH continued to drop on each day with the lowest pH being 5.93 on Day 7.

Significance: The use of ATP measurement is a very rapid, sensitive and extremely efficient method for detecting very low-level contamination levels compared to pH when a testing regime is implemented.

P3-50 Advantages of Rapid ATP Sterility Testing Using the HygienaTM Innovate System for Rapid Detection of *Geobacillus stearothermophilus* in Non-Dairy Milk Alternatives

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Introduction: Risks to UHT treated products and the very nutritious non-dairy milk alternatives is sporadic contamination with gram-positive thermotolerant spore-formers. The subsequent growth and senescence of these organisms needs to be considered when designing a rapid, ATP-based systems detection program.

Purpose: Demonstrate the rapid growth of *Geobacillus stearothermophilus* in non-dairy milk alternatives and best methods for rapid detection using ATP-based methods.

Methods: Wild-type *Geobacillus* was isolated from customer supplied non-dairy milk, this was confirmed to 99% ID using the Riboprinter system.

The bacteria were grown overnight and inoculated into 1 L product packs at <10 CFU per pack. Growth followed for 7 days at 55°C. Triplicate packs were incubated and the resultant RLUs measured. Plate confirmations were run at each incubation time alongside pH measurements.

The bacteria were also inoculated at each dilution from -1 through -9 into 100mL of milk decanted into sterile vials. These were then incubated at 55°C and assayed for RLU values at 4, 8, 12, 24 and 48 hours.

Results: The mean RLU for in-pack incubation is greatest at 24 hours at 7,001 RLUs. This value declines daily until the baseline RLU is reached. This baseline RLU would be considered negative but since the packs have gone through the growth cycle, this will be 100% spores with no vegetative cells.

The out-of-pack incubation shows that growth proceeds rapidly and significant RLUs are measurable above the baseline at 4 hours for higher dilutions (-1), (-2) and (-3) with the lower dilutions (-5), (-6) and (-7) being detected at 8 hours incubation time. Stationary phase was reached at 8 to 12 hours in this matrix.

Significance: ATP measurements for contamination monitoring of UHT products needs to consider the possibility of senescence in the product when measuring at high temperatures over several days' incubations.

P3-51 Manual or Automated: A Comparison of DNA Recovery Methods for Shiga Toxin-Producing *Escherichia coli* from Environmental Samples

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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, and air samples. This multi-matrix field sampling effort necessitated the assessment of high-throughput sample processes that yield DNA products compatible with PCR detection of STEC genes.

Purpose: Evaluate the recovery of STEC DNA from air, sediment, and water samples using manual extraction and an automated, spin-column based processor.

Methods: Two aliquots of 1-ml each were obtained from 135 samples enriched using the FDA BAM Chapter 4A method. DNA was manually extracted from one set of aliquots using the DNEasy Blood and Tissue kit following the manufacturer's protocol for Gram-negative bacteria. The eluates from sediment and water samples were additionally cleaned using the Zymo OneStep PCR inhibitor removal kit. DNA was extracted from the second set of aliquots using the DNEasy Blood and Tissue kit in the automated Qiacube Connect processor. DNA products were analyzed for *stx1*, *stx2*, and *wzy* genes using an ABI 7500 qPCR machine, according to BAM Chapter 4A method.

Results: Manual and automated DNA extraction methods were used to evaluate 135 samples. Negative results and same gene targets were identified in 79% of samples (41 air, 30 sediment, and 35 water, with an average C_t deviation of ±2.29, ±0.69 and ±1.76, respectively) using both extraction methods. In the 29 samples with mismatched results, at least one target was detected by the automated method (n=20) versus the manual extraction method (n=9). For these samples, C_t values were late (average 38.21).

Significance: The automated and manual extraction methods yielded comparable results in 79% of the samples. However, when mismatches occurred, gene targets were amplified 3x more often from DNA extracted with the automated rather than manual method. Qiacube Connect could be an alternative method for DNA extraction from various environmental samples.

P3-52 Head to Head Sensitivity Comparison of Four Commonly Used PCR Methods in Food Pathogen Testing

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Introduction: Comparisons of rapid foodborne-pathogen detection platforms usually involves overall method performance, which provides little information about the source of observed differences in method sensitivity. We hypothesized that the lysis and detection chemistry of each method should be roughly equivalent and that the primary driver of detection sensitivity is volume of sample delivered to the final molecular detection reaction.

Purpose: To compare the sensitivity of the post-enrichment lysis and detection steps of four commonly used molecular foodborne-pathogen detection methods.

Methods: *Salmonella Abaetetuba* was diluted to extinction in 2-fold steps in buffered peptone water (BPW), with dilutions tightly clustered around the theoretical limit of detection (LOD). For each method, four technical replicates (duplicate lysates with duplicate detection reactions) were prepared from each sample, and tested exactly as instructed by its manufacturer including data analysis. Statistical comparisons used the paired sample method described in AOAC "Appendix J".

Results: In the range of 180–2,900 CFU/mL, representing five dilutions (20 total tests/method), significant differences in method sensitivity were observed. The two qPCR methods returned 7/20 (BAX® System) and 13/20 (BAC*Gene*) positive results. Neither method detected all replicates at any of the five dilutions. The BAX end-point and 3M MDS methods performed significantly worse, returning 2/20 and 0/20 positive respectively. The lowest concentration at which all four technical replicates were detected was 26,000 CFU/mL for real-time BAX, 8,700 CFU/mL for BAC*Gene* and 78,000 CFU/mL for end-point BAX and 3M MDS.

Significance: The results showed significant differences in method performance. The observed differences did not support the volume to detection hypothesis. The rare strain of *Salmonella* tested may have been a more important determining factor in the overall results. Results with a more common strain of *Salmonella* will be instructive.

P3-53 Norovirus Capsid P Domain Detection Using a Real-Time OMPG Nanopore

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Introduction: Human noroviruses are the leading cause of foodborne illness globally. Numerous properties of norovirus make them difficult to control, including low infectious dose, stability, and diversity, making rapid detection and subtyping crucial. Nanopore outer membrane protein G(OmpG)-based sensing has numerous advantages, such as high sensitivity, tolerance of inhibitors, rapidity, portability, and the ability to subtype; however, it has not been utilized for microbial targets.

Purpose: The purpose of this study was to develop an OmpG nanopore technology for detection and subtyping of noroviruses.

Methods: Norovirus capsid protein was cloned and expressed in *E.coli* BL21. Peptide sequences that bind target were presented on OmpG in two different ways; tethering or sequence replacement of peptide in an OmpG loop. Initial work resulted in detectable signal of norovirus target; however, signal needed to be improved for more repeatable and sensitive signal. Thus, optimization of bait peptide location in OmpG was performed using a FLAG tag and 3 anti-FLAG antibodies.

Results: The electrical current of peptide-tethered OmpG after adding target norovirus protein exhibited a 20pA drop in signal, showing the potential of OmpG to detect norovirus, however aforementioned optimization was needed to increase sensitivity and subtyping availability. We thus studied the binding motif presentation in OmpG with a FLAG tag. As a result, sequence replacement in $OmpG^{Q222FLAG}$ not only detected multiple target antibodies, but also was capable of discriminating different isotypes of monoclonal antibodies, and different isotypes of antibodies in a polyclonal antibody, each with characteristic signal at the tested antibody concentration of 17nM. Based on this study, further work to clone an anti-norovirus peptide into the Q222 position for optimized detection of norovirus is being performed.

Significance: This work reports the first successful detection of norovirus by OmpG nanopores, and further optimization demonstrates the potential of the OmpG nanopore sensing to rapidly, sensitively, and portably detect target protein, as well as sensitively discriminate even closely related proteins and mixtures thereof.

P3-54 Evaluating the Potential of Magnetic Ionic Liquids to Capture Non-Enveloped Virus Versus Viral RNA for One-Tube Capture, Concentration, and Genomic Extraction

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❖ ***Developing Scientist Entrant***

Introduction: Magnetic ionic liquids (MILs) are a class of hydrophobic solvents that are effective as capture reagents for foodborne bacterial pathogens, though data on viruses is limited. Since they are nonspecific, it is important to evaluate their binding affinity for intact viral particles versus free nucleic acid, especially when used with endpoint detection methods such as PCR.

Purpose: In this study, we optimized and compared the capture and purification of bacteriophage MS2, a human norovirus surrogate, versus free MS2 RNA using MILs.

Methods: MS2 or purified RNA was diluted into 1X PBS (pH 7.4) to 10^5 PFU/mL or copies/mL, respectively, and extracted using cobalt-, manganese-, or nickel-based MILs. MIL was then added to the suspension, vortexed and separated using a magnet. Supernatant was removed and samples were washed to remove unbound MS2 and/or free RNA. Captured MS2 or RNA was eluted by vortexing with modified LB broth, then RNA was purified and quantified by RT-qPCR.

Results: MILs with similar cationic structure and different metal anions showed similar recovery efficiency for MS2 (3.9±0.99%-7.4±4.4%). Each showed higher recovery for purified RNA (8.1±0.99%-23.7±5.3%), though the difference was only significant ($p>0.05$) for Co-based MILs. Higher MIL volume (30 µL) did not improve recovery and increased time required for separation by ~10 min. Optimal MIL volume was determined to be 7.5-15 µL, with a 10-15 min separation time. Further research is needed on effects of suspension pH and use of surfactants to increase MIL dispersion and target binding.

Significance: MILs demonstrated favorable affinity for both intact virus and free RNA, and could potentially be optimized for recovery of either target. If combined with a wash and lysis step, this could facilitate development of a one-tube viral concentration and RNA purification method without the need for heavy instrumentation, which would inform future work on portable sample preparation for in-field pathogen detection.

P3-55 Detectability of Novel *Listeria* Strains Using Alternative PCR and Chromogenic Methods

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Introduction: New *Listeria* species continue to be discovered over the years and their detection and characterization by classical and alternative methods currently available for food testing can be called in question.

Purpose: The study evaluates the detectability of new *Listeria* species and sub-species, discovered by Carlin et al in 2021, by alternative molecular and cultural methods proposed for *Listeria* detection in food: iQ-Check *Listeria* spp. kit, AL, Palcam, RAPID'*L.mono* and RAPID'*Listeria spp.*

Methods: Six *Listeria* strains were ordered from BCCM collection (LMG31917, LMG31919, LMG31918, LMG31920, LMG31921, LMG31922). Inoculum <20 CFU were spiked in 225 ml of LSB and incubated at 30°C for 24 hr. After growth, cultures were streaked on chromogenic media and incubated 24 hr and 48 hr at 37°C. In parallel, cultures were diluted up to ~10^3 CFU/ml and tested using PCR method for *Listeria* spp. with Easy II extraction.

Results: All strains are detected by the molecular method at a level of ~10^3 CFU/ml at the end of the enrichment. While most of the species and sub species (4 out of 6) grow on chromogenic media after 24 hr incubation with typical morphology, the 2 species classified in the sensu lato clade (*L. portnoyi* and *L. rustica*) showed absence or low growth at 37°C. Additional testing demonstrated growth on chromogenic media when incubated at 30°C, confirming their sensitivity to high temperature (as mentioned in Carlin et al. publication) but not to media formulation.

Significance: The results of this study confirmed that the alternative methods tested can detect all Listeria species and sub-species discovered by Carlin et al. Temperature sensitive species can be detected at 30°C when using cultural methods.

P3-56 Suretect *Listeria monocytogenes* PCR Assay and Suretect *Listeria* Species PCR Assay Awarded AOAC Official Methods of Analysis First Action

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Introduction: *Listeria monocytogenes* is a major foodborne pathogen, therefore detecting *Listeria* in food and environmental surfaces is crucial to helping to prevent infections. The Thermo Scientific™ SureTect™ Listeria species PCR Assay and the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay (candidate assays) enable rapid detection of *Listeria* species and *Listeria monocytogenes* respectively, in a broad range of foods and environmental surfaces.

Purpose: To demonstrate the accuracy and reproducibility of the candidate Assays and gain AOAC Official Methods of Analysis (OMA) First Action.

Methods: An unpaired interlaboratory study was performed according to AOAC Appendix J, comparing both candidate Assays to the United States Food and Drug Administration/ Bacteriological Analytical Manual (FDA/BAM) Chapter 10 Listeria reference method. Eighteen participants from 10 laboratories located within the US and Europe were solicited for the study, with 12 participants submitting valid data. The matrix selected was full-fat cottage cheese (25 g). Statistical analysis was conducted according to the Probability of Detection (POD) statistical model.

Results: The difference in POD results indicate equivalence between the candidate Assays and reference method. The candidate Assays demonstrated acceptable inter-laboratory reproducibility as determined in the collaborative evaluation.

Significance: The two candidate Assays demonstrated they are accurate, reliable and reproducible methods for the detection of *Listeria* species, or *Listeria monocytogenes* specifically. Both Assays were awarded AOAC OMA First Action.

P3-57 Validation of a Real-Time PCR Workflow for the Detection of *Staphylococcus aureus* in Dairy Matrices for AOAC PTM Approval

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Introduction: There are approximately 240,000 cases of *Staphylococcal* food-poisoning reported annually in the United States. A major zoonotic source of the pathogen is the dairy industry, with *Staphylococcus aureus* the most significant etiological agent of mastitis infection in dairy cattle. Rapid detection of *S. aureus* is of marked importance in protecting public health and preventing disease burden.

Purpose: To evaluate the Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay (candidate method) for detection of *S. aureus* in dairy matrices according to the AOAC® Performance Tested Methods℠ program.

Methods: A matrix study consisting of 100 g powdered infant formula (with probiotics), 100 g whole milk powder, 100 g whey protein concentrate, 100 g edam cheese and 100 g mozzarella cheese was conducted according to AOAC Appendix J requirements. The candidate method was compared against the ISO 6888-3:2003 reference method and the FDA BAM Chapter 12 reference method which was modified to a detection principle. Product stability, robustness and inclusivity/exclusivity studies were also completed according to Appendix J guidelines.

Results: Matrix studies showed no statistically significant differences between the candidate and reference methods through probability of detection (POD), with all confidence intervals containing '0.00' as stipulated in Appendix J. Robustness studies showed no significant difference in assay performance after set parameter deviations through POD analysis (intervals contained 0.00), and product consistency and stability studies showed consistent performance across the assay stipulated shelf life (POD analysis intervals contained 0.00). The inclusivity/exclusivity study correctly detected/excluded all strains analyzed.

Significance: The data shows that the candidate method constitutes a rapid and reliable alternative workflow for the detection of *S. aureus* from dairy matrices, with results obtained in as little as 80 minutes post enrichment. This is the first AOAC PTM approved singleplex PCR assay for *S. aureus* detection, which will support the dairy industry.

P3-58 Rapid Detection of *Listeria monocytogenes* Using an Oligonucleotide-Based Flow-Through Electrochemical System

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Introduction: The inability of many nucleic acid-based detection methods to handle large sample volumes limits their capacity to accurately detect low-abundance organisms without enrichment.

Purpose: A flow-through detection platform capable of examining large sample volumes was adapted for the detection of nucleic acid sequences.

Methods: The platform consisted of a transducer made of graphite felt, which served as both the capture surface and working electrode within the electroanalytical system. Utilization of this porous, electrically conductive material allowed for the passage of large volume samples while providing a large surface area that, when conjugated with neutravidin, supported the attachment of biotinylated oligonucleotides and permitted the binding/capture of complementary sequences from the target organism. A second oligo, composed of a DNA sequence adjacent to that of the biotinylated oligonucleotide and crosslinked with horse-radish peroxidase, was employed to detect the captured sequences. In the presence of substrate (3,3',5,5'-Tetramethylbenzidine), the reaction product was detectable and *Listeria monocytogenes* could be differentiated from *L. innocua* using this electrochemical system.

Results: Initial investigations with the flow-through electrochemical biosensor found that when blocked with a mixture of bovine serum albumin and sheared salmon-sperm DNA, the sensor could distinguish *L. monocytogenes* from *L. innocua*. Total assay time was <2.5 h and the limit of detection (LOD; differentiated from blank using Student's t-test, $p<0.0076$) was ~2 × 10e4 cells/mL, which was significantly different than the LOD (~2 × 10e6 cells/mL) for an equivalent assay that measured the response optically instead of electrochemically.

Significance: A novel adaptation to a previously developed biosensor platform capable of detecting low quantities of bacteria in large sample volumes has allowed for the genetic identification of *L. monocytogenes* via oligonucleotides. This adaptation not only expands the detection capabilities of the flow-through electrochemical biosensor to other foodborne pathogens for which effective but relatively inexpensive antibodies are not currently available but also enhances other aspects such as the ability to be easily modified and storage stability.

P3-59 An Impedimetric Method of *Listeria monocytogenes* Detection for Food Safety Applications

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a prevalent foodborne bacterium that has been linked to several recent outbreaks of foodborne illness. Molecular-based diagnostic methods are widely used for *Listeria* contamination detection in food products. So far, no established inexpensive and rapid diagnosis methods are available. We present a bacteriophage-based electrochemical biosensor for selective identification and quantification using impedimetric detection.

Purpose: We have developed a charge-directed immobilization method for aligning phage particles as bio receptors on the electrode surface for selective detection of *L. monocytogenes* using electrochemical impedance spectroscopy (EIS).

Methods: A phage-based electrochemical biosensor was developed to detect *L. monocytogenes* Scott A using commercially available Listex phage (P100 phage) as a bioreceptor biomolecule. Phages were immobilized onto a functionalized multiwall carbon nanotube using an electric field-induced, charge-directed orientation strategy. The sensitivity of the biosensor was evaluated using different concentrations of *L. monocytogenes* Scott A (10^1-10^8 CFU/mL). In addition, the specificity of the biosensor was evaluated using non-target analytes *E. coli* O157:H7 and *Salmonella enterica* subsp. *enterica* serotype Typhimurium 291RH. The stability of the biosensor was evaluated by storing the phage immobilized electrode at 4 °C in 1X phosphate-buffered saline (pH 7.4). Lysogeny broth medium was used to culture *E. coli* O157:H7. Brain Heart Infusion Broth medium was used to culture *L. monocytogenes* and ser. Typhimurium-291RH.

Results: The biosensor, under optimal conditions, gave a linear response from 10 to 10^4 CFU/mL, with good reproducibility. Further, the sensor showed a detection limit of 8.4 CFU/mL. The biosensor exhibits good specificity for *L. monocytogenes* Scott A, but more work needs to be done on non-selective binding between the electrode and non-target analytes. The developed biosensor was found to be stable for up to one week.

Significance: We have developed a novel ultra-sensitive biosensor to detect *L. monocytogenes*. The developed biosensor exhibited a low detection limit, and this capability makes it a competitive tool for detecting foodborne pathogens.

P3-60 Evaluation of Multiplex Nanopore Sequencing for *Salmonella* Serotype Prediction and Antimicrobial Resistance Gene and Virulence Gene Identification

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Introduction: In our previous study, multiplex-nanopore-sequencing based whole genome sequencing (WGS) method for *in silico* serotype prediction of *Salmonella* isolates has been evaluated. Both SISTR and SeqSero2 results indicated that accurate serotype prediction can be achieved within one day.

Purpose: Since only ten serotypes were tested in our previous study, the above conclusions were yet to be validated in a larger scale of test. Moreover, we would like to explore the feasibility of this method on antimicrobial resistance (AMR) gene and virulence gene identification.

Methods: In current study, we validated this method with 69 *Salmonella* serotypes and identified AMR/virulence genes with Abricate and RGI for both Nanopore-multiplex-sequencing data and Illumina sequencing data.

Results: We found that accurate serotype prediction with multiplex-nanopore-WGS data was achieved within about five hours of sequencing at a minimum of 30 × *Salmonella* genome coverage per isolate with SeqSero2 as the prediction tool. The total number and profiling of AMR/virulence genes of the 69 Salmonella serotypes were identical between using Illumina and Nanopore data, with Abricate as the identifier, while small variations were observed per isolate between the gene numbers and profiles from the two sequencing platforms. Taking results generated by using Illumina data as the benchmark for AMR and virulence gene identification, the average false negative probability per isolate was 1.37% for AMR genes, and 1.18% for virulence genes when using Abricate. Meanwhile, we found that RGI identified resistance genes with nanopore data at a much lower accuracy compared to using Abricate, possibly attributed to RGI's less stringent minimum similarity and coverage by default for database matching.

Significance: This study is a starting point for future validation and verification of multiplex-nanopore-sequencing based WGS as a cost-efficient and rapid *Salmonella* classification and resistance/virulence gene profiling tool for the food industry, and paved the way for its application in surveillance, tracking, and risk assessment of *Salmonella* across the food supply chain.

P3-61 Magnetic Extraction and Detection of *Salmonella* typhimurium

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❖ ***Developing Scientist Entrant***

Introduction: Non-typhoidal Salmonella enterica has been identified as the third leading cause of foodborne illnesses globally, leading to approximately 78 million illnesses and 59,000 deaths per year. In the US, is also one of the most common foodborne pathogens and annually causes around 1.35 million infections, 26,500 hospitalizations with 420 deaths, resulting in approximately $400 million in direct medical cost. Many salmonella infections are associated with contaminated food products or from contact with feces from infected people or animals. Appropriate treatment and prevention of *Salmonella* infection are bases on diagnostic methods. Many current diagnostic technologies are effective, but they are costly, time-consuming, and need a pure culture. There has been continuing efforts to develop to rapid concentration and detection of the bacteria. Recently, magnetic nanoparticles (MNPs) are gaining much attention as effective agents to rapidly concentrate pathogens.

Purpose: The purpose of this study is to use glycan-coated magnetic nanoparticles (GMNPs) to concentrate bacteria from food matrices and followed by a biosensing technique.

Methods: After successfully concentrating the MNP- bacteria complex, detection of the pathogens is conducted by using a colorimetric DNA biosensor. The specific probe was designed for Salmonella and functionalized gold nanoparticles (GNP) were used. Once Salmonella is detected, the red color of GNP remains stable. Absorbance spectrum of the negative control, target and non-target samples were also confirmed the detection.

Results: In preliminary trials, the glycan coated MNPs can highly concentrate the bacteria in 5-10 min from water and food matrices and the biosensor is able to detect the pathogen in 30-40 min. The detection limit of DNA pathogen is lower than 10 ng/ml.

Significance: This study shows that the magnetic extraction and detection can be used to quickly identify pathogens compared to the traditional method which takes 18- 24 h. Detailed results and discussion will be presented.

P3-62 Transition from Relative (qPCR) to Absolute (dPCR) Quantification and the Potential Food Safety Applications

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Introduction: Digital PCR (dPCR) is a novel technology used for quantification of microorganisms and vastly differs from traditional quantitative, (qPCR). In qPCR, genomic targets are quantified per reaction while dPCR separates sample DNA into thousands of micro-partitions, allowing for attributing amplification of target(s) to a specific molecule.

Purpose: The objective of this study was to evaluate the COVID-19 infrastructure and dPCR technology for food safety research applications via detection of multiple genomic targets, comparing the advantages and limitations, data generated, and quantified results of dPCR to qPCR.

Methods: Wastewater samples (n=24) were collected (50ml) in November 2021 from treatment facilities (New Castle County, Delaware) and processed using modified CDC's National Wastewater Surveillance System methods including incubation, filtration, centrifugal-ultrafiltration, RNA extraction, and quantification. SARS-CoV-2 (N1 and N2 targets) and pepper mild mottle virus (molecular control) detection were performed. Data output from qPCR and dPCR were compared qualitatively.

Results: dPCR data are presented as a reading of positive partitions, copies/µl and confidence intervals; while qPCR data are reported as copies/µl or cycle threshold values per sample and often associated with a standard curve. Thresholds differentiating background from amplified fluorescence varied by well (~18-44RFU) in dPCR, while qPCR had one threshold (0.05NFU) for all wells/assay. The dPCR 2-D scatterplots were shown as -/+, +/-, +/+, and -/- for N1/N2 detection, while qPCR data were totaled per well. These scatterplots display the partitions potentially containing multiple targeted sequences within a single genomic (DNA) molecule.

Significance: These findings suggest that dPCR will be significantly impactful in detection of foodborne illness causing microorganisms by providing absolute quantification that can be compared across laboratories and countries when using standardized methods. Detection of multiple segments within microorganisms' genomes and scatterplot analyses will allow for genetically similar organisms (*e.g., Cyclospora* and *Eimeria*) to be differentiated and antibiotic-resistance genes to be attributed to specific bacteria.

P3-63 Evaluating, among Food Workers, the Impacts of Vaccination, Testing, and Non-Pharmaceutical Interventions on SARS-CoV-2 Transmission: A Novel Integrated QMRA-IDT Modeling Approach

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❖ ***Developing Scientist Entrant***

Introduction: The SARS-CoV-2 pandemic poses heightened infection risk to essential workers in the food supply chain whose jobs may preclude infection control measures (physical distancing).

Purpose: To evaluate, among essential food workers in food processing facilities, vaccination, and non-pharmaceutical intervention (NPI) risk reduction strategies for controlling SARS-CoV-2 transmission.

Methods: This model combined an infectious disease transmission (IDT) model with a quantitative microbial risk assessment (QMRA). The model leveraged the QMRA framework to simulate SARS-COV-2 food worker exposure risk (8h-shift) in an indoor food processing facility and the IDT model to translate this risk to facility-wide transmission. The QMRA model consisted of distance-mediated transmission modes (aerosol, droplet, fomites) and incorporated NPIs aimed at reducing exposure (physical distancing, masking). Integrating the QMRA with the IDT model, we simulated the impact of these NPIs in addition to vaccination (low to high coverage) and testing (daily, biweekly, weekly frequency) on SARS-CoV-2 outbreak size and probability at a 50-person food facility. All scenarios were evaluated under the assumption of good manufacturing practices (surface disinfection, handwashing).

Results: High vaccination coverage plus masking of all food workers or daily testing of all unvaccinated workers were the most effective strategies at preventing a COVID-19 outbreak within the food facility. With vaccination alone, coverage above 85% was necessary to prevent outbreaks. Vaccination combined with masking had a synergistic effect, with enhanced impact of each intervention. With more effective masks (double masking, N95s), vaccination coverage of 70% or above was sufficient to prevent COVID-19 outbreaks. With testing alone and lower vaccination coverage (<50%), daily testing was necessary to prevent outbreaks. Testing frequency could be reduced with higher degrees of vaccination coverage or with the addition of effective masking.

Significance: This novel QMRA-IDT approach found that essential food worker populations can be effectively protected from COVID-19 outbreaks with masking combined with higher vaccination coverage or higher testing frequency.

P3-64 Evaluation of the Performance of Different *Listeria* Enrichment Broths Using Bioscreen Automated System

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Introduction: *Listeria* monocytogens is an important foodborne pathogen. The recovery of low numbers of *Listeria monocytogenes* from foods and environmental samples requires the use of pre-enrichment/enrichment step. A variety of enrichment media and methods have been developed for the detection of *Listeria* spp. and *L. monocytogenes*. The Bioscreen system can automatically perform microbiological growth curve tests and, thus, is a useful tool to evaluate the growth media performance.

Purpose: Use Bioscreen automated system to assess the most commonly used *Listeria* enrichment broth media to grow *Listeria* and suppress closely related non-*Listeria* organisms.

Methods: Seven *Listeria* species and six non-*Listeria* bacterial strains were tested with eight commonly employed *Listeria* selective enrichment broths and one non-selective medium. The lag phase duration times in the growth curves were calculated to evaluate the performance of each medium.

Results: For *Listeria* cultures, LESS Plus, LSB and LX are growth permissive, had similar lag times (12.1 – 14.5 hr) to non-selective medium TSB (12.5 hr). 24LEB, LEB and BLEB showed medium range lag times (17.8 – 19.2 hr). The longest lag times (21.3 – 24.1 hr) were found in UVM, Demi-Fraser. *L. grayi* ATCC 25400 didn't grow in UVM and Demi-Fraser, and its growth was also delayed in BLEB medium. For non-*Listeria* cultures, LESS Plus suppressed the growth of all six exclusive organisms, LSB and 24LEB suppressed two, UVM, LX and Demi-Fraser inhibited one of them. *Listeria* selective broth BLEB and LEB and non-selective medium TSB supported the growth of all six exclusives.

Significance: Among all eight selective *Listeria* enrichment broths tested, LESS Plus is the best medium which supports the growth of *Listeria* species, and selectively inhibits the competitive organisms. Our results show that LESS Plus medium is a superior option for enrichment and detection of *Listeria* species including *L. monocytogenes* relative to the other *Listeria* enrichment broths tested.

P3-65 Evaluation of the Pathfinder™ *Listeria* Broth Method for the Detection of *Listeria monocytogenes* and *Listeria ivanovii*

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Introduction: PathFinder™ Listeria broth is a selective and differential culture media to detect and confirm the presence of *L. monocytogenes* and *L. ivanovii* from surfaces with the use of a darkening indicator (esculin reaction) and a green fluorescent indicator.

Purpose: The purpose of this analytical study was to evaluate the performance of the broth when challenged with a low concentration of target (inclusivity) organisms and a high concentration of non-target (exclusivity) organisms.

Methods: *L. monocytogenes* and *L. ivanovii* (n = 46) were tested at $1.5x10^3$ CFU/mL and non-target organisms (n = 30) were tested at $1.5x10^8$ CFU/mL in the broth media. All inoculated broth tubes were incubated at 35°C for 48 hours. Tubes were examined for esculin hydrolysis and fluorescence at 30 and 48 hours of incubation. Only samples with both esculin hydrolysis and green fluorescence were considered a positive result. All organisms evaluated were blind coded after the organism suspensions were prepared in order to avoid biased result interpretation.

Results: 46/46 (100%) of *L. monocytogenes* and *L. ivanovii* were detected after 48 hours of incubation. Of the 30 non-target strains, one *L. seeligeri* strain tested yielded a false positive. *L. seeligeri* is a closely related species to *L. monocytogenes* and *L. ivanovii.* This cross-reactive result was likely due to the high inoculum tested. At a lower concentration, this organism appears inhibited by the media.

Significance: This broth is an accurate method for the detection of *L. monocytogenes* and *L. ivanovii* at low concentrations and will be further validated for environmental surface testing in the near future.

P3-66 Assessment of Multi-Toxin Detection with Myco 7 Array on the Evidence Investigator Analyser According to the Association of American Feed Control Officials Method Performance Criteria

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Introduction: The use of reliable multi-analytical methods is beneficial for testing laboratories and is particularly indicated for the detection of mycotoxins as the occurrence of mycotoxins is widespread throughout the world in different cereals and feed types. Cereals may present single or multiple mycotoxin contamination and multicomponent feed -a mix of various cereals with additives- also contributes to the co-occurrence of mycotoxins. The current

non-uniformity of methods and corresponding coefficient of variation of Proficiency Test (PT) scheme results are raising concerns over sample analysis to Association of American Feed Control Officials (AAFCO).

Purpose: The aim of this study was to assess the precision and reliability of multi-mycotoxin, semi-quantitative Myco 7 Array according to AAFCO Method Performance Criteria, across nine AAFCO feed testing laboratories following ISO5725, 1994 and IUPAC: Horwitz, 1995.

Methods: For the simultaneous detection of predominant mycotoxins, the reported biochip array was used and applied to the biochip analyser Evidence Investigator. The system enabled the analysis of up to 54 biochips at a time. Mycotoxins were extracted from feed by a single generic liquid/liquid extraction. Sixteen PT samples were assessed: 15 from AAFCO, 1 from Food Analysis Performance Assessment Scheme (FAPAS) as well as 1 control material (Randox Food Diagnostics). Reproducibility assessment: Z-scores (-2 ≤ Z ≤ +2) and HorRat values (0.3<HorRat≤2).

Results: The Z-score pass was as follows: 100% (Deoxynivalenol), 99% (Fumonisins, Ochratoxin A, Aflatoxins G1, B1 and Zearalenone) and 97% (T-2/HT-2 toxins). The HorRat values were within the acceptable ranges for all 17 samples (Deoxynivalenol and T-2/HT-2 toxins), 16 samples (Fumonisins, Aflatoxin B1 and Zearalenone) and 15 samples (Ochratoxin A and Aflatoxin G1).

Significance: Data generated under the study conditions, indicates that this biochip array met the performance criteria and therefore is *fit-for-purpose* for use in the official feed control program for mycotoxins.

P3-67 New Convenient Medium for the Simultaneous Recovery and Rapid Detection of *Salmonella* and *Cronobacter sakazakii* in Powdered Infant Nutritional Products

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Introduction: Strong desiccation tolerance is an outstanding feature of both *Salmonella* and *Cronobacter sakazakii* that enables their long-term survival in powdered infant nutritional products. Despite many technological and food safety improvements, the industrial manufacturing of powdered infant formula (PIF) still needs to acquire and implement a reliable detection of these bacteria so as to not jeopardize the health of infants. A method allowing for the simultaneous recovery of both bacteria would offer up considerable advantages for reducing the overall cost of testing, time, and labor requirements.

Purpose: The objective of this study was to develop a new enrichment medium enabling the simultaneous recovery of *Salmonella* and *C. sakazakii* followed by rapid PCR-based detection.

Methods: Optimizing a one-step enrichment, 200 PIF and baby cereal samples were contaminated with 0.5-1.0 MPN/sample of *S.* Agona and *C. sakazakii* and stabilized for two weeks. Each 100 g sample was incubated with 700-900 mL of Actero Multiplex medium at 35°C for 14-18h, and then analyzed using different PCR-based assays. For the inclusivity study, 30 *Salmonella* and 20 *C. sakazakii* strains were examined for growth while 20 non-target strains were tested to determine the selectivity of the medium. The alternative method was validated using 80 fractionally inoculated PIF samples in comparison with ISO 6579-1:2017/ISO 22964:2017.

Results: As short as 16h of enrichment with Actero Multiplex medium allowed for the PCR-based detection of both target bacteria that was confirmed by direct plating. All the target strains were grown-up while the non-target bacteria were completely or partially inhibited. The Probability of Detection confirmed the equivalent performance of the alternative method *vs* the reference methods. No false positives and one false negative were detected.

Significance: A one step-enrichment using the new medium enabled the simultaneous recovery of *Salmonella* and *C. sakazakii* in powdered infant nutritional products for as short as 16h followed by PCR-based detection.

P3-68 Detrimental Effects of Adding 0.5% K2SO3 to *Salmonella* Enrichments Containing Onion Powder

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Introduction: Per the FDA BAM, Chapter 5, Section C. 10, onion powder should be pre-enriched in a non-selective broth with added K2SO3 (final concentration of 0.5% K2SO3) in order to neutralize the inhibitory effects of onion on *Salmonella* growth. The use of K2SO3 is often extended to matrices containing onion powder in addition to other spices. However, for some matrices, such as Mexican Chili Powder, enriching in BPW with 0.5% K2SO3 can be detrimental to *Salmonella* recovery.

Purpose: The purpose of this study was to demonstrate that it is not prudent to use 0.5% K2SO3 for onion powder when it is mixed with certain other spices.

Methods: Limit of Detection (LOD_{50}) studies were performed on two onion mix samples: 64% onion powder + 36% paprika, and 64% onion powder + 36% black pepper. Per ISO 161410-2:2016, LOD_{50} determinations were made by weighing 24 x 25g samples of each matrix. The 24 samples were divided into four spike levels, ranging from ~ 0.5 cfu to 4 *Salmonella* cfu. Each matrix was tested in both BPW and BPW + 0.5% K2SO3. Both PCR and ELFA platforms were utilized. An LOD_{50} was calculated using the Wilrich and Wilrich (2009) spreadsheet. An LOD_{50} of ≤ 1 cfu/sample is desirable.

Results: The onion/paprika mix gave an LOD_{50} of 1.8 cfu/25g when enriched in BPW, but *Salmonella* was not detected in any of the 24 test samples when enriched in BPW + 0.5% K2SO3. Similarly, the onion/black pepper mix gave an LOD_{50} of 0.42 when enriched in BPW but was not detected in BPW + O.5% K2SO3.

Significance: 0.5% K2SO3 should not be used indiscriminately to neutralize the inhibitory effects of onion powder on *Salmonella* recovery.

P3-69 Independent AOAC Validation Study of the Detectx Combined Assay for the Detection of *Aspergillus*, *Salmonella*, and STEC (*stx*1 and/or 2) in Dried Cannabis Flower and Dried Hemp Flower

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Introduction: The PathogenDx Detect^{x} Combined assay uses microarray technology to simultaneously detect the presence of bacterial and fungal pathogens in cannabis and/or cannabidiol (CBD) products.

Purpose: The validation study evaluated the PathogenDx Detect^{x} Combined assay for the detection of *Aspergillus, Salmonella,* STEC (*stx*1 and/or 2) species. The validation consisted of two matrix studies in dried hemp flower and dried cannabis flower (≥ 0.3% delta-9 tetrahydrocannabinol) , product consistency, stability, robustness and inclusivity and exclusivity for two targets: *Aspergillus* and STEC.

Methods: The PathogenDx Detect^{x} Combined assay was evaluated per AOAC CASP *Aspergillus*, *Salmonella*, and STEC PTM for cannabis and hemp flower. The study included inclusivity, exclusivity, robustness, stability and consistency as well as a matrix study with 30 replicates (non-inoculated, 0.7-1.5 CFU/g, and 5 CFU/g).

Results: In the inclusivity and exclusivity study 50 out of 50 *Aspergillus* isolates were detected, 50 STEC isolates were detected. For the exclusivity study 26 out of 30 *Aspergillus* and 30 out of 30 STEC non-target strains were correctly excluded. In the matrix study, the PathogenDx Detect^{x} Combined assay showed no significant statistical differences between confirmed results for dried hemp and cannabis flower. Robustness testing indicated small changes to the method parameters did not impact the performance of the assay. Stability and consistency studies verified the assay's shelf-life claims were appropriate and manufacturing of the assay was consistent.

Significance: The PathogenDx Detect^{x} Combined assay is able to detect both bacterial and fungal pathogens in a single reaction with a LoD of 1 CFU/g and is an effective method for the qualitative detection of *Aspergillus*, *Salmonella*, and STEC in a multiplex assay from dried hemp and cannabis flower.

P3-70 AOAC® Performance Tested MethodSM Validation for the Detection of *Salmonella* and Shiga-Toxin Producing *E. coli* (STEC) from Matrices Containing Delta-9-Tetrahydrocannabinol Using a Loop-Mediated DNA Amplification (LAMP) Bioluminescent Based Rapid Detection Method

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Introduction: The use of rapid molecular methods may enable faster detection of foodborne pathogens in food matrices. Matrices containing tetrahydrocannabinol are rich in terpenoids which may interfere with the recovery and detection of foodborne pathogens, thus optimizing protocols to verify method performance for application into routine analysis is important.

Purpose: To evaluate the performance of LAMP-based bioluminescent methods to detect *Salmonella* and STEC in food products containing tetrahydrocannabinol following AOAC standard method performance requirements (SMPRs) for STEC and *Salmonella*.

Methods: Two sets of ten-gram portions of matrices containing high and low levels of tetrahydrocannabinol were artificially inoculated targeting 0CFU/10g (n=5), ~2CFU/10g (n=20) and ~10CFU/g (N=5) of E. coli O157 or E. coli O26; both portions were also spiked at the same levels with *Salmonella enterica* sv Typhimurium or Enteriditis, so that each set had both microbial targets. Samples were enriched with 90mL of BPW-ISO and incubated at 41.5°C for 28 and 32h. Samples were analyzed with the LAMP-based methods for the detection of *stx* and *eae* genes and for *Salmonella*. All samples were confirmed by culture; the probability of detection (POD) was calculated following AOAC Appendix J guidelines.

Results: The LAMP-based bioluminescent methods recovered and detected *Salmonella* and STEC in the evaluated matrices. After POD analysis, no statistically significant differences were observed between the number of presumptive positive results detected by the candidate methods and the confirmed positive results determined by the reference method for any of the samples tested, at any of the time points tested.

Significance: Methods have been granted AOAC® Performance Tested Method℠ certification offering testing laboratories a rapid and accurate method for the dual detection of *Salmonella* and STEC in matrices containing delta-9-tetrahydrocannabinol.

P3-71 Comparative Evaluation of Loop-Mediated Isothermal Amplification (LAMP) Bioluminescent Assay and FDA BAM Procedure for Detection of *Salmonella* in Probiotic Products

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Introduction: Probiotic microorganisms are widely known for their inhibitory actions against pathogens, and may interfere with the recovery and detection of microbial contaminants present in probiotic products and ingredients. For successful detection of low levels of *Salmonella* in the presence of probiotic microorganisms, it is critical that appropriate sample preparation, enrichment procedures, and detection methods are employed to allow the growth and detection in matrices with high probiotic background.

Purpose: This study evaluated a LAMP assay for the detection of *Salmonella* in 375-g probiotic products and compare its performance against FDA BAM procedure.

Methods: The method comparison study was performed according to ISO 16140-2 and used probiotic pet food as test matrix. Different sample sizes (375-g and 25-g) were spiked with *Salmonella* at levels ranging from <0.1 CFU to $2.0x10^3$ CFU per test portion. Samples were enriched in Buffered Peptone Water (BPW) + 10 mg/L vancomycin for 375-g samples and in BPW without vancomycin for 25-g samples, then incubated at 37°C for 18-24 h. Analyses for *Salmonella* were performed using both LAMP assay and biochemical confirmation.

Results: *Salmonella* LOD50 for the 375-g probiotic petfood was 1.397 using LAMP method and 0.850 for 25-g sample using FDA BAM method. The resulting RLOD50 (1.70) for this unpaired study was <2.5, indicating equivalency between the two methods. For the same sample size, *Salmonella* recovery was higher in the presence of vancomycin.

Significance: The results show that LAMP-based bioluminescent assay can be an effective method for the rapid detection of low levels of *Salmonella* in probiotic products. Successful detection requires the selection of appropriate sample preparation and enrichment procedures to enable pathogen recovery and detection against a high probiotic background.

P3-72 Independent Laboratory Study for the Nutraplex™ PRO Assay for the Detection of *Escherichia coli, Staphylococcus aureus, and Salmonella* spp.

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Introduction: NUTRAPLEX™ PRO is a real-time PCR assay for the simultaneous detection of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* species from one universal enrichment in 24 hours. This assay utilizes a multiplex detection method that targets generic *E. coli* on the HEX channel, *S. aureus* on the ROX channel, and *Salmonella* spp. on the Cy5 channel. Additionally, the assay has an internal amplification control (IAC) on the Cy5.5 channel. This method couples the advantages of the real-time PCR format with a streamlined enrichment and sampling protocol which requires no DNA purification, complicated end user manipulations, or data analysis, in a user-friendly format.

Purpose: The purpose of this AOAC® Independent Laboratory Study was to compare the alternative method to FDA/BAM Chapters 4, 5, 12, and USP <2022> for the detection of *E. coli*, *S. aureus* and *Salmonella* species in whey powder.

Methods: The assay was compared to FDA/BAM Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria, Chapter 12: *Staphylococcus aureus*, and Chapter 5: *Salmonella* reference methods. The assay was also compared to the U.S. Pharmacopeia (USP) <2022> Microbiological Procedures for Absence of Specified Microorganisms – Nutritional and Dietary Supplements reference method following an unpaired study design.

Results: Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. No statistically significant difference was observed between the two methods.

Significance: This novel assay allows for fast, reliable detection of *Escherichia coli, Staphylococcus aureus,* and *Salmonella* species with presumptive results obtained in as little as 2.5 hours post enrichment.

P3-73 Validation of the GENE-UP® Nutraplex PRO™ Assay for the Simultaneous Detection of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* Species in Select Foods with PCR-Based Culture Confirmation

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Introduction: Testing for nutraceuticals and their ingredients is often performed by traditional culture procedures and is time consuming. The NUTRAPLEX assay is a real-time PCR multiplex assay that detects *Escherichia coli, Staphylococcus aureus* and *Salmonella* species from select food products and ingredients vital to the nutraceutical industry.

Purpose: To validate the performance of the NUTRAPLEX method against the US Pharmacopeia <2022> and FDA BAM Chapters 4, 5, and 12 reference methods and direct PCR confirmation from culture as part of the AOAC Performance Tested Methods℠ (PTM) program.

Methods: The candidate method was evaluated using 25g unpaired test portions of whey powder, pea powder, turmeric, and a vitamin mineral pre-mix at three different levels of contamination of at least two of the target organisms: 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 and doing PCR colony confirmation directly from an isolated colony. PCR colony confirmation was also performed on all Inclusivity and Exclusivity strains.

Results: Statistical analysis was performed using the Probability of Detection model. No statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference methods for all matrices for the Salmonella target. For *S. aureus* and *E. coli*, statistically significant differences were observed in: *S. aureus* – whey powder and pea powder; *E. coli* – pea powder, turmeric, and vitamin pre-mix. The BAM methods are not designed to be low level qualitative screening assays like the candidate method. The USP method is designed to be a qualitative screening assay, similar to the candidate method, and only one statistical difference was observed with turmeric powder. All PCR colony confirmations were accurate.

Significance: These data supported the certification of the candidate method as an AOAC Performance Tested Method℠ (PTM 082103).

P3-74 Enhancing the Isolation Capability of *Salmonella* Isolates Using ISO 6579-1: 2017Reference Method in Combination with Dynabeads Anti-*Salmonella*

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Introduction: *Salmonella* isolation from poultry samples constitute a huge challenge due to the high background flora. This could cause the isolation process to be less accurate, time consuming, and more laborious, which eventually might lead to incorrect decisions made by the decision makers.

Purpose: This study aimed to perform immunomagnetic separation (IMS) technique using Dynabeads® anti-Salmonella after the pre-enrichment step to enhance the performance of the method, reduce the efforts, save the time, and reduce the cost.

Methods: Fifty-seven samples of chicken (Caracas chicken, Shawarma and seasoned chicken) were collected from the local markets from different brands and were tested following two protocols; Protocol number one (Gold standard method) and Protocol number two (Enhanced method).

Results: The results of the two protocols showed total agreement in either the positive samples or the negative ones. Out of 57 samples, 32 samples were identified as positive for *Slamonella* using both protocols and 25 samples were identified as negative using both protocols as well. Protocol two had the advantage of being much clearer, faster and higher confidence in the detection and isolation of *Salmonella*.

Significance: The developed protocol (protocol # 2) with the use of immunomagnetic separation (IMS) has yielded better results with higher confidence. The newly developed protocol has notably reduced the competitively behavior of other microorganisms allowing for Salmonella to be isolated.

P3-75 Productivity and Accuracy Comparison Study between the 3M™ Petrifilm™ Plate Reader Advanced and a Trained Technician, Using Two 3M™ Petrifilm™ Plate Types

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Introduction: Manually counting indicator tests can be a major challenge in food testing laboratories due to the time consuming nature of the task, and in turn this has driven the demand for automated enumeration systems.

Purpose: Demonstrate how automated colony counting systems can increase productivity while achieving accurate results comparable to a human technician manually counting indicator tests.

Methods: Orange Juice, Café Latte, and a snack were spiked using pure strains of *Escherichia coli* at two different levels and were plated on to 3M™ Petrifilm™ Aerobic Count (AC) Plates and 3M™ Petrifilm™ Rapid Aerobic Count (RAC) Plates. One 3M™ Petrifilm™ Plate Reader Advanced (PPRA) and 4 trained technicians in 2 labs counted 25 replicates for each sample and inoculum level on AC and RAC Plates. The time was recorded based on the first to last colony counted on a plate.

Results: The average reduction in time to enumerate plates was calculated as a metric of productivity. The average time savings rapidly increased from 20 to 60 CFU/mL, and then became relatively constant around 85-95%. Technician counting times per one single colony across all inoculum levels remained constant between 0.26 to 0.36 seconds while PPRA counting times ranged from 0.10 to 0.17 seconds. The accuracy was considered equivalent if the percent difference between the log of the human count and the log of the PPRA count was within 10%. Results between the two methods showed an equivalency of 99 - 100%, with the exception of the snack sample plated onto AC at 140 CFU/mL (81% equivalency).

Significance: Accurate automated enumeration systems can increase productivity while maintaining the accuracy of a human technician, which has become more and more critical in a world dealing with COVID-19.

P3-76 Detection of *Lactobacilli*, Yeast, and Moulds in Kraft Heinz Tomato Ketchup Using the Hygiena™ Innovate Rapiscreen™ System

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Introduction: Lactic acid bacteria are contaminants of low pH condiments causing severe spoilage and burst containers. The low CFU levels can mean that traditional methods may miss the spoilage.

Purpose: Rapidly detect lactic acid bacteria (LAB) and low pH tolerant yeast in Kraft Heinz tomato ketchup.

Methods: *Lactobacillus fermentum, L. casei* and *L. brevis* were cultured in MRS Broth at 25°C for 5 days. *Zygosaccharomyces bailii* was cultured in Yeast Growth Broth at 25°C for 5 days. The tomato ketchup was sterilely decanted from original packaging, diluted 1:4 into MRS broth for LAB detection and 1:4 in Yeast Growth broth for yeast detection. The dilution allows for rapid growth and detection using ATP.

The LAB cultures were diluted to <10, <5, <2.5 and <1.25 per 25 mL of diluted sample. LoD are estimated using 3 replicates at each dilution series.

After 96 hours incubation samples were drawn from each bottle and tested for ATP content using the system and plated on PDA and SDA. The confirmation plates were grown for another 5 days.

Results: At 96 hours, the ATP system detects all dilutions of the LABs and the yeast. The lower dilutions down at <1.25 were fractionally detected meaning that 1 or 0 CFU were inoculated or grown in each 25 mL tomato ketchup dilution. The mean RLUs for positive growth was 140,375 RLUs and for negative growth was 818 RLUs. This gives a Z-score of 251 which is an overwhelming detection positive. For *Zygosaccharomyces* in the 1:4 dilution of tomato ketchup in YGB, the positive RLU was 56,172 and negative growth was 27 RLUs giving a Z-score of 9,925.

Significance: Early and significant detection of lactic acid bacteria and *Zygosaccharomyces* in tomato ketchup using an out-of-pack dilution in selective media is rapid using ATP. This allows quicker release of products and shorter hold times.

P3-77 Detection of Commercial Sterility in Non-Dairy Milk Alternative Products Using the Hygiena™ Innovate Rapiscreen™ System

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Introduction: Non-dairy milks are becoming popular; this increase in demand is driving testing efficiency. Detection panel of 7 microorganisms is demonstrated using the Hygiena™ Innovate system compared to traditional plating.

Purpose: Rapidly detect spoilage organisms and allow rapid release of products, Adez Almond Milk and Adez Bevanda Vegetale (Oat Milk), by Coca Cola.

Methods: Culture mixes (Non-Bacillus Cocktail – *Streptococcus salivarius*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*), *Geobacillus stearothermophilus*, *Bacillus coagulans* and Heat-Resistant Mould cocktail (*Talaromyces pinophilus*, *Byssochlamys fulva*) were inoculated into product bottles through a sterile closure at <10 CFU per pack, control packages were uninoculated. 10 replicate bottles were used and incubated at 30°C and 55°C.

At the following time points, 24, 48, 72, 96, 120 and 240 hours, samples were drawn from each bottle and tested for ATP content using the Innovate System and plated on PDA and SDA using a Miles and Mizra plating technique.

Results: Minimum detection from almond and oat milk for each group was as follows: Non-Bacillus Cocktail – 48 hours and 48 hours; *Geobacillus stearothermophilus* – 24 hours and 24 hours; *Bacillus coagulans* – 72 hours and 72 hours; and heat-resistant spores – 72 hours and 72 hours, respectively.

Confirmed negative and positive RLUs for each group were as follows: Non-Bacillus Cocktail - 4 to 9 and 2 to 5, 45 to 143,980 and 2,214 to 252,373; *Geobacillus stearothermophilus* - 4 to 6 and 3 to 5, 1,026 to 66,601 and 404 to 19,641; *Bacillus coagulans* – 1 to 10 and 3 to 7, 158 to 923 and 3338 to 36392; and HR spores – 3 to 6 and 2 to 5, 133 to 1705 and 58 to 7644, respectively.

Significance: Early confirmed detection of risk organisms from non-dairy milk alternatives is rapid using ATP detection, allowing quicker release of products and shorter hold times.

P3-78 Comparison of Three Methods for Enumeration of *Bacillus coagulans* (BC30) from Pet Food Ingredients and Products

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Introduction: Very few methods exist for enumeration of BC30 from food products. With the incorporation of this novel probiotic in supplements and food, a need arose to be able to enumerate this specific organism.

Purpose: The objective of this study was to compare three methods (USP *Bacillus coagulans* GBI-30 6086, EN15784:2009-12 – Isolation and enumeration of presumptive *Bacillus* spp., EN15787:2009 – Isolation and enumeration of *Lactobacillus* spp.) for enumeration of BC30 from pet food products.

Methods: Two lots each of pure BC30 probiotic powder (10^{10} CFU/g, n=10), tallow (10^{7} CFU/g, n=10), powdered meat-based flavor mixed with BC30 powder (10^{8} CFU/g, n=10), and kibble formulated with BC30 powder (10^{6} CFU/g, n=10) were produced by the Nestle Purina Product Technology Center. Each of these matrices were processed and enumerated as described for all three methods. Data for all samples were analyzed via accuracy profile to determine method equivalency, and a two-sample T-Test for significant difference between methods.

Results: For comparison purposes, the USP method was considered as the reference and the EN15787:2009 method was the alternative. EN15784:2009-12 was unable to produce detectable growth of BC30 after 24 hours of incubation at 37°C, and was excluded. The levels of BC30 enumerated with both methods agreed with the intended formulation levels. The 2-sample T-Test indicated that there was a significant difference in recovery of BC30 between the two methods ($p < 0.05$) for all matrices, except the meat flavor + BC30 ($p > 0.05$). Data from EN 15787 had much higher standard deviations than USP, which indicated higher variability between the data points for all matrices. A majority of data points fell outside of the acceptability limit of the accuracy profile, thus indicating that the USP and EN15787 were not equivalent.

Significance: The USP method may be more suitable for enumeration of BC30 from pet food ingredients and products.

P3-79 Detection of Beverage Spoilage Organisms in Low pH Juice Products Using the Hygiena™ Innovate Rapiscreen™ System

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Introduction: Low pH juice products have a unique set of issues when contaminants are tested. This study demonstrates a wide variety of organisms that were spiked and recovered from 6 beverage products.

Purpose: Rapidly detect a variety of organisms from 6 beverage products, Sports Drink, Mixed Berry, Apple, Orange 10%, Orange 85%, and Red Berry using the Hygiena™ Innovate System.

Methods: *Dekkera bruxellensis, Talaromyces pinophilus, Lactobacillus fructivorans, Saccharomyces cerevisiae, Alicyclobacillus acidoteresstris, Candida orthopsilosis* and *Byssochlamys fulva* were acid adapted by dilution in TSB to strengthen the organism to recreate natural contamination events. The organisms were then individually growth in media and spiked into each product individually at <50 CFU per pack. The packs were run in triplicate and incubated at 25°C, after 48 to 168 hours samples were drawn, ATP measured, and confirmation plates produced.

Results: *Saccharomyces* and *Candida* were detected in all beverages by 48 hours with RLUs of 9,120, 49, 21,629, 38, 46,291, 2,026, 50, 160, 1,361 and 33 respectively. Sports Drink did not support the growth of *Saccharomyces* or *Candida*. The negative RLUs ranged from 1 to 5. *Talaromyces*, *Byssochlamys* and *Dekkera* were detected at 96 to 120 hours in all products with RLUs of 75, 54, 5,198 and 116, 77, 16,838 and 136, 572, 216 and 154, 56, 3255 and 643, 174, 64. Sports Drink only supported the growth of *Byssochlamys* at 168 hours with an RLU value 59,099. *Lactobacillus* and *Alicyclobacillus* did not grow in any of the products except *Lactobacillus* grew in Orange 85%, detected by ATP and plate methods at 168hr incubation. Confirmation plates used typically showed growth 48 hours behind the detectable ATP levels.

Significance: Early and significant detection of acid-adapted organisms in low pH beverage products is rapid using ATP methods. This allows quicker release of products and shorter hold times.

P3-80 Reduced Enrichment Time for Detection of *Listeria* at 37°C

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Introduction: Interest continues to grow for the enrichment and detection of *Listeria monocytogenes* and *Listeria* spp. at 37°C with the possibility of reducing enrichment times.

Purpose: This study evaluated the incubation time necessary at 37°C to achieve *Listeria* detection results equivalent to incubation at 30°C.

Methods: Raw milk, raw milk cheese, salami, spinach, and turkey lunch meat were inoculated with *Listeria monocytogenes* and *Listeria grayi* at <10 CFU per test portion. The 25 g samples were then enriched with 225 ml *Listeria* Special Broth (LSB) and incubated for 18, 21, and 24 hr before testing with the iQ-Check *Listeria spp.* and/or iQ-Check *Listeria monocytogenes* II test kits. Results were confirmed culturally by plating to chromogenic media.

Results: Enrichment at 37°C reduced the enrichment period necessary (for results equivalent to 30°C) from 24 hr to 18-21 hr for raw milk and raw milk cheese along with 18 hr for salami, spinach, and turkey lunch meat. Improved results were seen at 37°C with PCR in some instances, especially with raw milk products, but also increased the presence of competing bacteria making plate culture confirmation more difficult.

Significance: These data show that incubation of *Listeria* enrichments at 37°C can reduce enrichment times compared to incubation at 30°C.

P3-81 Evaluation of New Matrixes with Perkinelmer Solus One *Listeria* and Automated Dynex DS2 System

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Introduction: Finished product pathogen testing is a key program for validation of HACCP and food safety within food safety programs, but individual matrixes must be verified prior to use on new methods and equipment to ensure valid results.

Purpose: The purpose of this study was to evaluate the PerkinElmer Solus ONE *Listeria* immunoassay-based test kits with new matrixes including cream cheese, ketchup, deli meat, and chocolate which were not previously documented in their AOAC certifications.

Methods: Commercially available samples of cream cheese, deli meat (ham), and chocolate were portioned into 25g samples and inoculated with a cocktail of *Listeria monocytogenes* outbreak strains at multiple levels targeting <1000 CFU, <100 CFU, and <10 CFU. Samples were enriched with SOLO+ medium at a 1:10 dilution and incubated at 30°C for 22-30h. The samples were analyzed following the AOAC Performance Tested method 051802 utilizing the automated system DS2. Controls including media controls and inoculated media controls were utilized in addition to the kit required controls. Organisms were plated on modified Oxford media to enumerate inoculation levels. Organisms were confirmed from plates used for enumeration of the inoculum as *L. monocytogenes* via Gram stain and bacterial identification via Vitek 2 following BAM Chapter 10.

Results: Initial inoculation levels were 525 CFU/g, 55 CFU/g, and 7 CFU/g, for inoculation targets <1000 CFU, <100 CFU, and <10 CFU respectively. The Solus ONE *Listeria* kit was able to detect all levels of inoculation including targets of <1000 CFU, <100 CFU, and <10 CFU for all matrixes. Positive and negative controls were acceptable.

Significance: Solus ONE *Listeria* immunoassay-based test kit, DS2 automated system, and AOAC Performance Tested method 051802 may be appropriate for additional matrixes outside of the original AOAC validation and useful tools for finished product testing and specifically for cream cheese, ketchup, deli meat, and chocolate.

P3-82 Verification of Fit for Purpose of the 3M Molecular Detection Assay 2- *Salmonella* spp. and 3M Molecular Detection Assay 2- *Listeria monocytogenes* Following ISO 16140-3 Guidelines for Their Application in Cheese and Ham Matrices

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Introduction: Before a method is implemented for routine testing, verifying fit for purpose and proficiency are important tasks, especially for pathogen testing. In 2021, ISO published the 16140-3 guidelines for method verification in a laboratory. The guidelines enable the generation of data of fully validated methods to be implemented within the scope of a laboratory, providing information related to the proficiency of the lab to perform the method and whether is fit for purpose for a specific application.

Purpose: To perform verification of two molecular methods for pathogen detection in ham and cheese following ISO 16140-3 scheme.

Methods: Implementation verification and food (item) verification were followed according to ISO 16140-3 'Protocol 3' to test pasteurized double-cream cheese and pre-sliced chicken both part of the scope of the testing laboratory. Seven 25g test portions were spiked with 5CFU/sample or 4 CFU/sample of reference strains of Salmonella and *Listeria monocytogenes,* respectively. One non-spiked sample for each set was included. Each test portion was enriched with 225 mL of buffered peptone water or 225 mL of Demi-fraser for *Salmonella* and *L. monocytogenes* respectively. Samples were incubated at 37°C for 26h for *Salmonella* and 30h for *Listeria monocytogenes*. After incubation samples processed following validated procedures described for the 3M Molecular Detection Assays. Each set of samples was tested by two different technicians.

Results: All the contaminated samples with *Salmonella* or *Listeria monocytogenes* were reported as positive when ham and cheese were evaluated. Differences among technicians were not found. According to ISO 16140-3, 6 out of the seven spiked samples should render a positive result, thus the verification study meets the acceptance criteria defined in the standard.

Significance: The application of the 16140-3 enable faster implementation of rapid methods for pathogen detection, demonstrating method's fit for purpose before routine use.

P3-83 Solid Phase Reversible Immobilization Bead Concentration Combined with PCR for the Detection of *E. coli* O157:H7 in Foods

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❖ ***Developing Scientist Entrant***

Introduction: Real-time PCR is an effective technique for rapid and simultaneous detection of foodborne pathogens, however, it is limited by the concentration of target DNA that can be isolated from a food matrix. Solid phase reversible immobilization (SPRI) paramagnetic beads are designed to specifically bind to DNA and help remove unwanted materials in food matrices, providing better binding capacity and exhibiting higher efficiency for food samples having low concentrations of DNA.

Purpose: This study aimed to develop a novel upstream bacterial DNA concentration using SPRI beads and compare its efficacy with immunomagnetic separation (IMS) for the detection of Shiga toxin producing *Escherichia coli* O157:H7 in food samples.

Methods: Food samples were spiked with 10, 100 and 1000 CFU/mL of *E. coli* O157:H7. Two DNA concentration methods were compared, *viz*., (1) DNA isolation followed by concentration using SPRI beads, and (2) IMS followed by DNA isolation, prior to performing a high-resolution melt-curve multiplex PCR to detect *eae*A, *stx*1, *stx*2 genes. PrepMan Ultra Sample Preparation Reagent was used for DNA isolation. The efficacy of these methods was compared by measuring the 1) yield 2) purity and 3) detection limit and time of the target pathogen following the PCR.

Results: DNA isolated and concentrated using the SPRI method showed better purity and yield compared to the IMS-based method. Moreover, a minimum detection limit of 10 CFU in the majority of the spiked food was obtained within 4 h of enrichment for samples concentrated using SPRI as compared to an enrichment time of 6 h or higher required for the IMS-based method.

Significance: SPRI paramagnetic beads in combination with multiplex HRM real-time PCR can be applied for the rapid concentration and detection of *E. coli* O157:H7 that are present in low concentrations in food samples, making this a useful tool to improve food safety.

P3-84 Evaluation of the GENE-UP® *E. coli* O157:H7 2 (ECO2) for the Detection of *Escherichia coli* O157 (Including H7) in a Variety of Foods

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Introduction: The GENE-UP® *E. coli* O157:H7 2 (ECO2) is a FRET Technology & Melt Analysis based real-time PCR system used for rapid, specific detection of *E.coli* O157 (including H7) from food and environmental samples.

Purpose: The performance of the alternative method was compared to Canadian culture-based reference method MFHPB-10 and evaluated according to Microbiological Methods Committee (MMC) guidelines for relative validation of qualitative microbiological methods for consideration as a laboratory procedure.

Methods: Unpaired samples spiked with *E.coli* O157:H7 (and co-inoculated with 10 times L_1 with *E.coli* as an interference organism) were analyzed by the alternative and reference method. Raw Meats (Frozen, processed and Unprocessed) , Raw and raw processed fruit and vegetables and Raw Dairy were each inoculated at three inoculum levels: 20 samples at a level (L_1) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2), approximately 10 times L_1, and 5 un-inoculated samples. Alternative samples were enriched in BPW broth (Raw dairy supplemented with Acriflavine 10mg/L), incubated at 42 ± 1˚C, tested at 8h 10h and 20h of incubation. Reference samples were enriched in m-TSB supplemented with 20 g/ml Novobiocin for 18-24h at 42˚C. All analytical outcomes were culture confirmed by the reference method.

Results: Collectively from the analysis of 630 samples, a Probability of Detection (POD) statistical model determined the alternative method exceeded the criteria outlined by the MMC, obtaining relative sensitivity of ≥98%, relative specificity of ≥ 90.4%, false positive rate of <9.6%, false negative rate of <2% and test efficacy of ≥ 94%.

Significance: The GENE-UP® *E. coli* O157:H7 2 (ECO2) is a suitable method for detecting *E. coli* O157 (including H7) in various matrices after 8h (Raw Meat-25g), 10h (Raw Meat 375g, Fruit & Vegetables- 200g), and 20 h (Raw Dairy-25g) of incubation, thereby significantly reducing presumptive reporting times over the reference method.

Poster

P3-85 Validation of the 3M™ Petrifilm™ Rapid Aerobic Count Plate for the Enumeration of Total Aerobic Colony Counts in a Variety of Foods Against the Canadian Reference Method (MFHPB-18)

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Introduction: The 3M™ Petrifilm™ Rapid Aerobic Count Plate is a sample-ready-culture-medium system which contains nutrients, cold-water-soluble gelling agent, and dual-sensing indicator technology that facilitates colony enumeration. This alternative method is used for enumeration of total aerobic bacteria in food samples.

Purpose: The objective of this study was to evaluate the performance of the alternative method against the Health Canada Compendium of Analytical Methods method MFHPB-18 in a variety of food matrices for inclusion as a Laboratory Procedure (MFLP).

Methods: The alternative and comparative reference method (MFHPB-18) were analyzed by testing 5 food categories (Raw poultry, Heat-processed Dairy Ready-to-Eat, Ready-to-reheat meat products, Fresh Produce and Fruit, Multi-component foods). Naturally contaminated samples were subjected to spoilage, and supplemented with fresh matrix to obtain counts across five logarithmic levels, ranging from uncontaminated to 6 Log CFU/g, in order to encompass matrix-related regulatory limits and the counting range of the alternative method.

Results: Linearity was evaluated using the Chi^2 test, followed by linear regression analysis; analysis of variance and bias was conducted as outlined in "The Procedure for the Development and Management of Food Microbiological Methods" (Microbiological Methods Committee (MMC)). A one-tailed t-test was used to evaluate if there is significant bias between the two methods. For most food items, the critical F-value was greater than the calculated F-value, illustrating that the relationship between the two methods was linear. Analysis of variance results also yielded favorable data, based on comparison of the two methods, for most food items ($\alpha = 0.05$). On average, considering all food items, the bias value is 0.0157, which is within the acceptability criteria.

Significance: The 3M™ Petrifilm™ Rapid Aerobic Count Plate met the Canadian requirements of the MMC. This method offers the capability of enumerating total aerobic colony counts in foods after 24 ±2 h of incubation at 35ºC, reducing presumptive reporting times.

P3-86 Rapid and Direct Identification of Pathogens from Food Matrices Using Magnetic Nanoparticles and Supervised Machine Learning Algorithms Applied to Near Infrared Spectroscopy Data

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❖ ***Developing Scientist Entrant***

Introduction: While Near Infrared spectroscopy (NIRS) has often been proposed as a sensitive and reliable technique for pathogen detection, the time required for bacterial growth and separation processes is a common concern.

Purpose: In this work, glycan coated magnetic nanoparticles were used to concentrate *B. cereus* and *E. coli* from various food matrices and detected using Near Infrared Spectroscopy.

Methods: Following magnetic separation, Near Infrared spectra of the pathogen was acquired without any sample modification. A program was written in MATLAB for data preprocessing, dimensionality reduction and pathogen classification with raw NIRS data as an input. Preprocessing of the data was followed by dimensionality reduction using Principal Component Analysis. The Principal Components were then fed to machine learning models including Linear Discriminant Analysis, Naïve Bayes, Neural Networks, and Support Vector machine to test and compare their accuracy

Results: Neural Network was found to be the best classifier for *B. cereus* with an efficiency (n=16) of 93.8 % followed by Gaussian (87.5%). For *E. coli (n=16)* , 100% classification was achieved using Naïve Bayes followed by Linear Discriminant Analysis and Neural Network (91.7%).

Significance: The results demonstrated the ability of magnetic nanoparticles to extract pathogens from complex food matrices and confirmed the suitability of NIR spectroscopy to classify important food pathogens. This technique can help in preventing food-borne outbreaks.

P3-87 Effect of Bed-Depth on Inactivation of *Enterococcus faecium* NRRL B-2354 during Hot-Air Drying of Fresh Cut Apple Cubes

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Introduction: Previous research evaluated the effect of hot-air drying of apple cubes on the lethality of two microorganisms at two temperatures. However, the effect of the drying bed depth on the lethality of the drying process is still unknown.

Purpose: To study the thermal inactivation of *E. faecium* in apple cubes during air drying as effected by bed depth and temperature.

Methods: *E. faecium* was grown on TSAYE. Culture was harvested in buffered peptone water. Cored, peeled Gala apple cubes (~6.35mm) were inoculated at 8.32±0.19 log CFU/sample. Apple cubes were dried at 104°C for up to 70 min or 135°C for up to 32 min in ~0.75 kg batches using a hot-air dryer with a vertically directed heat source and a fixed bed height. Three subsamples consisting of 4 inoculated cubes were enumerated, at each time point (n≥5) from top of the bed (4.5 cm), on modified TSAYE. Water activity (a_w) measurements were taken at corresponding time intervals.

Results: Drying at 104°C led to a 0.44±0.14 and 2.56±0.72-log CFU/sample *E. faecium* reduction after 30 and 70min, respectively. During 135°C drying, there was a 1.15±0.38 and 3.71±0.27-log CFU/sample *E. faecium* reduction after 20 and 32 min, respectively. Higher ($p<0.05$) microbial reduction was achieved with a 4.5cm bed depth at 70 and 32 min during 104 and 135°C drying compared to drying a thicker bed (9 cm) of apple cubes as obtained in from a previous study. Significantly higher *E. faecium* reduction ($p<0.05$) during 135°C than 104°C was observed at the same drying time (~30 min). Inoculated apple cubes' a_w dropped from 0.98±0.01 to 0.37±0.07 (104°C, 70 min) and 0.64±0.09 (135°C, 32 min).

Significance: A reduced bed depth led to higher *E. faecium* reduction within the same drying time. Under the conditions tested, less than 4-log CFU/sample reduction was achieved at the end of drying.

P3-88 Inactivation of Foodborne Pathogens in a Nectarine Drying Process

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Introduction: Moderate thermal dehydration processes at temperature ≤140°F aim to provide specific quality attributes to dried fruits and/or are often used in small-scale dried fruit manufacturers. Although there are limited reports of outbreaks in dried fruits, it is crucial to understand pathogen inactivation efficacy of fruit drying processes to ensure safety of the finished product.

Purpose: The objective of this study was to evaluate pathogen lethality in sliced nectarines using a hot-air drying process.

Methods: Product temperature and water activity profiles of sliced nectarines were collected during a moderate hot-air drying process. Worst-case scenarios within the first 5 hours and at the end of drying time were determined based on the lowest product temperature, the lowest water activity (Aw) and the shortest residence time. Isothermal treatment (n = 3) at selected worst-case conditions: (a) 120°F for 2 hours at Aw of 0.7, and (b) 135°F for 4 hours and 10 minutes at Aw of 0.4, were carried out to determine inactivation levels of *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Results: Results showed that heat treatment for 2 hours at 120°F resulted in 1.29, 1.80, and 1.37 log reductions for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively, in nectarine at Aw of 0.7. In nectarine at Aw of 0.4, 4.38, 5.39 and 4.72 log reductions for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively, were achieved after a treatment of 4 hours and 10 minutes at 135°F.

Significance: In this case, pathogen lethality achieved during production of dried nectarines using a moderate temperature hot-air drying process was cumulatively greater than 5 logs. As we continue to strengthen food safety programs and compliance with the preventive controls for human food rule,

Poster

the dried fruit industry must validate moderate temperature drying processes to ensure inactivation of foodborne pathogens and safety of the finished product.

P3-89 Regulatory Considerations for Small-Scale Produce Drying Operations: A Multi-State Perspective Obtained through Inspector Interview

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❖ ***Developing Scientist Entrant***

Introduction: Rising foodborne outbreaks implicating low-moisture foods highlighted the need to evaluate the food safety practices related to produce drying. The local and state regulations specific to dried produce production vary widely between states, which can complicate food safety regulatory compliance for small-scale processors in the US.

Purpose: To assess the impact of regional variability on dried fruit regulatory oversight and identify food safety training needs for small-scale produce drying operations.

Methods: Participant recruitment was conducted in four states – California, Indiana, Massachusetts and Rhode Island – through a local point of contact. We targeted 30 food safety inspectors. The interview script was reviewed by three university food safety specialists and pilot tested with three regulatory professionals. Two researchers in addition to the interviewer took notes during the interview (via Zoom). Areas of inquiry included food safety controls during drying process, regulation interpretation, processor interactions, and potential technical support from university extensions.

Results: From the participants interviewed, none had inspected small to very-small scale produce drying facilities. Participants were unsure whether dried fruits were considered a time-temperature control for safety (TCS) or non-TCS food. Drying produce that were peeled, ground, or sliced would require more inspection criteria, such as proper refrigeration, than whole-dried produce. Participants did not indicate any defined critical limits for process preventive controls of the drying process during inspection. All participants mentioned inspecting for "very basic" Good Manufacturing Practice compliance, such as availability of handwashing sinks, and receiving or storage temperatures. A common theme was participants viewing themselves as educators more than enforcement authorities thereby employing a strong inspector-processor relationship for effective communication and resolution of food safety violations. All participants agreed that more technical support on best practices and information for produce drying from University Extension can benefit both processors and inspectors.

Significance: Results from this work provide clarity on regulatory interpretation variation and identify a need for produce drying technical support and guidance for industry.

P3-90 Factors Influencing Desiccation Tolerance of *Salmonella* and Enterohemorrhagic *E. coli*

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✦ ***Undergraduate Student Award Entrant***

Introduction: Outbreaks of *Salmonella* and Enterohemorrhagic *Escherichia coli* (EHEC) in low-moisture foods have increased interest in evaluating how pathogens tolerate desiccation. Previous studies have found that EHEC and *Salmonella* have varying desiccation tolerance, but there is limited data on the impact of prior growth conditions on desiccation tolerance in a osmolyte-free environment.

Purpose: The goal of this study was to quantify how various serotypes of *Salmonella* and EHEC respond to desiccation and if their tolerance is influenced by initial growth in liquid or on agar media.

Methods: A total of 21 strains belonging to EHEC serotypes O121, O157, O111, O121, O45 and O103 and *Salmonella* serotypes Agona, Enteritidis, Montevideo, and Tennessee were evaluated in triplicate**.** Following growth at 37C in LB broth and on LB agar, cells were suspended in sterile DI water and inoculated onto a plastic surface at an initial density of 8 log CFU/mL. Plates were incubated at 22C, 40% RH for 48 hours. Cells were rehydrated using sterile DI water and plated onto LB agar for enumeration. Viable cell counts were performed at 0 and 48 hours and differences calculated.

Results: Desiccation tolerance varied significantly among strains dependent on growth method ($p < 0.01$). *Salmonella* Agona strains grown in broth were significantly more tolerant ($p < 0.001$) than those on agar with an average log reduction of 0.36 ± 0.2 and 0.83 ± 0.3 respectively. Differences among serotypes were also observed. 0111:H8 strains grown in broth were significantly more sensitive ($p < 0.05$) to desiccation (average log reduction 1.31 ± 0.25) compared to 045:H2 grown in broth (average log reduction 0.78 ± 0.33).

Significance: The results show that the response to desiccation stress among enteric pathogens is not uniform and also emphasized the importance of using a broad range of strains when testing desiccation characteristics.

P3-91 Needs Assessment of the Low-Moisture Food Industry: The Next Steps to Advance Food Safety Research and Extension

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❖ ***Developing Scientist Entrant***

Introduction: Low-moisture food safety is getting attention due to increasing foodborne recalls and outbreaks. However, limited studies have assessed the food safety challenges and research and extension need of the low-moisture food industry.

Purpose: This needs assessment aimed (1) to identify the food safety challenges and data gaps; (2) to assess the perceptions of adopting food-safety-enhancing technologies; (3) to explore the food safety culture and education need of the low-moisture food industry.

Methods: In Study 1, participants from low-moisture food industry upper management were interviewed. For Study 2, an online survey was developed based on the findings of Study 1. A different group of participants with experience in the low-moisture food industry participated in the survey. Qualitative data was analyzed using Nvivo 12 and quantitative data was analyzed using Microsoft Excel.

Results: The studies had 25 participants (n=12 for Study 1; n=13 for Study 2). Many participants worked with commodities such as nuts and seeds, spices, flour, and dried fruits and vegetables. Microbial food safety was perceived to be a continuing challenge in the low-moisture food industry. Although many microbial risk reduction control measures have been implemented, three primary food safety concerns were identified: cleaning, sanitation, and hygienic design; human factors; and pathogen reduction. The top three data gaps that need to be filled were (1) validation study design; (2) equipment cleaning/sanitation timing and efficacy; and (3) customers' food safety knowledge, attitudes, and handling practices of low-moisture foods. Participants perceived the major barriers to adopting food-safety-enhancing technologies were production feasibility and efficiency (85%), budgetary priorities (77%), and product characteristics such as quality, sensory, and further processing property (77%).

Significance: The findings will provide guidance for future research and extension programs to enhance food safety in the low-moisture food industry.

P3-92 Validation of Biltong (Dried Beef) Process Lethality Using Non-Pathogenic Surrogate Organisms Associated with Beef

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❖ ***Developing Scientist Entrant***

Introduction: Validations studies conducted within a food processing facility using surrogate organisms could better evaluate a manufacturer's ability to achieve desired microbial reductions than controlled laboratory studies with pathogenic bacteria.

Purpose: The objectives of this project were to isolate and identify potential surrogate bacteria during biltong processing, conduct biltong surrogate validation lethality studies, and measure critical factors and intrinsic parameters during processing.

Methods: Bacteria were collected during biltong processing and identified using 16S rRNA PCR and DNA sequencing. Beef pieces (1.9-cm x 5.1-cm x 7.6-cm; 85-105g) were inoculated with either *Carnobacterium* spp. or *Lactobacillus sakei* (300mL), vacuum-tumbled in a marinade of spices, 4% (v/w) 100-grain red wine vinegar, and 2.2% NaCl and dried in a humidity-controlled oven for 8 days (25°C; 55% relative humidity). Surviving surrogate bacteria were enumerated by stomaching beef pieces in a fixed volume of diluent to account for weight loss during processing. Evaluation of water activity, pH, and salt concentration were performed after 6 timepoints during processing. Trials were performed in duplicate replication with triplicate samples per time period and analyzed by RM-ANOVA.

Results: After 8 days of drying, beef reached >60% moisture loss, salt concentration >4%, a_w <0.85, and pH 5.2. Trials conducted with *Carnobacterium* achieved a >5-log (5.85-log) reduction over a drying period of 8 days and were comparable to reductions observed with *Salmonella, L. monocytogenes, E. coli* O157:H7 and *S. aureus* under the same processing conditions. Trials with *Lb. sakei* failed to achieve a 5-log reduction (2.03-log) over the same drying period and was significantly different from pathogenic trials ($p<0.05$).

Significance: *Carnobacterium* lethality aligned with the reductions observed with previous pathogenic biltong validation studies indicating *Carnobacterium* could be an effective in-plant surrogate to monitor the microbial safety of biltong processing within a manufacture's own facility. This project helps to fill USDA-FSIS knowledge gaps in air-dried, shelf-stable dried beef.

P3-93 Time, Temperature, and Antimicrobial Dosage Have Varying Impact on the Reduction of *Escherichia coli* Populations in Wheat Berries

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Introduction: Recent microbial outbreaks in flour have prompted the milling industry to evaluate addition of antimicrobial solutions at the tempering step. Milling temperatures and tempering time tend to vary from season to season which impacts antimicrobial efficacy and may necessitate modified antimicrobial dosages.

Purpose: Evaluate the reductions of *Escherichia coli* populations in wheat berries at varying tempering temperatures, tempering times, and antimicrobial dosages.

Methods: Irradiated wheat berries were dried to a moisture of 8.50% w.b. to compensate for inoculum addition. Wheat was portioned (200 g), inoculated (6 log CFU/g with 5-strain cocktail of *E. coli* surrogate), and permitted to attach for 24 h. Post-attachment, wheat was tempered with consistent volume but increasing concentrations (5, 10, 15%) of lactic acid (Purac® FCC88) to achieve ca. 14.5% w.b. moisture. Bags were intermittently shaken for 2 h and stored flat at varying temperatures (12.8, 20, 37°C) for varying times (8, 16, 24 h). Post-storage, three 50-g samples were transferred into sterile stomacher bags and diluted 1:2 with buffered peptone water and serially diluted in Butterfield's buffer. Samples were plated on sorbitol MacConkey agar (35°C for 24 h).

Results: Tempering time, temperature, and antimicrobial concentration had a significant effect ($p<0.05$) in reducing *E. coli* population in wheat berries. The results showed that as the tempering time and temperature increased, *E. coli* reduction increased. Similarly, an increase in the antimicrobial concentration led to increase in *E. coli* reduction. Also, a significant interactive effect ($p<0.05$) of time, temperature, and antimicrobial was seen. The highest concentration of lactic acid, highest temperature, and longest duration resulted in the greatest *E. coli* reduction.

Significance: Understanding the interaction of time, temperature, and dose can assist millers in design of tempering protocols based upon environmental conditions. Increasing temperature, antimicrobial dose, and tempering time can decrease *E. coli* population in wheat berries making flour safer.

P3-94 *Salmonella* Survival on Whole Wheat Berries during Storage

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Introduction: Outbreaks due to pathogens in wheat flour have exposed knowledge gaps in wheat safety. Numerous publications state conditions needed to prevent spoilage of whole wheat by mold and pests, but less is known about pathogen survival during storage and drying of whole wheat at such conditions.

Purpose: Therefore, the objective of this study was to determine the survival of *Salmonella* during storage of whole wheat.

Methods: Whole wheat berries were inoculated with a liquid cocktail inoculum containing six *Salmonella* strains (8mL inoculum/400g wheat), hand massaged for 3 min, and dried in a biosafety cabinet for 24 hr. Inoculated wheat was placed in sealed containers within incubators set at 4°C or 25°C and held at relative humidity of 33% or 43% using saturated salt solutions (4 total conditions). Triplicate 2g samples per condition (n=3) were removed at timepoints (post-desiccation, 1-, 7-, and 21-day storage) for enumeration of survivors and measurement of water activity/moisture content. ANCOVA was conducted using MATLAB to estimate population decline over storage duration, then pairwise comparisons were completed to determine statistical differences between conditions.

Results: Initial *Salmonella* population in inoculated, desiccated wheat was 8.60±0.04 log CFU/g. No statistically significant decline in *Salmonella* population ($P\geq0.05$) was observed after 21 days of storage under any condition. The greatest rate of population decline was 0.011±0.005 log CFU/g per day, which occurred at 25°C and 43% humidity ($P=0.054$). No significant differences ($P\geq0.05$) in rate of population decline were observed in pairwise comparisons of each condition tested. Water activity of wheat equilibrated to target within 7 days of storage.

Significance: No significant population decline during early storage highlights the importance of preventing contamination of wheat on-farm and the utility of reduction strategies during wheat processing. This research can expand upon guidance regarding whole wheat spoilage and quality during storage to include information regarding foodborne pathogens as well.

P3-95 Survivability and Thermal Resistance of *Salmonella* and *Escherichia coli* O121 in Wheat Flour during Extended Storage of 360 Days

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne pathogens like *Salmonella* and *Escherichia coli* O121 can endure the harsh low water activity (a_w) environment of wheat flour for extended periods of time and can proliferate upon hydration for baking

Poster

Purpose: This study determined the survivability and thermal tolerance (D- and z-values) of *Salmonella* and *Escherichia coli* O121 in wheat flour during the ambient temperature storage of 360 days.

Methods: The *Salmonella* and *E. coli* O121 studies were conducted as two independent experiments. Studies were randomized complete block designs with three replications as blocks and analyzed using one-way ANOVA and Tukey's ($P \leq 0.05$ was considered significant). The wheat flour (375 g) was spray inoculated individually with 7-isolate *Salmonella* or 3-isolate *E. coli*O121 cocktail, mixed, and then dried back to the original a_w levels at 37°C in an incubator. On each analysis day, inoculated wheat flour (~5 g) was placed inside the thermal-death-time (TDT) disks, heat treated at set temperatures in hot water baths, and sampled at predetermined time intervals for determining the survival microbial population. The initial inoculation population in wheat flour for *Salmonella* and *E. coli* O121 was ~ 8log CFU/g.

Results: The D-values of *Salmonella* and *E. coli* O121 cocktails in inoculated flour were determined on days 1, 30, 90, 180, 270, and 360. The population of *Salmonella* and *E. coli* O121 in wheat flour decreased by 5.0 and 5.6 log CFU/g, respectively, during the storage period of 360 days. The $D_{70°C}$, $D_{75°C}$, and $D_{80°C}$ values of *Salmonella* in wheat flour remained similar during the storage period. Whereas, for *E. coli* O157:H7 in wheat flour, the $D_{70°C}$ value decreased from 20.3 to 7.1 min, and $D_{75°C}$ decreased from 10.2 to 2.7 min, during the storage period of 180 days. The z-values of *Salmonella* and *E. coli* O157:H7 remained similar during the storage period.

Significance: The D-and z-values from this research can be employed for validation of thermal processes to ensure microbiological safety of wheat flour.

P3-96 Survival of Shiga Toxin–Producing *E. coli* in Various Wheat Flours during Storage

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Introduction: While wheat flour does not support microbial growth due to its low water activity, it can still serve as a vector for foodborne pathogens and numerous outbreaks of *E. coli* have been linked to flour. While previous studies have shed light on the persistence of STEC populations, it is unknown if compositional differences among varieties of consumer wheat flour impact the survivability of resident STEC populations.

Purpose: The purpose of this study is to compare the survival of STECs O121 and O157:H7 in different varieties of wheat flour.

Methods: Five different types of consumer wheat flours were examined: all-purpose unbleached, all-purpose bleached, bread, whole wheat, and self-rising. The flours were inoculated with either STEC O121 or O157:H7 and stored in a humidity-controlled chamber at 25˚C and 50% relative humidity. Samples were taken in triplicate at various timepoints before being serially diluted and plated onto both TSAYE and R & F® non-O157 STEC Chromogenic Plating Medium. After incubation at 35˚C, plate counts were used to determine reductions in CFU.

Results: For both strains, the largest reduction in population occurred within the first 24 hours post-inoculation in all flours tested. After 42 days, the population of O121 had decreased by an average of 2.95±0.15 log(CFU) across all flour types, while O157:H7 populations were undetectable, having dropped by 5.74±0.50 log(CFU) by day 29.The average rate of reduction of O157:H7 across all flour types (0.0196±0.0026 log(CFU)/day) was significantly higher than that of O121 (0.0068±0.0007 log(CFU)/day), indicating different survival and death kinetics of O121 and O157:H7 in wheat flour. However, the observed reductions in bacterial populations were independent of flour type.

Significance: This study elucidated how different varieties of wheat flour impacts the persistence of STEC strains, aiding to a more complete understanding of the fitness of STEC populations in wheat flour.

P3-97 Thermal Resistance of *Enterococcus faecium* NRRL B-2354, *Escherichia coli*, and *Salmonella* in Chocolate Chip Cookies at Three Moisture Levels

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Introduction: *Salmonella* is a pathogen of concern in low moisture ingredients such as wheat flour. *Enterococcus faecium* NRRL B-2354 is commonly used as a surrogate for foodborne pathogens in low moisture products.

Purpose: This study investigated the thermal resistance of *E. coli, Salmonella* and *E. faecium* in chocolate chip cookies at three moisture levels.

Methods: Wheat flour was inoculated with freeze dried *E. faecium,* a cocktail of *E. coli*, or *Salmonella* at approximately 7 log CFU/g, and acclimated at 25°C for 24 h.

Inoculated flour was combined with other ingredients to achieve target moistures. Samples were dispensed in to pouches, vacuum sealed, exposed to 65 to 85°C and pulled at predetermined intervals. Surviving organisms were counted, averaged, then transformed to log CFU/g.

Results: The dough moistures were 7.86% (a_w 0.647), 12.10% (a_w 0.765), and 17.10% (a_w 0.796). *E. faecium* was the most heat resistant, followed by *Salmonella* and *E. coli*. *E. faecium* was at least three times more heat resistant compared to *Salmonella* under the same moisture and temperature condition. The *E. faecium* to *Salmonella* ratio was larger at higher moisture levels. *Salmonella* D values at 70, 75, and 80°C for 17.10% cookie moisture were 2.36, 0.90, 0.48 min, respectively, while the D values at 75, 80 and 85°C for 12.10% moisture were 1.17, 0.78, and 0.44 min, respectively, and for 7.86% moisture were 2.39, 1.07, and 0.48 min, respectively.

Significance: *Salmonella* resistance increased as cookie moisture level decreased. Thermal resistance data showed that *E. faecium* would be a suitable surrogate for in-plant validation studies of chocolate chip cookies during baking. This data can be used as a scientific basis for thermal validation of similar soft cookies with similar moisture.

P3-98 Impact of Chlorinated Water on Pathogen Inactivation during Wheat Tempering and Resulting Flour Quality

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❖ ***Developing Scientist Entrant***

Introduction: Outbreaks of enteric pathogens linked to wheat flour have led the wheat milling industry to seek solutions addressing this food safety concern. Chlorinated water at 400-500ppm has been used in the flour milling industry as a tempering aid to control growth of yeast and mold in tempering bins. However, the effectiveness of chlorinated water on inactivating enteric pathogens on wheat kernels remained unknown.

Purpose: Quantify pathogen reduction on wheat kernels and changes in flour quality when chlorine is added to wheat tempering water.

Methods: Five strains of Shiga-toxin producing *Escherichia coli* (STEC) and two strains of *Salmonella* were inoculated onto hard red spring wheat at 7 log CFU/g and stored at room temperature for 1-month. Inoculated wheat was tempered with four concentrations (0, 400, 800, 1200ppm) of chlorinated water (pH 6.5). The effect of chlorine was determined by calculating the change in cell density for each level using the response at 0ppm as a reference. Uninoculated wheat tempered with chlorinated water was used to measure flour quality parameters such as gluten functionality.

Results: Changes in pathogen density over 18 hours ranged from -1.93 to -0.35 log CFU/g at 400ppm chlorine, and changes at 800ppm and 1200ppm were not significantly ($p>0.05$) different from those at 400ppm. Significant ($p< 0.05$) differences in the extent of reduction were observed among strains. However, the impact of chlorinated water in reducing native microbes on wheat kernels was minimal, ranging from -0.69 to -0.22 log CFU/g for all concentrations. No significant ($p>0.05$) changes occurred in flour quality and gluten functionality during the breadmaking process.

Significance: The data show chlorinated water at concentrations currently used in industry for tempering could reduce enteric pathogen numbers by 0.85 log CFU/g for STEC, and 1.71 log CFU/g for *Salmonella*, with no significant effects on flour quality and gluten functionality.

P3-99 Inactivation of *Salmonella* and Shiga-Toxin Producing *Escherichia coli* on Soft Wheat Kernels Using Vacuum Steam Pasteurization

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❖ ***Developing Scientist Entrant***

Introduction: Recent foodborne outbreaks of Shiga toxin-producing *E. coli* (STEC) and *Salmonella* traced back to low moisture foods have highlighted the need for treatments to control these pathogens in foods such as flour. In previous lab-scale studies, vacuum steam treatment was capable of reducing microbial loads on soft wheat kernels while maintaining the flour quality and gluten functionality. While demonstrating the potential for pathogen reduction, the previous system was limited in capacity and fine temperature control. This study used a redesigned lab-scale vacuum steam pasteurizer to assess how effective the system could inactivate the pathogens on wheat kernels. The improvements include greater capacity, an improved insulated treatment chamber, a computer-controlled steam and vacuum system, and continuous sample temperature recording.
Purpose: Quantify pathogen reduction on wheat kernels using vacuum steam pasteurization.
Methods: STEC O26:H11 and *Salmonella enterica* Agona were inoculated onto soft red wheat at 8 log CFU/g and water activity adjusted to 0.51 ± 0.02 after inoculation. The treatment chamber was filled with 1.9 kg of wheat kernels and was preheated to 45°C with steam. Three technical replicates of 25g inoculated samples were placed in gauze bags individually and buried in the pre-heated wheat before sealing the chamber and activating the vacuum to 272mbar. A thermocouple placed in the middle of the filler wheat continuously recorded the temperature. Samples were enumerated at 0, 2, 4, 6 mins at the target temperature of 75°C to quantify changes in cell density over time.
Results: A log reduction of 4.47±0.14 log CFU/g was observed for *Salmonella* Agona, and reduction of 4.64±0.43 log CFU/g was observed for *E. coli* O26:H11 after 6 minutes at 77 ± 1°C.
Significance: Vacuum steam treatment of soft wheat for 6 minutes effectively leads to a 4.5 log reduction of *Salmonella* and *E. coli* O26:H11.

P3-100 Determination of the Thermal Inactivation Kinetics of *Salmonella* and a Surrogate in Milk Powder as Impacted by Water Activity and Protein Content

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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 powder to then evaluate the suitability of the chosen surrogate.
Methods: Non-fat dry milk (NFDM) was co-inoculated with *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 (ca. 9.0 log CFU/g). Inoculated NFDM was then equilibrated to the desired a_w of 0.20 ± 0.02. After five days, NFDM was loaded into thermal-death-time disks and heated in a water bath (75°C, 80°C, or 85°C), randomly removed at predetermined time points, and immediately cooled to halt inactivation. NFDM was removed from the disks and plated on tryptic soy agar supplemented for enumeration of *Salmonella* and *E. faecium*. Experiments were replicated at least three times. Similar experiments at other water activity levels and a second milk powder (85% protein content) are currently in progress.
Results: The *D*-values at 75°C, 80°C, and 85°C for *Salmonella* in NFDM were 17.1 ± 1.1 min, 7.4 ± 1.8 min, and 3.4 ± 0.5 min (R^2=0.89-0.99), respectively. The *D*-values at the same temperatures for *E. faecium* were 16.2 ± 1.1 min, 7.5 ± 2.1 min, and 5.0 ± 2.7 min (R^2=0.74-0.96), respectively. The z-values for *Salmonella* and *E. faecium* in NFDM (a_w=0.2) were 14.5 ± 2.1°C (R^2=0.9474) and 20.6 ± 4.2°C (R^2=0.751), respectively.
Significance: Overall, *E. faecium* exhibited greater thermal resistance than *Salmonella* in most experiments, lending to its potential suitability as a surrogate for *Salmonella* in NFDM. This data will be key in understanding the effects of water activity on thermal resistance of pathogens in milk powders of varying composition.

P3-101 Current Inactivation Strategies for *Cronobacter sakazakii* in Foods: A Systematic Review

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Introduction: *Cronobacter sakazakii* (CS) is of major concern for immunocompromised individuals and critically dangerous amongst newborns. CS has been identified in cases of meningitis, enterocolitis and septicemia of neonates, with mortality rates of 40 to 80 percent. CS is a gram-negative non-spore forming bacterium found in a wide range of products and production environments. CS forms biofilms, presents resistance to osmotic stress and low water activity and it is able to survive up to 2 years in sealed dry infant formula.
Purpose: To conduct a systematic review to investigate current inactivation strategies for CS.
Methods: Research basis such as Scopus and Web of Science were used, with the keywords "inactivation", "drying", "thermal inactivation" and "*Cronobacter sakazakii*". The search returned a total of 188 articles and 70 were selected. Articles selected were from a 10-year time period (2011-2021), that evaluated quantitatively the reduction of CS in foods.
Results: Approximately half of the studies focused on foods of animal origin, mainly focused on dairy products (dry milk, infant formula), followed by vegetable products (spices, infant tea, flour, cut fruit, mushrooms, lettuce and vegetable ingredients for infant formulas). Studies focused on emergent technologies as pre or post-treatment strategies, either chemical, with use of natural extracts or organic acids, or physical, ranging from high pressure, vacuum, freeze drying, ultrasound, pulsed light, Near-Infrared (NIR) and UV radiation and Cold Atmospheric Plasma (CAP), isolated or combined in synergistic behavior, and as alternative thermal treatments, such as microwave and radio-frequency heating. Reductions obtained ranged from 1 to up to 5 total logarithmic cycles.
Significance: The systematic review allowed to outline a trend towards emergent technologies, identifying current challenges in CS control: strategies of reducing the intensity of inactivation steps, minimizing undesirable changes, reducing the initial contamination load, combining alternative treatment, as well as evaluating storage survival conditions.

P3-102 Levels and Distribution of *Salmonella* in Naturally Contaminated Cashews

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❖ ***Developing Scientist Entrant***

Introduction: Naturally contaminated raw materials associated with foodborne illness outbreaks are rarely available for evaluation of concentration and distribution of the outbreak pathogen.
Purpose: To evaluate the levels and distribution of *Salmonella* present in naturally contaminated raw cashews from a fermented cashew "cheese" outbreak.
Methods: Two unopened 22.7-kg boxes from a single lot of cashew kernel pieces linked to a salmonellosis outbreak were each divided into seven approximately equal units. Three 10-g subsamples per unit were evaluated for aerobic plate count (APC) and coliform counts, and 10 50-g subsamples per unit were enriched for the presence of *Salmonella.* Presumptive-positive colonies were confirmed using CHROMagar Salmonella and real-time PCR (*invA*) and then serotyped using standard methods; most probable number (MPN) was determined.

Results: APC and coliform counts ranged from 1.91 to 4.95 (mean 2.49 ± 0.56 log CFU/g) and 0.60 to 4.92 (mean 1.82 ± 0.76 log CFU/g), respectively. *Salmonella* was recovered from four (Box 1) or seven (Box 2) units. One of 10 subsamples were positive in all but four cases; one (Box 1) and three (Box 2) units had two positive subsamples. MPN values were 0.0014 MPN/g (95% CI: 0.00063–0.0036; Box 1) and 0.0031 MPN/g (95% CI: 0.0017–0.0057; Box 2). *Salmonella* Urbana was identified in Box 1 (three subsamples) and Box 2 (eight); *Salmonella* Fresno (one), II28:m,t (one), and an untypeable isolate (one) were unique to Box 1, and *Salmonella* Nima (two) was unique to Box 2. Two serotypes (Urbana, untypeable) were recovered from a single Box 1 subsample. Of the four serovars recovered from the cashews, *Salmonella* Urbana is the only one in common with the four outbreak-associated serovars.

Significance: Understanding distribution and concentration of *Salmonella* in naturally contaminated products provides important information for outbreak investigations, root cause analysis, hazard analysis and risk assessments.

P3-103 High Level and Heat Resistance of Natural Microflora Contaminated in Peppers in Sichuan Province, China

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Introduction: Pepper spices are often sun-dried and may be severely contaminated by microorganisms from harvest to consumption. Microflora in dry peppers can cause food-borne illness and food spoilage at adequate conditions, posing threat to consumers and food industries. Understanding the contamination level of microflora and their heat resistance provide essential information to detect and control the microorganisms in spices.

Purpose: We aimed to quantify the amount and heat resistance of natural microflora in local pepper spices.

Methods: A total of 180 dry peppers (60 each of Sichuan pepper, white pepper and chilli pepper samples) from retail stores in 22 districts in Sichuan Province, China, were investigated their natural microflora contamination. On this basis, the three samples with the highest bacterial counts of either pepper were selected for isothermal inactivation studies at 80-100℃, respectively. We also investigate the effect of water activity at 25℃ ($a_{w,25℃}$ in white pepper) on iso-thermal inactivation efficacy. And other six chilli pepper samples with the same water activity (adjusted $a_{w,25℃}$ to 0.5) were used to observe microbial heat resistance at at 95-105℃ .

Results: The data showed severe contamination of natural microbial in all spice samples (powdered pepper are even worse than granule peppers). According to the index, a sum of 85.00%, 98.33% and 50% of Sichuan pepper, white pepper and chilli pepper were off-specification. Completely different inactivation kinetics of the total-plate-count (TPC) were observed in three pepper samples, indicating that the control of natural microflora in spices are hardly standardized. The heat resistance parameters of total-plate-count in spices were very high. For instance, D_{105} value of TPC in chili powders are 8-22 min.

Significance: The microbial safety of spices in retail market raises our concerns. Heat resistance of TPC in tested samples were extremely high (even by adjusting the $a_{w,25℃}$), raising difficulties to reduce the contamination level of dried peppers effectively by heat.

P3-104 Heat Resistance and Transcriptome Sequencing of *Salmonella enterica* Enteritidis PT 30 at Different Degree of Desiccation

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is known for its long-term survivability and high heat-resistance in low-moisture foods (foods with water activity a_w at 25°C less than 0.85). We previously have reported that decreasing $a_{w,T}$ can exponentially increase the heat resistance D_{80} value of *Salmonella*, yet how z_T value changes with $a_{w,T}$ and the specific molecular mechanisms underlying its persistence to desiccation is not clear.

Purpose: We aimed to quantify the change of heat resistance parameters and transcriptomes of *Salmonella enterica* Enteritidis PT 30 (*S.* Enteritidis) at different levels of $a_{w,T}$.

Methods: *S.* Enteritidis was inoculated on filter paper and equilibrated at different $a_{w,25}$ levels via saturated salt solution jars. The survivors was tracked up to 70 d, and the heat resistance parameters of bacterial cells were determined by high-temperature water-activity cells previously developed by Washington State University. The transcriptomes of cells at $a_{w,25}$ 0.11, 0.32, 0.65, and 0.85 for 24 h, 72 h, and 168 h were compared by the RNA-seq approach.

Results: *S.* Enteritidis exhibited good survival at a_w 0.11 to 0.80, yet the most reduction was > 3 log at $a_{w,25C}$ 0.65. The D_T values of *S.* Enteritidis increased at reduced $a_{w,T}$ values (same as the reported trend); while the z values were independent from a_w. For instance, the z value of *S.* Enteritidis at $a_{w,T}$ 0.30, 0.60, and 0.80 were 14.3, 13.5, and 13.8°C, respectively. The transcriptome of *S.* Enteritidis was significantly altered under different desiccation stresses, but there was no linear change with the decrease of a_w, which may explain the different survival curves observed in the actual experiment. Genes involved in heat and cold shock response, DNA protection and regulatory functions likely play roles in cross protecting *Salmonella* from desiccation and starvation stresses.

Significance: Our study reported the survival, heat resistance, and the molecular mechanisms of *S.* Enteritidis at different degrees of desiccation.

P3-105 Guaiacol Production is Confirmed in One of Three Novel Species of *Alicyclobacillus*

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❖ ***Developing Scientist Entrant***

Introduction: Some species of *Alicyclobacillus* can spoil products like juice through production of the metabolite guaiacol; however, there is a gap in understanding the distribution of guaiacol production across the genus among newly designated *Alicyclobacillus* spp.

Purpose: This study assesses guaiacol production in species of *Alicyclobacillus* commonly found in juice products and uses whole genome sequencing (WGS) to reassign the identities of isolates previously classified as *A. acidoterrestris* or *A. acidocaldarius*.

Methods: WGS data was collected on 49 isolates and compared to 17 *Alicyclobacillus* type strain genomes using Average Nucleotide Identity Based on BLAST analysis (ANIb) with pyani version 20171222. Core single nucleotide polymorphisms (cgSNPs) were extracted using kSNP version 3.1. Both 16S rRNA and *rpoB* gene sequences were extracted from genome assemblies through BLAST and used to create RAxML phylogenetic trees in Geneious Prime using the GTR gamma nucleotide model and 1000 bootstrap replicates. Guaiacol production was assessed using a CosmoBio peroxidase assay and Biomerieux's VeriPro Assay across 26 *Alicyclobacillus* isolates, tested in triplicate.

Results: The genomic analysis and phylogenetic trees revealed multiple strains clustered away from existing type strains in this genus. The ANIb values were <95% compared to type strains, which is the cutoff for species delineation, and three new species relevant to the food industry were proposed. These three species are *A. mali*, *A. suci*, and *A. fructus*. Of these new species, *A. suci* was able to produce guaiacol. *A. suci* is closely related to and has previously been identified as *A. acidoterrestris*. All guaiacol-testing methods indicated that *A. suci* and *A. acidoterrestris* can produce guaiacol, while *A. acidocaldarius, A. mali,* and *A. fructus* cannot.

Significance: This study reassigns the identities of industrially relevant species of *Alicyclobacillus* and confirms high variability in guaiacol production among species, which must be considered in industrial testing.

Poster

P3-106 Sensitivity of Planktonic Cells and Spores Suspension of *Bacillus amyloliquefaciens, Bacillus atrophaeus, Alicyclobacillus acidoterrestris,* and *Geobacillus stearothermophilus* to Elevated Hydrostatics Pressure Augmented with Mild Heat and Acidic Bactericidal Compounds

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Introduction: As one of the leading commercial methods for non-thermal processing of food commodities, application of elevated hydrostatic pressure continues to gain importance in the private industry. Utilization of acidic antimicrobials could augment the decontamination effects of the technology, assist in optimizing the processing costs, and provide residual bactericidal effects during shelf-life.

Purpose: Current study compared pressure-sensitivity of planktonic cells and endospores of *Bacillus amyloliquefaciens, Bacillus atrophaeus, Alicyclobacillus acidoterrestris,* and *Geobacillus stearothermophilus* treated by hydrostatic pressure in presence of malic and citric acids.

Methods: Pressure intensity of 650 MPa was used at 60.0 °C with and without 1% malic and citric acids for treatments lasting up to seven minutes. The temperature was precisely controlled using a water jacket surrounding the pressure processing chamber that is mechanically linked to a circulating water bath. The data are statistically analyzed using Tukey- and Dunnett's-adjusted ANOVA at type I error level of 0.05.

Results: The pressure treatment at 650 MPa resulted in reductions of ($P < 0.05$) nearly 6 and <3 logs CFU/mL of *Bacillus amyloliquefaciens* planktonic cells and endospores, respectively. Addition of 1% malic acid provided about 90% further reduction ($P < 0.05$) of the microorganism's endospores. Among the endospores studies, *Geobacillus stearothermophilus* exhibited highest resistance to pressure-based treatments. The D-values for inactivation of this microorganisms at 650 MPa and 60.0 °C were 2.34, 24.21, 9.98, and 11.40 mins for planktonic cells, endospores without any antimicrobial, endospores with 1% malic acid, and endospores with 1% citric acid, respectively.

Significance: Pressure treatments of 650 MPa at 60.0 °C resulted in > 5 logs of reductions of the planktonic cells. Treatments had appreciably lower efficacy against the endospores of the same microorganisms. Addition of acidic antimicrobials could further enhance the decontamination efficacy of the pressure-based treatment for decontamination of planktonic spoilage microorganisms and the bacterial endospores.

P3-107 Performance Evaluation of bioMérieux VERIFLOWTM *Alicyclobacillus* Assay and Japan Fruit Juice Association Method for the Testing of *Alicyclobacillus* Species in Juice Raw Materials and Finished Products

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Introduction: Juice manufactures commonly use Japan Fruit Juice Association (JFJA) method for the testing of *Alicyclobacillus* species in Japan. It is easy to use; however, it takes up to 10 days to get the final result, which is time-consuming.

Purpose: This study compares the performance of bioMérieux VERIFLOW™ *Alicyclobacillus* (ACB) assay with JFJA method to evaluate the use of rapid methods for testing *Alicyclobacillus* spp. in juice raw materials and finished products.

Methods: A total of fourteen samples, including three types of juice products (orange, apple, and pineapple), their concentrates, and syrup (glucose/fructose mixture) from two different suppliers were selected. Samples were firstly evaluated for the natural contamination of *Alicyclobacillus* species. For those without any natural contamination, culture suspensions of *Alicyclobacillus acidoterrestris* ATCC 49025^T were inoculated into each sample at two different levels, 1-19 CFU/ml or 20-60 CFU/ml. After homogenization, samples were then tested using ACB assay and JFJA in parallel. Finally, the performance of ACB assay was analyzed for its sensitivity and relative trueness.

Results: All 14 samples tested were negative of *Alicyclobacillus* species. They were subsequently used for the inoculation experiment. Overall, the sensitivity of ACB assay was 88% and the relative trueness was 83.3%. For juice finished products, the sensitivity and relative trueness were both 100%. For juice concentrates and syrup, the sensitivity was 81% and the relative trueness was 70.8%. In summary, ACB assay can accurately detect *Alicyclobacillus* species in juice finished products, even at low levels of contamination (2 CFU/ml), while it may have limitations on testing raw materials, such as pineapple juice concentrate and syrup. Labs should perform method comparisons prior to its routine use.

Significance: The ACB assay provides a 52 h rapid solution that enables juice producers to release juice finished products 8 days earlier compared with using JFJA method. This rapid solution will help juice producers reduce costs associated with inventory, in-process contamination, production inefficiencies, and holding products at risk.

P3-108 Heat and Sanitizer Resistance of *Sporolactobacillus* spp. Causing Deterioration in Acid Foods

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Introduction: *Sporolactobacillus* spp. have been implicated in souring and swelling of acid foods such as fruit juices and canned fruits. However, little is known about their control method, partly due to the difficulty of spore formation by these bacteria.

Purpose: This study presents the heat and sanitizer resistance characteristics of *Sporolactobacillus* spores to help preventing the quality abnormalities caused by them.

Methods: *Sporolactobacillus putidus* tokai37 and *Sporolactobacillus* sp. tokai38, identified as related species of *S. terrae*, were used in this study. Both strains were isolated from foods. First, conditions for the spore formation of the strains were investigated. Then, the spore suspension was prepared for each strain, and heat and sanitizer resistance tests were performed. Sanitizers were obtained from Ecolab.

Results: For spore formation, 1/2 Potato Dextrose Agar containing 3% $CaCO_3$ and 0.46% Murashige & Skoog Plant Salt Mixture was used and cultured for 3 weeks at 35 °C under anaerobic condition. In the heat resistance test, strain tokai37 had $D_{95°C}$ = 6.1 min, $D_{92°C}$ = 17.9 min, $D_{89°C}$ = 50.6 min, and z-value = 6.5 °C, and strain tokai38 had $D_{95°C}$ = 5.2 min, $D_{92°C}$ = 16.3 min, $D_{89°C}$ = 32.7 min, and z-value = 7.7 °C. In the sanitizer resistance test at 50 °C and 1 min contact, the reduction rates of strain tokai37 in 0.5% Oxonia Active 110, 0.5% vortex, and 0.01% sodium hypochlorite were >5.5D, >5.5D, and 3.2D, respectively. Similarly, the reduction rates of strain tokai38 in 0.5% Oxonia Active 110, 0.5% vortex, and 0.01% sodium hypochlorite were >4.5D, >4.5D, and 3.8D, respectively.

Significance: This study provides novel inactivation conditions of *Sporolactobacillus* spores, the acid food spoiler. Identifying the sterilization indices for pasteurization and production-line cleaning is critical for reducing the risk of *Sporolactobacillus* spoilage in food production and preventing deterioration of product quality by overheating.

P3-109 Survival of *Lactobacillus acidophilus* 5 and *Lacticaseibacillus casei* 01 in Alginate Edible Coatings in Fresh-Cut Mango and Melon during Storage

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Introduction: Probiotics are beneficial microorganisms that may antagonize the growth of pathogens. Edible coatings have been suggested as alternatives for the preservation of fresh-cut fruits and probiotic vehicles, increasing the shelf-life and safety of this ready-to-eat food. Few is known about the survival of lactobacilli probiotics in alginate coatings when applied to fresh-cut fruits.

Purpose: To evaluate the survival of lactobacilli probiotics in isolated or mixed culture in alginate edible coatings during refrigerated storage in fresh-cut mango and melon.

Poster

Methods: Coatings were formulated by adding alginate (1.5 g/100 mL) in sterile distilled water (70 ºC, 240 rpm for 30 min). Glycerol (0.75 g/100 g) and sunflower oil (0.04 g/100 g) were used as plasticizer and water-barrier agents, respectively. Tween 80 (0.05 g/100 g) was added to improve fruit adherence. *Lactobacillus acidophilus* 5 (La-5) or *Lacticaseibacillus casei* 01 (Lc-1) were incorporated isolated (~8 log CFU/mL) or mixed (1:1; ~8 log CFU/mL) in coatings. All coatings were applied on mango and melon pieces by immersion (2 min) followed by a new immersion in the crosslinking solution (1 min). Samples were stored at 7 ºC for 16 days. The viability of probiotics was assessed after application (day zero) and 4, 8, 12 and 16 days by plating in modified-MRS (La-5) and MRS-vancomicin (0.05 mg/ mL; Lc-1).

Results: La-5 counts did not change in mango or melon during the storage period evaluated. Lc-01 counts decreased ~1 log CFU/g after 8 days in melon but increased ~1.5 log CFU/g after 16 days, reaching counts similar to day zero. When tested in mixed culture (1:1 ~ 8 CFU/mL) counts did not change in mango but increased ~2 log CFU/g in melon after 16 days.

Significance: Results show the viability of probiotics in alginate edible coatings in fruit during storage and highlight that survival varies with the strain and the fruit tested.

P3-110 Improve Taste and Microbial Quality of Plant-Based Meat Analogue Thanks to Food Cultures: The Example of Soy-Minced Meat Analogue

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Introduction: The plant-based meat alternative (PBMA) market has experienced high growth mainly driven by flexitarians (from 14% in 2018 to 36% in 2020). They aim for healthier, high quality food at an affordable price. Because of too short shelf life, overly processed and sometimes poor sensory experience, flexitarians are not always satisfied by the PBMA available at retail.

Purpose: The purpose was to evaluate if the addition of specific food cultures (*Leuconostoc carnosum Latilactobacillus curvatus, Latilactobacillus sakei*) can improve the microbial quality offering longer shelf life while substituting additives (acetic acid …). The focus was also on the evaluation of sensory characteristics (intensity of beany note and umami flavor).

Methods: Minced meat analogue from soy was inoculated with food cultures (concentration between 6.5 and 7.0 Log cfu/g) and MAP packed. Shelf-life tests (study of the main microbial spoilers) and challenge tests (*L. monocytogenes* and *B. cereus*) were initiated considering a shelf life of 21 days at 7°C. Metagenomics analyses (D0, D16, D21) were carried out over the shelf life. Physicochemical parameters (pH, sugars, organic acids) evolution was also measured. Finally, sensory evaluation (D0, D7, D16, D21) was performed by a panel (hedonistic approach) and results compared with volatile organic compounds. All analyses were carried out in triplicate.

Results: Food cultures significantly modified the bacterial ecosystem of the minced meat analogue helping suppress the growth of yeasts, *B. cereus* and *L. monocytogenes*. The drop in pH was faster when culture was added, but final pH difference between the control and the batches with culture was less than 0.2 pH U. From a sensory point of view this pH difference was not negative.

Significance: The addition of a properly selected food culture can improve the overall quality of PBMA. This ingredient helps food suppliers develop a PBMA that meets flexitarian demands.

P3-111 Evaluation of the Microbial Quality of Plant-Based Meat Analogs

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Introduction: Meat analogs mimicking traditional meat in its functionality, bearing similar texture and sensory attributes receive raising interest of consumers. However, limit information is available about the microbial quality of meat analogs (MA).

Purpose: This study aimed to assess the microbial quality of MA during storage by investigating the behavior of native bacteria and *Pseudomonas fluorescens*.

Methods: Ground beef (GB) and pea-based (PBM), and soy-based meat analogs (SBM) were purchased from grocery stores and stored at 4 °C for 10 days. Their total aerobic bacteria (APC), lactic acid bacteria (LAB), yeast and mold (Y/M), *Enterobacteriaceae*, and native *P. fluorescens* were monitored on days 0, 3, 5, and 10 by plating onto non-selective and selective agar. Part of the samples were also artificially inoculated with 3 Log CFU/g of *P. fluorescens*, changes of which were also studied during storage.

Results: The initial APC, LAB, Y/M, *Enterobacteriaceae*, and native *P. fluorescens* in three types of meats ranged from 3.10 to 5.62, 2.05 to 4.39, < 2.00 to 3.46, < 2.00 to 2.90, and < 2.00 to 3.87 Log CFU/g, respectively. GB had significantly higher ($P < 0.05$) initial microbial load than PBM and SBM. During storage, native and inoculated *P. fluorescens* increased faster in GB and PBM than in SBM, while APC and LAB increased faster in MA than in GB. Greater than 3-log of *Enterobacteriaceae* growth was observed in PBM, as compared to < 1-log increase in GB and SBM. APC and inoculated *P. fluorescens* exceeded 6-log on day 5 in GB and PBM, with off-odor detected in GB on the same day. All meats were spoiled on day 10 due to the over-growth of spoilage microorganisms.

Significance: This study provides insight into the microbial quality of MA, which is needed for establishing quality assurance and food safety plans for them.

P3-112 Using Computer Modeling to Evaluate Suitability of Intervention Targets for Reducing Food Spoilage: Example of Raw Milk Somatic Cell Count as Target for Reducing Spores Responsible for Spoilage of Fluid Milk

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Introduction: Cost is usually the main factor when deciding on an intervention for reducing food spoilage while the benefit is often neglected or hard to determine precisely. Computer modeling using existing research data offers a useful tool for precise estimation of the benefits.

Purpose: Introducing an example of using computer modeling and existing research data to evaluate suitability of the intervention target.

Methods: Previously published data, from a cross-sectional farm study, were re-evaluated using a newly developed Monte Carlo simulation model that predicts growth of psychrotolerant sporeformers during refrigerated storage of pasteurized milk. Data on Somatic Cell Counts (SCC) together with correlated Psychrotolerant Spore Counts (PSC) in raw milk from 92 farms were used to predict expected shelf-life of pasteurized milk made from raw milk produced at these farms. This baseline prediction for pasteurized milk was used to evaluate increase in shelf-life in different "What-If"' scenarios that evaluated three possible interventions targeting SCC to reduce PSC in raw milk: (i) Using raw milk from only the 10 farms with the lowest SCC (<100,000 cells/mL), (ii) Excluding the raw milk from the 10 farms with the highest SCC (>630,000 cells/mL), (iii) Using raw milk from only farms with SCC below the limit of 400,000 cells/mL

Results: The model predicted that eliminating raw milk from 10 farms with the highest SCC or from farms with SCC above the limit of 400,000 cells/mL would only extend the shelf-life from 18.4 to 18.6 and 18.9 days, respectively. The model also predicted that using raw milk from the 10 farms with the lowest SCC would extend the shelf-life by 1.7 days.

Significance: Our example demonstrates how low-cost computer modeling can inform decision making and potentially prevent investments where cost surpasses the expected benefits.

P3-113 Methods for Conducting Challenge Studies in White Pan Bread Inoculated with *Penicillium roqueforti*

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Introduction: There is no universally accepted method for conducting filamentous fungi challenge studies in food products.
Purpose: Two methods (visual inspection or microcalorimetry) were used to assess the efficacy of mold inhibitors in bread inoculated with *Penicillium roqueforti.*
Methods: *Penicillium roqueforti* culture was grown on malt extract agar for 7 d. Spores were harvested and diluted to 100 spores/µL. Inoculated bread included a no-add control, pH 5.16, and 0.75% calcium propionate ("CalPro"), pH 5.17. For studies monitoring visual growth, two loaves of bread were spot inoculated on the sides and bottom of the loaf. Each loaf was inoculated at 36 sites with 10 µL of inoculum at each site. The loaves were placed in a clear bread bag, kept at room temperature (ca. 20°C), and checked daily for signs of visual *Penicillium* growth. For studies using microcalorimetry, the bread was trimmed into cubes to fit into a 20 mL ampule. Each cube was inoculated on the crust surface with 3 µL of inoculum, sealed closed, and placed into a TAM III microcalorimeter set at 25°C. Heat output was measured on a µW scale.
Results: Two ampules of CalPro began showing activity at 200 h and reached a maximum (80 µW) by 375 h. One ampule showed no activity. Two ampules of control began showing activity at 50 h with a maximum (80-90 µW) at 150 h, and one began showing activity at 100 h with a maximum (70 µW) at 175 h. Control began showing visible mold at day 5 with 100% of sites (n=36) molded by day 7. CalPro began at day 13 with 100% (n=36) of sites molded by day 20.
Significance: Microcalorimetry shows promise in the ability to quickly screen fungal growth in inoculated bread products and warrants further method development.

P3-114 Clean Label Preservation System to Control Ropiness Spoilage in Bread

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Introduction: Outgrowth of *Bacillus subtilis* spores during storage of contaminated bread results in the formation of rope spoilage, causing deterioration of bread by off-odors and sticky bread crumb leading to economic loss.
Purpose: The purpose of this study is to show the efficacy of conventional organic acid salts and clean label preservation solutions in bread baking to prevent spoilage by *Bacillus subtilis* spores outgrowth.
Methods: A 6 $\log_{10}$ CFU/mL spore solution of *Bacillus subtilis* ATCC 8473 was used to inoculate bread dough (100% wheat flour, 0.62% instant yeast, 1.62% salt, 3.78% sugar, 2% sunflower oil and 70% tap water (baker's percentages)). Control breads without preservatives were compared with bread containing 0.3% Probake CP (calcium propionate), 0.5% Progusta CA (calcium acetate) or 0.5% IsoAge Ca (natural vinegar) (based on flour weight). The doughs were mixed, divided into 70 g portions and baked for 20 minutes at 210°C in a convection oven, after which breads were cooled for 1 hour, transferred to plastic bags and incubated at 30°C for a maximum of 14 days. Buns were inspected on smell and texture and *Bacillus subtilis* was enumerated in triplicate by surface plating on BHI agar plates after 4, 7, 10 and 14 days of storage. Significance of the differences between treatments was determined using one-way ANOVA.
Results: Growth of *Bacillus subtilis* in control breads reached 8.2±0.2 $\log_{10}$ CFU/g after 14 days. Addition of 0.3% Probake CP, 0.5% Progusta CA and 0.5% IsoAge Ca reduced counts significantly ($P<0.05$), to respectively 2.4±0.4, 2.7±0.7 and 2.9±0.6 $\log_{10}$ CFU/g after 14 days. None of the treated buns showed any sign of spoilage, while the control treatment showed texture loss and clear off-odors.
Significance: Incorporating conventional organic salts or natural vinegar in bread can significantly reduce the growth of *Bacillus subtilis* during storage, decreasing the economic loss due to ropiness.

P3-115 Withdrawn

P3-116 Machine Learning-Based Classification of *Salmonella enterica* Serovar Typhimurium Isolates Based on Transcriptomics Data Identifies Signatures of Stress Response

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Introduction: *Salmonella enterica*, a major enteric foodborne pathogen with a highly variable pathogenicity profile, undergoes numerous stresses in its route to infection, such as under various food processing-related stressors, antibiotics stress, and infection-related stress. 'Omics' data can help identify signatures of *Salmonella* survival under various infection and environmental conditions, which in turn could help improve the accuracy of microbial predictive models.
Purpose: The aim of this study was to use machine learning to identify genes associated with *Salmonella* stress response using transcriptomic (RNA-Seq) data from *in-vitro* studies simulating bacterial stress conditions.
Methods: RNA-Seq transcriptomics data for *Salmonella enterica* serovar Typhimurium subjected to 32 infection and environment-related stressors was obtained from public repositories and transcriptomics studies. Supervised machine learning algorithms (random forest (RF), support vector machine (SVM), and Elastic Net) were used to classify *Salmonella* into stress response classes based on differential gene expression. Subsequently, genes were ranked according to their impact on classification to create a genetic signature indicative of *Salmonella* stress response.
Results: Elastic Net showed the best classification statistics (Area under the Receiver Operating Characteristic curve; AUC-ROC = 0.73; classification accuracy = 0.68) compared to RF and SVM (AUC-ROC = 0.66 and 0.64; accuracy = 0.60 and 0.62, respectively). A large number of genes reported as being associated with stress response in prior studies were identified as important indicators of *Salmonella* stress response. These included transcriptional and stress regulators, and genes associated with virulence.
Significance: Machine learning and RNA-Seq expression data were found to be good at classifying bacterial sequences based on stress response-related gene expression. The genes identified as important in this study could be used as predictive biomarkers in 'omics'-informed microbial predictive models, which in turn could help re-evaluate its overall survival ability in food systems and infectivity in host systems.

P3-117 Evaluating the Growth of Spoilage Relevant Bacterial Isolates on Baby Spinach

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Introduction: Food spoilage contributes to food waste. Measuring the growth of spoilage relevant bacteria on baby spinach can provide insights into microbial spoilage, allowing for the development of models that predict the product's shelf life.
Purpose: The aim of this study is to measure the growth of spoilage relevant bacteria on baby spinach.
Methods: Packaged baby spinach (n = 3) was stored at 4˚C and tested over 20 days of shelf life during September and October 2019. Bacterial isolates, collected during testing, were characterized by 16s rDNA sequencing, followed by sequencing the *ileS* gene if an isolate was identified as *Pseudomonas* sp. Isolate sequence data was used to construct maximum-likelihood phylogenetic trees that were used to identify a subset of representative isolates. Rifampicin-resistant mutants were selected from this subset for growth experiments, to allow for differentiation of the inoculated strain from the microbiota of the spinach. Mutants were individually spot inoculated onto baby spinach that was previously washed with acidified sodium chlorite. The inoculated samples were stored at 6˚C and tested over 24 or 30 days, depending on the isolate.

Results: Among 17 of the initially selected isolates, rifampicin-resistant mutants could be selected for 16 isolates. For 10 isolates, growth characteristics of the rifampicin-resistant strain were substantially different from their respective parent strain; hence only 6 strains were used to obtain strain specific growth data on inoculated spinach. Absolute log growth ranged from 5.8 log (*Pseudomonas rhodesiae*) to 3.33 log (*Pseudomonas* sp.) among the different strains (average of 3 replicates). Overall microbial growth in spinach ranged from 4.8 to 7.1 log.

Significance: Our study provides initial growth data that can be used for modeling spoilage in baby spinach, but also illustrates the challenges associated with obtaining strain specific growth rates for products with large and diverse microbiota.

P3-118 Evaluation of Sources for *Salmonella enterica* Infections Using Genomic Data and Machine Learning

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Introduction: Salmonellosis, which is caused by the bacterium *Salmonella enterica*, is one of the most frequent foodborne infections in the world. Effective interventions necessitate determining the infection source, which is difficult since transmission can occur through several routes, including contaminated environments, and different foods. Variation in virulence genes has allowed source tracking, allowing isolates from infected individuals to be traced to specific animal food sources or environmental reservoirs.

Purpose: This study was aimed at developing a model for source attribution of human salmonellosis cases using machine learning modeling of *Salmonella enterica* whole-genome sequences.

Methods: The model was based on virulence genes data for clinical *Salmonella enterica* isolates and isolates extracted from animal sources (chicken and swine) and the agricultural environment. Source attribution was performed using a predictive machine learning approach in R (v.4.1.2).

Results: As compared with logit boost, stochastic gradient boosting, and support vector machine radial kernel, the random forest algorithm provided the best performing model with an accuracy of 0.728. The random forest algorithm predicted the source of 44 human salmonellosis cases. The most important sources of the pathogen were environmental sources (40.9%) followed by chicken (36.4%), and swine sources (22.7%). The model revealed several important genes including tetracycline and sulfisoxazole resistance genes, indicating there was the dissemination of antimicrobial-resistant (AMR) strains.

Significance: Based on whole-genome sequencing data, machine learning has the potential to improve source attribution modeling. This indicates that WGS coupled with machine learning-based predictive modeling would improve our capability to track *Salmonella enterica* and other foodborne pathogens from different food sources.

P3-119 A Machine Learning Approach to Identify Key Drivers Influencing Populations of Generic *Escherichia coli* in Surface Waters in Florida

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Introduction: Foodborne contamination by pathogens, such as *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC), in fresh produce is a major food safety concern that has received significant attention in the United States. Agricultural water is a known pathway for pathogen contamination of fresh produce. However, there is large variation in the microbial quality of agricultural waters using surface water sources because they are open to the environment.

Purpose: The purpose of this study is to identify key drivers of microbial quality in surface waters in Florida.

Methods: Here, we investigate the relationship between generic *E. coli* (pathogen indicator organism, n=909) concentrations in various types of surface waters (canal, pond, lake, river, stream) and their respective physicochemical water characteristics (pH, redox potential, water temperature, conductivity, turbidity), climatological conditions (air temperature, precipitation, ET, radiation, daylength, vapor pressure deficit), and landscape features (elevation, land use, NDVI, proximity to waste water treatment facilities) across the state of Florida. Our approach was to implement a machine-learning framework (random forest) for variable dimension reduction and to identify the main drivers influencing the prediction of generic *E. coli* concentration levels.

Results: Rainfall intensity, turbidity, conductivity, redox, and pH were identified as important processes which suggests that rain events are driving the transport of bacterial sources via runoff, this is particularly the case for pond sites in central FL. Other potential covariates to consider include organic carbon and NDVI.

Significance: Future directions include integration of the reduced set of key drivers to inform challenges of predicting the magnitude and frequency of generic *E. coli* populations mechanistically in surface waters, that are potential sources of irrigation and a pathway for food-borne contamination in agricultural waters.

P3-120 Linking the Genome Data of *Salmonella enterica* Strains Isolated from Chicken Meat in Mexico with Their Virulence Capacity

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❖ ***Developing Scientist Entrant***

Introduction: Association between the genotypic and phenotypic characteristics of *S.enterica* strains is critical to predicting their virulence level. Recently, whole-genome sequencing (WGS) has been used to link genome data with a phenotypic response.

Purpose: To link the genome data of *S.enterica* strains with their virulence capacity.

Methods: WGS was performed on six *S.enterica* strains isolated from chicken, previously classified into high-virulent (2), moderate-virulent (2), and low-virulent, according to their genotypic and phenotypic print. The genome data was used to assess the presence of virulence and antimicrobial genes, predicting the sequence type (ST) and serotype. The virulence capacity was estimated using a dynamic *in vitro/ex vivo* gastrointestinal model and histopathological analysis to calculate each strain's infection probability (Pinf) and tissue damage. A multiple factorial analysis (MFA) was conducted to link WGS with virulence.

Results: The two strains classified as having high-virulence belonged to Enteritidis serotype, the two moderate-virulent strains corresponded to Infantis and Saintpaul, and the two low-virulence strains to Schwarzengrund. The ST was serotype-dependent. The serotypes: Enteritidis, Infantis, Saintpaul, and Schwarzengrund, belonged to ST11, ST32, ST50, and ST96, respectively. All the isolates have the *aac(6')-Iaa gene,* associated with tobramycin and amikacin resistance. Regarding the 361 virulence genes tested, 74.2% were observed in all strains, 0.5% were not observed in any, and 25.3% varied among them. No statistical differences in the Pinf were observed among the strains. The MFA showed a distribution of the isolates according to their previous classification, and a high connection (Lg=0.81) between the epithelial integrity and the presence of *sefABC, pefBACD, steABC, sopE, sseI/srfH, gtgE, spvRABCD, mig-5, rck, sseK3,* and *SodCI* genes, which were only found in the *S.* Enteritidis strains, and are mainly mobile genetic elements (MGE).

Significance: These data support the idea that specific virulence genes, mainly MGE, could be associated with virulence capacity.

P3-121 How Do the Survival Kinetics of Cross-Contaminated *Escherichia coli* O157:H7 Differ in Ground Beef during Thermal Inactivation Process?

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Introduction: Since cross-contamination of bacterial pathogen to food surfaces would be one of the main sources of foodborne illness, many studies on cross-contamination of bacterial pathogens have been conducted. However, there are few studies on inactivation kinetics of cross-contaminated pathogenic bacteria on foods due to complicated experimental setup. The inactivation kinetics of bacterial pathogen on food surface after cross-contamination would play an important role in evaluation of bacterial behavior for quantitative microbial risk assessment.
Purpose: The objective of this study was to investigate inactivation kinetics of *Escherichia coli* O157:H7 in ground beef after cross-contamination from stainless steel surfaces.
Methods: Pre-grown *E. coli* O157:H7 were inoculated on a stainless steel bowl surfaces and dried in a safety cabinet for 3 h, and then the contaminated stainless steel bowl were stored at 15°C and 50-60% relative humidity for up to 24 h. Ground beef (ca. 100 g) were kneaded in the contaminated stainless steel bowl to realize cross-contamination. The ground beef sample (10 g) was placed into a plastic film bag and then heated in a water-bath at 55.0 to 62.5°C for certain period. The observed inactivation kinetics was described by Weibull model using second-order Monte Carlo (2DMC) method.
Results: The inactivation kinetics of cross-contaminated *E. coli* O157:H7 in the ground beef showed almost same the inactivation behavior with directly inoculated *E. coli* O157:H7 in ground beef under all temperatures examined. There is no difference in the estimated D-values between with and without cross-contamination. The 2DMC model successfully simulated the inactivation kinetics of *E. coli* O157:H7 with its variability and uncertainty under dynamic heating process.
Significance: The inactivation kinetics of *E. coli* O157:H7 in ground beef would not be influenced by prior exposure to desiccation stress on stainless steel surface and the cross-contamination process.

P3-122 Finding the Underlying *Salmonella* Concentration Distribution in Ground Beef in the U.S. for Quantitative Microbial Risk Assessment Purpose

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Introduction: Characterizing the initial prevalence and concentration of pathogens in raw material is key for Quantitative Microbial Risk Assessments (QMRA).
Purpose: This study aimed at empirically identifying the probability distribution of *Salmonella* concentration in ground beef in the US.
Methods: We obtained FSIS microbiological sampling data on *Salmonella* detection test and Most Probable Number (MPN) enumeration collected on ground beef *via* FSIS program MT43. Maximum likelihood (ML) methods were applied jointly to detection results and MPN number of positive tubes per dilutions, considering missing data and adjusting for establishment production volume. The fit of various parametric distributions, including gamma, Weibull, lognormal, their zero-inflated derivation and mixtures of those distributions, were visually inspected and formally evaluated based on Likelihood Ratio Test (LRT), Akaike Information Criterion and bootstrap LRT, as appropriate. We compared ML methods to Bayesian MCMC methods.
Results: Samples collected from April 2016 to February 2021 resulted in 34,775 detection tests: 627 were positive (percent positive: 1.8%; adjusted to establishment production volume: 2.6%). No positive tube was observed in 426 (83%) of the 512 samples with available MPN pattern. Other samples lead to MPN values ranging from 0.03 MPN/g to 93 MPN/g.
We couldn't reach stable fit using Bayesian MCMC methods without strongly informative priors. Using ML method, the *Salmonella* concentration distribution in ground beef fits best to a mixture of two log-normal distributions (meanln = -22.6 ln bact/g, sdln = 6.78, for 96.7% of samples; meanln = -6.15, sdln = 0.0225 for the remaining). The actual prevalence for 325g sample is estimated 2.6%, with a mean concentration in contaminated sample of -2.2 $\log_{10}$ bacteria/g.
Significance: The ML method allowed to test various sets of parametric distributions quicker than would comparable Bayesian methods. The observed distribution identified two potential patterns of contamination. The distribution can be used in QMRA.

P3-123 Modeling the Growth of Shigatoxigenic *E. coli* (STEC), *Salmonella*, and Generic *E. coli* in Raw Pork Considering Background Microflora at 10, 25, and 40°C

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❖ ***Developing Scientist Entrant***

Introduction: Pork products may contribute to STEC infections in humans. Background microflora has a significant effect on predicting pathogens' growth in pork products.
Purpose: To compare the growth of STEC (non-O157, O157, and O91) to *Salmonella* and generic *E. coli* in ground pork based on best performing primary models considering background microflora at processing (10 °C), room (25 °C), and sublethal (40 °C) temperatures.
Methods: Ground pork at 5 and 25% fat levels were inoculated with 3-4 log CFU/g nalidixic acid-resistant (50 ppm) *E. coli* and *Salmonella* strains. Five-gram pouches of each treatment temperature (10, 25, and 40 °C) were submerged in water baths for 25 to 440 h. Cells were recovered on MacConkey agar for *E. coli*, XLD for *Salmonella*, and 3M Aerobic plate count (APC) for background microflora. A 5 (bacteria) x 2 (fat content) x 3 (temperature) full factorial design was employed to compare the growth rate of each bacterium. Ten primary growth models were fitted using MicroRisk lab software. Maximum Growth Rate (log CFU/g/h) was compared at 95% level of significance.
Results: Competitive model incorporating no lag Buchanan fits better than other models. Fat content had a significant effect only at 10 °C for *E. coli*. Growth rate of STEC (except O91) and *Salmonella* were similar ($p > 0.05$) at 10 and 40 °C; however, *Salmonella* grew faster ($p < 0.05$) than STEC at 25 °C. Growth rate of non-O157 and O157 was similar at all temperatures. Growth rate of O91 was slower than other STEC and *Salmonella* at 10 and 25 °C but similar at 40 °C. A faster growth rate ($p < 0.05$) of generic *E. coli*than STEC and *Salmonella* at 10 °C could make it a potential surrogate at low temperatures.
Significance: Industry and regulators can use competitive models to develop appropriate risk assessment and mitigation strategies to improve the microbiological safety of raw pork products.

P3-124 *Salmonella* spp. in Peripheral Lymph Nodes of Bovine Origin: A Systematic Review and Meta-Analysis

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Introduction: The inclusion of peripheral lymph nodes (PLNs) contaminated with *Salmonella* in beef production may pose risks to public health. Considerable number of studies have reported *Salmonella* contamination in bovine PLNs, however, limited efforts have been made to study the epidemiology of *Salmonella* in these lymph nodes using aggregated data.
Purpose: To characterize the distribution of both prevalence and concentration of *Salmonella* in PLNs by lymph node type, geographical region, season, and production source using systematic review and meta-analysis approaches.

Methods: Two electronic databases and grey literature were searched with terms reflecting three concepts (i.e., bovine population, *Salmonella*, and lymph nodes). Relevance of articles was screened through a two-phase process, i.e., a preliminary screening based on title and abstract and advanced screening based on full texts. Then, data were extracted from identified articles including bibliographical information, study design, sampling approach, microbiological methods, and outcomes of interest. Random-effects meta-analysis was performed to compute composite prevalence and concentration of *Salmonella* in PLNs with quantified variability.

Results: A total of 22 articles were identified as relevant, with the majority from the U.S. (14/22, 64%). The overall prevalence of *Salmonella* in PLNs was 13% (95% CI: 9-18%), the variation of which was influenced by lymph node type, season, location, and production source. Higher prevalence was reported in subiliac lymph nodes (23%, 95% CI: 13-33%), compared to others. Samples collected in warm seasons tend to detect higher prevalence. Only four articles have reported concentrations in an extractable format. Anatum, Cerro, Mbandaka, Montevideo, and Typhimurium were the most reported serotypes in PLNs.

Significance: The present study synthesized quantitative evidence to investigate the source of variation in bacterial contamination in bovine lymph nodes, which facilitates the assessment of salmonellosis risks among ground beef consumers attributable to the inclusion of PLNs.

P3-125 Identification and Selection of Feed Safety-Related Risk Factors to be Included in the Canadian Food Inspection Agency's Risk Assessment Model for Inedible Rendering Plants

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Introduction: To support a risk-informed approach to oversight, the Canadian Food Inspection Agency (CFIA) is developing an Establishment-based Risk Assessment model for renderers (ERA-Renderer model) to allocate inspection resources according to the feed safety risks pertaining to inedible rendering plants.

Purpose: The objectives of this study were to identify and select risk factors pertaining to Canadian rendering practices that are associated to either an increase or a decrease in feed safety risks.

Methods: Between January and March 2020, a literature search was conducted to identify an exhaustive list of risk factors applicable to inedible rendering plants in Canada. Papers related to pet food, rendering production for industrial use, economical fraud or animal performance parameters were excluded from this study. A refinement process was then applied to retain only risk factors that met the inclusion criteria (e.g., availability of data sources, lack of ambiguity, measurability). Next, an expert panel reviewed the risk factors, conducted the final selection and defined the assessment criteria based on knowledge and experience in the Canadian rendering industry.

Results: A final list of 32 risk factors assessed by 179 criteria were selected: 21 extracted from the literature and 11 from expert advice. Overall, 4 risk factors pertain to the inherent risk of a plant (e.g., continuous cooking), 8 are related to risk mitigation strategies (e.g., feed safety certifications) and 20 refer to the compliance with regulations (e.g., inspectors' assessment of the plant process control program). As a next step, the relative risk of each assessment criterion will be estimated considering their impact on feed safety to design the model's mathematical algorithm.

Significance: By assessing the feed safety risk related to inedible rendering plants in Canada, this new tool will better inform the CFIA on the level of oversight required to manage this risk, enhancing both human and animal health protection.

P3-126 What is the Relative Impact of Evidence-Based Risk Factors on a Rendering Plant's Overall Feed Safety Risk? A Quest for Answers through an Expert Elicitation

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Introduction: The Canadian Food Inspection Agency (CFIA) is developing an Establishment-based Risk Assessment model for inedible rendering plants (ERA–Renderer model) to assess their feed safety risk based on 32 risk factors and 179 assessment criteria previously selected from the scientific literature and expert opinion.

Purpose: The objectives of this study were to estimate the relative risk (RR) of the assessment criteria according to their feed safety impact and to assess the maximum values for each risk factor cluster (i.e., inherent, mitigation and compliance clusters) when multiple criteria were identified in a given rendering plant.

Methods: In June 2021, a two-round Delphi expert elicitation was conducted with 20 Canadian feed experts. First, experts were asked to complete an online survey individually to provide their RR estimates. Then, aggregated results were shared with the expert panel and a virtual group discussion was held to tackle divergent results and exchange opinions. For the second round, experts reconnected to their survey and adjusted their results.

Results: No significant differences in estimated RRs were identified based on experts' profiles (e.g. years of experience, work sector). Criteria having the highest increase in risk included the use of both Specified Risk Materials (SRM) and non-SRM, the absence of segregation for imported and domestic products, and historical prosecutions or non-compliances related to the plant's process control, end-product control and import control programs. Mitigation criteria having the highest impact on decreasing the risk were the application of a complete segregation between raw materials and finished products and the implementation of a hazard sampling plan for finished products.

Significance: The median RR assigned to each criterion and cluster will be used to build the mathematical algorithm of the CFIA's ERA-Renderer model. This algorithm will be used to support better allocation of inspection resources according to the feed safety risk linked to each rendering plant.

P3-127 Evaluation of Cooling Rates of Foods in Home Refrigerators and Comparison with FDA Food Code Recommendations

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Introduction: Very little published data exists on cooling rates of foods in home refrigerators and associated risks.

Purpose: This study identifies factors which have a significant impact on cooling rates of foods in home refrigerators.

Methods: Ten users were given a USB Data logger with a temperature probe and provided with instructions on its use. Individuals were instructed to handle home-cooked or takeout foods as they would normally do, and then to insert the temperature probe prior to refrigeration of leftovers. Data loggers were set to record time-temperature points every five minutes during cooling. Data collection proceeded over 3 to 6 months. Data was analyzed using Excel and cooling rates were obtained. A total of 273 recordings were collected, and 30 were discarded for various reasons (e.g., logger malfunction, probe accidentally displaced, etc.). Statistical analysis was performed using RStudio to elucidate the effect of multiple variables on the cooling rate. Cooling rates were compared to FDA Food Code recommendations, 135°F (57.2 °C) to 70°F (21.1 °C) within 2 hours, and to 41°F (5 °C) or lower within the next 4 hours.

Results: Container material, food type, container volume, food volume, recording length and percentage of air all had a significant effect on the obtained cooling rates. Stainless steel containers had the fastest food cooling rates and cardboard containers the slowest. Food stored in containers with a volume under 2000 cm^3 or where the food volume was less than 1000 cm^3 cooled significantly faster than food in larger containers or with larger volumes. No significant difference was observed for fridge temperature (although all home refrigerators used were always under 7ºC), food or container depth.

Significance: This work helps characterize the risks posed by improper home cooling and can inform the development of best practice guidance and educational materials.

P3-128 Quantitative Microbial Risk Assessment for *Salmonella enterica* in Tomatoes

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Introduction: Foodborne disease outbreaks associated with fresh produce have been increasing over the past decade. Tomatoes are the second-most-consumed vegetable in the United States and have been implicated in more than 10 *Salmonella enterica* outbreaks since 2010. Quantitative microbial risk assessment could be useful in identifying potentially relevant sources of *Salmonella* contamination along the tomato production and supply chain as well as quantifying the number of illnesses that could arise.

Purpose: The purpose of this study was to develop a quantitative risk model to estimate the risk of illness caused by *Salmonella* in fresh tomatoes.

Methods: The factors considered in the farm-to-fork model were on-farm *Salmonella* contamination from soil and irrigation water and cross-contamination during washing of tomatoes. Other factors included time/temperature conditions during transportation and storage at retail stores and consumer homes. The model was run at 100,000 iterations in a Monte Carlo simulation using Palisade @Risk.

Results: Results from our initial simulations indicated low levels of risk of illness from the consumption of *Salmonella*-contaminated tomatoes. The median probability of illness was estimated to be 2 per 100 billion servings (5th percentile: 1.08×10^{-14}; 95th percentile: 1.22×10^{-6}). The estimated number of cases per year in the US has a wide range with a median value of less than one. A sensitivity analysis showed that the transfer of *Salmonella* from soil to tomatoes, *Salmonella* concentration in soil, cross-contamination due to wash water, *Salmonella* concentration in irrigation water, and home storage temperature are the major factors contributing to the estimated risk.

Significance: Although the risk of illness may be low, *Salmonella*/tomato outbreaks do occur. Mitigation strategies focused on reducing *Salmonella* concentrations in soil amendments and reducing the risk of cross-contamination during washing of fresh tomatoes could be key to preventing these outbreaks.

P3-129 Evaluation of Risk Factors for *Escherichia coli* O157:H7 Contamination in Leafy Greens Irrigated with Alternative Sources of Water

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Introduction: Many studies have identified irrigation water as a source of contamination in recent produce-linked outbreaks. With seasonal variations in climate changes, factors such as drought have a profound effect on agriculture. Recent studies have analyzed various wastewater sources to establish microbial and chemical profiles to determine if they are fit for use as irrigation in fresh produce fields.

Purpose: This study analyzed the risk factors associated with re-using three alternative water sources (return flow, harvested rainwater, reclaimed water) for potential use as irrigation water for fresh produce production.

Methods: A detailed search of scientific literature was done using Google Scholar and PubMed with the key words, 'irrigation water', '*E. coli* O157:H7', 'reclaimed water', 'return flow', 'harvested rainwater' and 'fresh produce'. This comprehensive study focused on scientific reports of the microbial and chemical profiles of the alternative sources and literature related to experiments done at the laboratory and field levels. Risk factors were identified during the farm to fork continuum and will be further examined for the implementation of control strategies.

Results: During the irrigation of the leafy greens, contaminated water being sprayed on the edible portions of the leafy greens, facilitates a direct transfer of pathogens. The presence of organic chemical compounds in sub-lethal conditions can increase the growth of and cause genetic variations such as antibiotic resistance in native microbiota such as coliforms, enterococci and *Aeromonas* spp. that could make them harder to treat if they infect consumers. The risk factors identified included prevalence of the pathogen in the water sources, at-harvest transfer from water to fresh produce, post-harvest processing, supply chain factors and consumer washing and consumption.

Significance: Alternative irrigation sources are being applied to crops not for human consumption but need an extensive health-based assessment before they can be applied to food crops in the field.

P3-130 Foodborne Illnesses from Leafy Greens: Attribution and Cost Estimates

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Introduction: There is a need to assess the burden and cost of illness from multiple pathogens associated with leafy greens; which are commonly identified as the cause of large multi-state foodborne illness outbreaks in the United States.

Purpose: This research constructs robust new estimates for the economic cost of foodborne illnesses associated with leafy greens based on multiple food source attribution models.

Methods: New source attribution and economic cost estimates were derived for illnesses associated with several common pathogens based on CDC's National Outbreak Reporting System (NORS) data. This analysis considered outbreaks occurring between 1998 and 2017 where food vehicles and pathogens were identified. Models used in the analysis included those of Painter and the Interagency Food Safety Analytics Collaboration (IFSAC). Attributions estimated for leafy greens and subcategories (e.g. lettuce, cabbage and spinach) were paired with incidence and economic cost data to estimate annual economic burden of illness for leafy greens in the United States.

Results: Results using a 'simple foods' attribution model (n=3164 outbreaks) found that leafy greens were associated with 7.11% of all outbreaks and 7.37% of illnesses from those outbreaks. Pathogens with higher attribution rates include Shiga toxin-producing *Escherichia coli* (STEC) (20.63% of leafy green outbreaks, 27.33% of illnesses) and norovirus (17.80% of leafy green outbreaks and 19.64% of illnesses). Illness-based attribution estimates yield 48,445 annual STEC cases and 1,072,829 annual norovirus cases attributable to leafy greens. This corresponds to annual health related costs of $256.7 million (90% CI: 109.1-554.5) for STEC and $677.0 million (90% CI:465.6-908.7) for norovirus. Lettuces account for 64.10% of leafy green STEC costs and 57.97% of leafy green norovirus costs.

Significance: Developing updated attribution and economic burden studies focused on specific foods helps risk managers identify and better target resources toward reduction of foodborne illness problems.

P3-131 Modeling the Growth and Survival of *Escherichia coli* on Fresh Strawberries Stored at Different Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: Strawberries have a limited shelf-life and are prone to bacterial contamination throughout the supply chain due to minimal processing. Predictive models built through inoculation of fruit and controlled storage can help forecast microbial safety and fruit shelf-life.

Purpose: The aim was to model the growth and survival rate of *Escherichia coli* on fresh strawberries stored at different temperatures.

Methods: *E. coli* (ATCC 35218) was used as a surrogate for *E. coli* O157:H7 and *Salmonella*. Strawberries, dip-inoculated with *E. coli*, were stored at 25 and 37 ℃ for 48 h, and 4 ℃ for 15 days. Bacterial survival and growth data were collected every 3 h for samples stored at 25 and 37 ℃ and every 24 h for 4 ℃ samples. Data are reported as log CFU/g, and CFU was determined by plating on selective media (Sorbitol MacConkey Agar). Microbial data (log CFU/g) were fitted using iPMP2013 software.

Results: The mean population of *E. coli* in the inoculum and inoculated samples (at start, 0 h) were 8.25 log CFU/ml and 5.91 log CFU/g respectively. A growing trend of *E. coli* was observed at both 25 and 37℃ storage temperatures. A decay trend was observed at 4℃, with 3.77±0.23 log CFU/g (mean ± standard error) of *E. coli* surviving on day 15. The mean population of *E. coli* increased (1.61±0.09) for up to 48 h at 25℃, whereas at 37℃, the mean population increased up to 21 h (1.21±0.15) and then slightly decreased over the remaining 27 h. The Baranyi model was fitted with acceptable goodness of fit statistics for both 25 and 37℃ samples (R^2 = 0.90 and 0.85, RMSE = 0.194 and 0.314, respectively).

Significance: Improved understanding of the growth and survival of *E. coli* on strawberries could help in the estimation of the risk of bacterial contamination due to variation in temperature across the supply chain.

P3-132 Identification of Risk Factors in Farms, Related to *Bacillus cereus* Foodborne Illness by Lettuce Consumption

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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* in lettuce from farm to table and to develop simulation models for *B. cereus* growth.

Methods: Growth predictive models were developed with the *B. cereus* cell counts in lettuce with respect to storage temperatures. Bacterial cell data for workers, hands, bed soil, and soil that cause cross-contamination were collected through the examination of *B. cereus* contamination in lettuce. The data for temperature and time during transportation, and consumption data were also collected. With these data, the risk of *B. cereus* due to ingestion of lettuce was simulated, and risk factors influencing B. cereus infection were identified.

Results: The initial contamination *B. cereus* in seeds was 0.9 Log CFU/g. The cumulative density in the production stage showed that the number of average *B. cereus* cells increased to 2.6 Log CFU/g. *B. cereus* contamination levels at the transportation stage were lowered from 2.6 to 1.1 Log CFU/g. Average consumption of lettuce was 39.9 g, calculated by Exponential distribution at 16.6% of frequency. The simulation showed that the foodborne illness by *B. cereus* occurred 3 times of 10,000 iterations, and consumption frequency, and soil contamination rate were identified as major risk factors, influencing *B. cereus* foodborne illness.

Significance: These results indicate that *B. cereus* should be controlled in seed, soil, workers' hands in the production stages to decrease *B. cereus* foodborne illness through the lettuce consumption.

P3-133 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 occasionally 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℃. 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 Monte carlo simulation in @RISK software.

Results: Of 247 samples, NoV was not detected and HAV 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 model $Risk=1-(1+\eta CV)^{-r}$ (η, 2.55×10^{-3}; CV, dose; r, 0.086) for NoV, and $Risk=1-(1+dose/\beta)^{-\alpha}$ (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 regarded as low in Korea.

P3-134 Exposure Assessment of *Salmonella* Species in Street Vended Fresh Cut Fruits and Vegetables in Ibafo, South-Western Nigeria

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Introduction: There is an increased concern that fruits and vegetables might be more important as a carrier for human enteric pathogens such as Salmonella spp. Microbes create a niche on the flora of vegetables such as cabbage and carrots and it has now become difficult to control them. In Nigeria, microbial quality of street vended fruits and vegetables generally consumed as mixed salads is questionable with evidence of fresh produce contamination.

Purpose: The focus of this study was to determine the exposure assessment of *Salmonella* spp. in street vended fresh cut fruits and vegetables used as mixed salads and the risk to consumers in Ibafo, Ogun-State, Nigeria.

Methods: A total of 250 samples of fresh cut cabbage, cucumbers and carrots were enumerated for presence of *Salmonella* spp. Similarly, data from completed studies in Nigeria and other published literature sources were obtained. A probabilistic exposure model was developed with Monte Carlo simulation in Excel add-in software using @Risk software.

Results: *Salmonella* spp were detected in 56% of the total sample analysed with a high prevalence in carrots (76%). The exposure per serving of *Salmonella* spp. in the mixed salad varied from minimum 0 Log CFU/serving to maximum 4.7 Log CFU/serving. In 95% of cases the consumers would be exposed to a dose lower than 5.8 log CFU of *Salmonella* spp. per serving. While in 99.5% of the time, the dose increases to 6.1 log CFU of *Salmonella* spp. per serving. The probability of illness per serving predicted by the model was 47% cases.

Significance: The developed exposure assessment model provided a framework for estimating risk associated with consumption of *Salmonella* spp. contaminated fruits and vegetables can guide the evaluation and development of intervention strategies.

P3-135 Buffer Models Linking pH Changes to Acid Concentrations during Cucumber Brine Fermentations

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Introduction: The safety of fermented vegetables depends on biochemically linked variables, pH and (protonated) acid concentrations. However, undefined buffering components of fermentation media limit the use of pH measurements alone for estimating fermentation safety.

Purpose: The objective of this research was to develop buffer models linking pH changes to acid concentrations in model vegetable fermentations.

Methods: Cucumber juice media (CJ) were made from pickling cucumbers (*Cucumis sativus*) having diameters of <27 mm (CJ1), 27-38 mm (CJ2) and 39-51 mm (CJ3). *Leuconostoc mesenteroides* (LA81) and *Lactiplantibacillus pentosus* (LA445) were used to anaerobically ferment CJ media for 24 and 48 h. A novel titration method was used to measure buffer capacity and acid concentrations were measured by HPLC. Buffer models that define pH for CJ with up to 100 mM lactic and 50 mM acetic acids were then developed using Matlab.

Results: CJ1 had greater buffering than CJ2 or CJ3, which had similar buffer models. Fermentation by heterolactic LA81 resulted in similar pH values (3.8 to 4.0), lactic (26 to 30 mM) and acetic (10 to 15 mM) acid concentrations for all CJ at 24 and 48 h. For homolactic fermentation by LA445, significant differences ($p < 0.05$) were observed for both acid concentration and pH. Smaller cucumbers and longer fermentation times (24 vs. 48 h) had lower pH (3.3 vs. 3.5) and higher lactic acid concentrations (46 vs. 75 mM at 48 h). Predicted pH values from buffer models for all CJ samples had a mean difference of 0.06 +/- 0.05 pH units compared to measured values.

Significance: Acid concentrations in fermented vegetables are difficult and expensive to measure. Buffer models may allow acid concentrations to be estimated from pH measurements for single and mixed acid fermentations, and therefore allow predictions of pathogen die-off.

P3-136 Developing an Agent-Based Model to Assess *Listeria* Control Strategies in Retail Stores

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Listeria monocytogenes* contamination can occur throughout the food chain, including in produce. However, limited tools are available for retailers to assess practices that could enhance control of *Listeria* transmission in produce.

Purpose: This study aimed to develop a simulation model of *Listeria* transmission in retail stores to improve pathogen sampling plans and control strategies.

Methods: A single retail store was used as a model environment, and an agent-based model (ABM) was developed using NetLogo 6.2.0. The floor plan of the retail environment was represented as uniform squares, and each agent, including display shelves with fresh produce that may be at higher risk of *Listeria* contamination and growth, forklift, runners, produce baskets, scales, employees, and tools used in cutting station was identified and located in the model. Transfer coefficients between agents were acquired from literature to simulate *Listeria* transmission and sanitation schedules of 3 distinctive areas were implemented to simulate the ability of different cleaning practices. Various what-if scenarios were further considered in each area and simulated to assess corrective actions.

Results: This model included 11 undirected links and 35 directed links of *Listeria* transmission between 39 agents that are commonly available in the retail store. *Listeria* concentration was collected hourly for a total of one month through the simulation. The sanitation schedule was predicted to be effective and to maintain the *Listeria* on the produce shelves at <0.05 CFU/cm^2 and on the dry shelves at < 0.01 CFU/cm^2. The model suggested that limiting the contamination from the incoming supply and improving the cleaning schedule can be effective in reducing *Listeria* concentrations in retail environment.

Significance: This model can be used as a foundation for developing a tool for predicting the level of *Listeria* transmission in retail stores to enhance control strategies.

P3-137 Withdrawn

P3-138 Compositional Changes in the WA 38 Apple Microbiome during Controlled Atmosphere Cold Storage

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❖ ***Developing Scientist Entrant***

Introduction: The long-term controlled atmosphere cold storage of fresh apples is necessary to ensure market quality apples are available to meet year-long demand; however, the conditions of the storage environment have been shown to impact food safety risks.

Purpose: To determine how and when the composition of bacterial and fungal microbiota of the apple surface change during long-term storage conditions.

Methods: Whole WA 38 apples (n=180) were harvested in September 2019 and the microbiota of the fruit surface evaluated at time of harvest, 1, 3, 6, 9, and 11 months into storage. Apples were treated with pyrimethanil and 1-methylcyclopropene before storage under controlled atmosphere conditions (1°C, 1% CO_2/2% O_2) to simulate conventional postharvest handling. At each time point, 10 individual apple subsamples were hand-massaged in 250 ml 1X Tris-EDTA buffer with 2% Tween 80 for 1 min before bath sonication for 5 min. Samples were centrifuged at 10,000 *x g* and pellet resuspended in 250 µl 1X TE before DNA extraction using the ZymoBIOMICS DNA Miniprep Kit. Genomic DNA was sequenced to identify bacterial and fungal microbiota composition using amplicon sequencing of the 16S rRNA V3-V5 and ITS1 sequences, respectively. Bacterial phyla and genera were compared at a relative abundance threshold of greater than or equal to 1%. Fungal phyla were similarly compared at a threshold of 0.1%. No fungal genera were identified at a threshold greater than or equal to 0.1%.

Results: Mothur v.1.44.3 identified 52,485 and 13,780 different bacterial and fungal OTUs, respectively. Six bacterial phyla and one fungal phylum were detected. Statistically significant increases in bacterial relative abundances occurred between one and eleven months in storage of Actinobacteria, Bacteroidetes, Firmicutes, and Tenericutes ($p \leq 0.05$).

Significance: This research has provided an example of market quality apple microbiota under industry-relevant storage conditions, which could be used to establish compositional targets for metagenomic surveillance in industry.

P3-139 Investigating Food Safety Process Parameters for Lacto-Fermented Sauerkraut

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❖ ***Developing Scientist Entrant***

Introduction: The rise in consumer interest for lacto-fermented foods has peaked the business opportunity for lacto-fermented foods, yet established critical limits that validate process control is limited.

Purpose: The aim of this project is to investigate the fermentation rate under different process conditions including fermentation vessel and change in formulation (salt concentration) over time.

Methods: Lab-scale sauerkraut was prepared with 4mm-shredded pieces of cabbage and mixed with sea salt for 15 minutes, filled with fermentation vessels with saltwater weight bags applied on top of cabbage below brine level and monitored over time (up to fourteen days). Experiments included change in sea salt concentration (1, 2, 3, 4, and 5%) and change in fermentation vessel (32, 16, 8-ounce glass jars). Physical characteristics (pH and water activity) were measured with statistical analysis using ANOVA and t-test/Kruskal-Wallis test to investigate the kinetic change over time.

Results: Results report that certain process parameters can change the fermentation process in some conditions. On day 2, 1 and 2% salt sauerkraut samples significantly lowered the pH (4.27 ± 0.13 and 4.15 ± 0.14 respectively) compare to the other concentrations. All samples reach pH below 4.6 after day 5. Fermentation vessel size reported that smaller vessels had a statistically higher pH level by day 3 (pH 4.65 ± 0.02) compared to large (4.11 ± 0.03) and medium vessels (4.00 ± 0.05). Water activity values changed over time depending on the salt concentration of sauerkraut samples; water activity readings for all salt concentration samples increase for the first two days, then significantly decrease after day 5.

Significance: Identifying the rate at which the pH is reduced below 4.6 in lacto-fermented foods such as sauerkraut, can help provide process controls that provide technical support for processors.

P3-140 Modelling the UV-C Inactivation Kinetics and Determination of Fluences Required for Incremental Inactivation of Several Strains of *Listeria monocytogenes*

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Introduction: There is a disagreement in the literature about the UV-C dose required to inactivate *L. monocytogenes* 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 strains of L. monocytogenes suspended in clear phosphate-buffered saline (PBS).

Methods: Ten *L. monocytogenes* dairy isolates belonging to serotype 1/2a, 1/2b, and 4b 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 254 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: For the 10 isolates tested a UV-C dose of 10 mJ/cm^2 could inactivate between 5.02 ± 0.273 and 5.95 ± 0.150 log cfu/ml. The required UV-C fluence to inactivate 1 log cycle (D_{10} value) of *L. monocytogenes* isolates in buffer were between 2.66 and 3.67 mJ/cm^2. The survival behaviours of all strains were best fitted to the Weibull+tail model with correlation coefficients between 97.42-99.64% was proposed to describe the incremental inactivation of all strains.

Significance: A clear understanding of the UV-C dose-response of dairy-associated *L. monocytogenes* in a clear buffer lays the foundation to determine the UV-C doses required to inactivate *L. monocytogenes* in milk and design successful UV based non-thermal milk pasteurization systems.

P3-141 Evaluation of Growth in Independent Submissions to the GenomeTrakr Network

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Introduction: The GenomeTrakr laboratory network collects genomic data on foodborne pathogens from a variety of non-clinical isolates for submission to the NCBI Pathogen Detection (NCBI-PD) surveillance platform, supporting the One Health approach to cross-disciplinary investigation. Since 2019, the FDA has provided tools for member labs to submit to NCBI independently.

Purpose: Evaluate the usage of independent submission protocols, and their effect on the quantity, variety, and timeliness of data submitted.

Methods: Using Tableau Desktop 2021.4, we imported metadata records for all GenomeTrakr submissions to NCBI-PD from 2019-present, including both submissions processed through FDA-CFSAN and those submitted independently. Aggregating the data by month of public release, we assessed the metadata for trends in submission behavior. This included an evaluation of differing sample source compositions across contributors to identify areas for improvement in collection efforts, a comparison of the volume of submissions, and the average collection-to-publication time across member labs.

Results: Member labs uptake of the independent submission protocols has been swift, going from 18% to nearly 96% of all isolates submitted independently in just three years. Analysis shows that FDA collection efforts are focused on environmental, or facility inspection swabs containing *Salmonella* and *Listeria* isolates, comprising 35% to 61% of the isolates submitted directly by FDA each year. Independent partner submissions provided valuable diversification to FDA's core effort through their greater sampling of animal source isolates, particularly for *Escherichia coli*. While measuring time from sample collection to publication, we observed that the metadata collector does not distinguish historical research from real-time surveillance. This shortcoming will be addressed in the upcoming release of the expanded One Health Enteric package.

Significance: The independent submission protocols have effectively decentralized the data collection procedures, demonstrating rapid scalability of public health surveillance using open data methods. We identified potential optimizations to further enhance the data collected under the GenomeTrakr program.

P3-142 Determining the Relevance of Factors for the Occurrence of Listeriosis by Consumptions of Pasteurized Milk, A Low Risk Product

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Introduction: The risk of foodborne illness due to biological hazards depends on the conditions within the food-chain and the pathogen characteristics. Elucidating the relevant factors for the risk in the food-chain makes it possible to control the foodborne illness risk effectively. However, it is difficult to traceback all the relevant specific conditions of the food-chain from occasional incidence of foodborne illness. Quantitative microbiological risk assessment (QMRA) can reveal the relevant factors in food-chains and the ones that determine whether the foodborne illness occurs or not.

Purpose: The objective of this study was to reveal the relevant environmental and/or intrinsic factors for the risk of listeriosis throughout milk production to consumption and the pathogen characteristics on the occurrence of listeriosis using the results of QMRA simulations.

Methods: The Spearman's correlation of environmental and/or intrinsic factors to listeriosis occurrences were derived from ten billion simulated QMRA scenarios of pasteurized milk. The listeriosis occurrences were described by binary data (0: no-illness; 1: illness) for indicating whether each simulated scenario caused a listeriosis or not. The QMRA simulation consisted of six sections (storage on farm, pasteurization, storage on distributing, retail, domestic, and dose-response). For comparison, the correlations to final concentration levels were also derived from the QMRA.

Results: Sixty-one listeriosis were estimated from ten billion QMRA simulations of pasteurized milk. The most relevant factors for listeriosis occurrence were estimated as being the pathogenicity, the thermotolerance, the initial level of *L. monocytogenes* strain and the domestic storage temperature conditions, while the thermotolerance and the pasteurization conditions had most relevance for the final contamination level of *L. monocytogenes*.

Significance: The sensitivity analysis on the final contamination level (or the illness probability), which is generally used for QMRA, can result in different results from sensitivity analysis than the sensitivity on the occurrence of listeriosis. The target of sensitivity analysis should be carefully selected based on the objective for appropriate QMRA and the risk management.

P3-143 Protection of the Essential Workforce from Occupationally-Acquired SARS-CoV-2: A Quantitative Risk Model on the Efficacy of Infection Control Interventions in Produce Production and Processing

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Introduction: Essential food workers experience an elevated risk of SARS-CoV-2 infection due to prolonged occupational exposures (e.g., 12h work shifts, frequent close contacts, enclosed spaces) in food production and processing areas, shared modes of transportation (car or bus), and employer-provided housing.

Purpose: The purpose of this study was to evaluate, among essential produce production and processing workers, the impact of combined food industry interventions and vaccinations on reducing the daily cumulative risk of SARS-CoV-2 infection.

Methods: Six quantitative microbial risk assessment (QMRA) models were created in R using a two-dimensional Monte Carlo package and 10,000 iterations to simulate the daily scenarios experienced by essential workers engaged in produce production and processing activities. These scenarios include shared transportation, an indoor processing facility and accompanying break room or an outdoor harvesting field, and shared residential housing. Viral exposures were combined from aerosol, droplet, and fomite-mediated transmission pathways to calculate infection risk. Combined interventions (2m physical distancing, handwashing, surface disinfection, universal masking, increased ventilation) and two-dose mRNA vaccinations (86–99% efficacy) were applied individually and jointly to assess risk reductions.

Results: Without any interventions, daily infection risks for an indoor worker ranged from 0.80 (95% CI: 0.47–0.98) at 2m distancing to 1.00 (95% CI: 0.99–1.00) at 1m distancing. For an outdoor worker, daily risks ranged from 0.22 (95% CI: 0.10–0.44) at 2m to 0.48 (95% CI: 0.26–0.82) at 1m. Relative to no interventions, combined food industry interventions reduced daily cumulative risks for an indoor worker by 93.0% and an outdoor worker by 87.5%. When combined interventions were integrated with vaccinations, the daily infection risk for indoor (0.001, 95% CI: 0.0001–0.005) and outdoor (0.004, 95% CI: 0.001–0.016) workers were reduced by ≥99.1%.

Significance: Without interventions, produce production and processing workers face considerable risk of occupationally-acquired SARS-CoV-2 infection. However, regular implementation of key infection control interventions, especially vaccinations, effectively mitigates these risks.

P3-144 Environmental Monitoring Data in Food Retail: Development of Analytical Approach for Benchmarking and Risk Assessment

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Introduction: Global food retail chains have challenges to prioritize their food safety intervention efforts throughout their stores/restaurants.

Purpose:

The optimal statistical analysis is not fully utilized in the monitoring data. The goal of this study was to develop analytical approaches applicable to the microbiological data collected from food retail to enhance improvement efforts.

Methods: Using descriptive statistic and type of distribution 1,000 observations were simulated from historical environmental monitoring data collected from food retail stores. The data included aerobic plate count collected from surfaces and visual inspection. Two methods were developed internal benchmarking and risk assessment. The simulated data was used to assess the performance of these methods.

The internal benchmarking consisted of two tools based on statistical indicators including cumulative average and quartiles. Gape analysis was conducted using ANOVA, Pairwise Pearson Correlations, and Logistic Regression modeling.

The risk assessment was adapted from the World Health Organization (WHO) quantitative microbial risk assessment. The risk assessment consisted of two tools including microbial data with/without the visual inspection. The risk level of incompliance was classified based on aerobic plate count level, surface type (food-contact or nonfood-contact), and department type (RTE/not-RTE). To assess the performance of the developed methods, statistical analysis was utilized.

Results: Both benchmarking tools distinguished the stores with significant lowest compliance (p-value <0.001). The gap analysis pinpointed the areas/departments need improvement. The correlation analysis revealed the association between incompliance and surface type, department, and cleaning method.

Both risk assessment tools distinguished the risk level of incompliance among the stores and provided comparable risk ranking (p-value 0.3), indicating this risk assessment tool could be used with or without the visual inspection data.

Significance: The developed benchmarking and risk assessment methods provide better insight to the environmental monitoring data and are likely to improve food safety intervention efforts.

P3-145 Performance Assessment of the Canadian Food Inspection Agency's Establishment-Based Risk Assessment Model Outputs for Feed Mills (ERA-Feed Mill Model)

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed an Establishment-based Risk Assessment model for feed mills (ERA-Feed Mill model) to allocate inspection resources based on feed safety risks.

Purpose: This study aimed at estimating the agreement between the risk assessments provided by the ERA-Feed Mill model and CFIA senior inspectors on the same feed mills, and to refine the model based on the identification of major discrepancies.

Methods: Between October 2020 and July 2021, data collected from 31 commercial and 27 on-farm randomly selected feed mills were used to conduct the performance assessment of the model outputs with the participation of 26 CFIA senior inspectors (experts). Each expert was presented the profiles from eight random feed mills and two control mills. Using an online survey, experts were asked to categorize mills according to their feed safety risk using a five-point risk score. Spearman correlation between results from the ERA-Feed Mill model and the experts' median scores were calculated and a sensitivity analysis was done to evaluate the impact of specific model criteria on correlation results.

Results: A moderate positive correlation (0.65, p<0.01) was found in general between experts' risk assessments and the model outputs. A higher correlation was seen in commercial feed mills (0.81, p<0.01) compared to on-farm feed mills (0.48, p=0.01). Criteria influencing experts' risk assessments the most included the volume of medicated feed, the implementation of measures for preventing cross-contamination, and the mill process control's

inspection results. The use of medically important antimicrobials for human health and the type of feed ingredients significantly contributed to decreasing the correlation when removed from the model.

Significance: Overall, no major discrepancies requiring adjustments to the model design were identified. Risk results from the ERA-Feed Mill model are expected to be implemented shortly as part of the CFIA's risk-informed oversight.

P3-146 Whole Genome Sequencing-Based Typing of *Listeria monocytogenes* Isolated from Seafood and Production Environments

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that is frequently isolated from seafood. To reduce prevalence in these ready-to-use products and ensure consumers' safety, monitoring of *L. monocytogenes* in seafood processing environments is important.

Purpose: This study aimed to gain a better understanding of the diversity of *Listeria* in the cooked shrimp and smoked fish sectors and appreciate the value of whole genome sequencing (WGS)-based typing for environmental monitoring.

Methods: Eighty-eight isolates from 8 different industries were characterized by WGS. Core genome multilocus sequence typing (cgMLST) analysis was implemented and compared to pulse-field gel electrophoresis (PFGE) data.

Results: Most isolates belonged to the group IIa (72%). The others were part of groups IIb (10%), IIc (11%) and IVb (7%). *L. monocytogenes* strains were distributed in 15 clonal complexes (CC). CC121, CC321 and CC9 were the most represented CC (n=34, 17 and 10, respectively). CC121 isolates were further subtyped and divided in 4 cgMLST types, each of which being specific to a production site. Most CC321 isolates are grouped into a single cgMLST type, demonstrating the survival of a *Listeria monocytogenes* strain in the manufacturing environment for at least 4.5 years. All the sequenced isolates carried at least 6 out of the 7 LIPI-1 genes. But LIPI-3 and LIPI-4 genes were detected in only 9 and 1 genomes, respectively, and were less frequently detected in not persistent strains. Isolates clustering achieved with cgMLST and predicted serotypes were shown consistent with previously obtained PFGE and molecular serotyping results.

Significance: WGS-based typing provided information on the large genetic diversity of *Listeria monocytogenes* strains in the cooked shrimp and smoked fish sectors and demonstrated the ability of this foodborne pathogen to persist in the production environment for many years. This observation supports the need for further research on mechanisms underlying persistence of *Listeria* in food industries.

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P3-147 Development of a Simplified Assurance® G.D.S. Workflow for Detection of *Salmonella* in Cocoa

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Introduction: Cocoa has antimicrobial properties and is a particularly difficult matrix for the detection of *Salmonella.* Cocoa testing workflows are therefore quite cumbersome relative to other food matrices.

Purpose: To create simple, robust methods for PCR-based detection of *Salmonella* in cocoa (AOAC PTM 050602).

Methods: First, a single enrichment showing low detection level (threshold cycle > 22 by established workflow) was used to compare qPCR signal between two sample preparation methods: *i)* A published protocol with two immunomagnetic separation (IMS) steps separated by a secondary enrichment and *ii)* a simplified workflow with a secondary enrichment initiated by a direct dilution of primary enrichment followed by a single IMS. Next, we compared the relative sensitivity of these methods to a validated immunodetection method (AOAC OMA 989.13). We analyzed samples of 325g cocoa powder and 25g cocoa nibs with a primary enrichment performed according to the FDA reference method. For each matrix, 2 samples were artificially contaminated at a high level (0.061 and 0.16 CFU/g respectively), 8 at low level achieving fractional positive (0.012 and 0.032 CFU/g respectively) and 2 uninoculated.

Results: We observed qPCR signal with our simplified protocol to be equivalent to the published protocol with *S. enteriditis* ($p = 0.223$) and to improve signal with *S. poona* ($p < 0.001$; paired two-tailed t-test; $n = 5$). Both workflows were then found to be equivalent to the validated method in recovery of positives for both 375g cocoa powder and 25g cocoa nibs (dPOD = 0 +/- 0.31 for both).

Significance: Our results support the use of a simplified *Salmonella* PCR detection workflow with equivalent performance to a validated *Salmonella* detection method.

P3-148 Evaluation of the Ability to Detect *Salmonella* Serovars by Immunomagnetic Concentration and Real Time PCR Detection

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Introduction: *Salmonella* is one of the most frequent cause of bacterial foodborne illness. Regulation requires the analysis of *Salmonella* spp. in several food commodities by reference or rapid alternative validated methods. Validation schemes requires the comparison of alternative methods with reference methods, providing some flexibility in the strain selection. Selection of challenging strains for validation may be crucial to guarantee method performance and reduction of false negative results.

Purpose: The purpose of this study was to evaluate the ability to detect different *Salmonella* serovars using an alternative method including immunomagnetic concentration and Real Time PCR detection.

Methods: A total of 69 pure strain suspensions belonging to different *Salmonella* serovars at concentrations between 2.5 Log_{10} CFU/mL and 4.5 Log_{10} CFU/mL were tested by AOAC, AFNOR and MICROVAL validated Assurance® GDS Salmonella method. Triplicates of 1 mL from suspensions of each strain were immunomagnetic concentrated and detected by Real Time PCR. When negative results were obtained for at least one of the triplicates, concentration was increased until all three replicates were positive.

Results: Positive results were obtained for 99 % (68 out of 69) of the strains tested at ≤ 4 Log_{10} CFU/mL. Salmonella Houtenae ATCC 43974 was the only strain requiring 4.7 Log_{10} cfu/mL to obtain positive results for the three replicates. Real Time PCR cycle threshold (Ct) values obtained showed values between 20 and 30, with no direct correlation with the *Salmonella* concentration tested. Repeatability measured as differences between triplicate Ct ranged 0.23 and 5.38.

Significance: Evaluation of high diversity of *Salmonella* strains for validation studies is crucial to confirm the ability of alternative methods to reduce false negatives. Challenging *Salmonella* strains should be included in inclusivity studies to evaluate ability of genetical detection systems.

P3-149 Characterization of *Salmonella* spp. in Finishing Pigs at Kansas Commercial Swine Farms by Whole Genome Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Recent multistate outbreaks have highlighted the importance of using rapid methods to trace contaminations in the food chain and the need of implementing these tools in accordance with new regulatory framework. The Interagency Food Safety Analytics Collaboration (IFSAC) estimated 12.7% of foodborne *Salmonella* illnesses attributed to pork products in the United States, as one of the top seven food categories that account for the majority of *Salmonella* illnesses reported in the recent years.

Purpose: The objective of this study was to characterize *Salmonella* spp. isolates in finishing pigs at 5 commercial swine farms across Kansas by Whole-Genome Sequencing (WGS).

Methods: A total of 186 samples were collected among farms and 28 selected isolates were analyzed following USDA guidelines and *Salmonella* confirmed by PCR. WGS was carried out using Oxford Nanopore MinIon. *De novo* genome assemblies were obtained with the Shovill pipeline version 0.9. NCBI Pathogen Detection was used to determine antibiotic resistance genes and SNP (single nucleotide polymorphism) clusters.

Results: A limited serotypes diversity was observed among our samples collection. The presence of pathogenic *Salmonella* was impacted by the farm, the health and age of pigs, and other environmental factors. Isolates were divided across SNP clusters and strains were matched with other environmental and clinical isolates from the NCBI database. AMR genes showed that 40% of the strains carried at least one antimicrobial resistance gene including those encoding for tetracycline, phenicol, aminoglycoside and beta-lactam resistance.

Significance: Our analysis shows the presence of pathogenic *Salmonella enterica* in finishing pigs at Kansas commercial swine farms and underscores their potential role as pathogen entry route into the pork production chain.

P3-150 Whole Genome Sequencing of *Salmonella* from Retail Meats in Chile Reveals Trade-Acquired Along with Locally Acquired *Salmonella* Serovars

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Introduction: The study of *Salmonella* in meat and poultry is relevant since they represent the primary sources attributed to salmonellosis cases globally. The well-established international trade of chicken meat means vehicles to spread *Salmonella.* Importantly, whole-genome sequencing provides the best resolution for evaluating the diversity of *Salmonella* isolates obtained from local and imported meats.

Purpose: This study evaluated the diversity of closely related *Salmonella* isolates from national (produced and distributed in Chile) and international meats (produced in Argentina and Brazil).

Methods: *Salmonella* isolates were obtained from the national surveillance program at the region of Valparaiso, in central Chile, during 2018. Isolates (n=63) were obtained from chicken and turkey meat from retail, restaurants, slaughtering houses, and importation trucks. Isolates were obtained from samples produced in Chile (n=51), Argentina (n=4), and Brazil (n=19). We characterized the antimicrobial resistance profiles using Kirby-Bauer, and genomes were sequenced with the MiSeq Illumina platform. Bioinformatics processing and analyses were performed with SPAdes, MLST2.0, SeqSero, and staramr in GalaxyTrakr (FDA).

Results: The most common serotypes found were *S*. Infantis (n=35) and *S.* Heidelberg (n=13), followed by *S.* Agona (n=6) and *S.* Minnesota (n=4). *S*. Infantis were closely related ST-32 isolates with 34 SNPs difference, all carrying from 4-8 antibiotic resistance genes, including 22 isolates encoding the beta-lactamase $bla_{CTX\text{-}M\text{-}65}$. All *S*. Infantis were obtained from samples produced and sold in Chile, except for one from Brazil. *S.* Heidelberg represented closely related ST-15, 13 from meat imported from Brazil and carrying the resistance gene $bla_{CMY\text{-}2}$; both *S.* Heidelberg and this gene are not commonly reported in Chile, which may indicate introduction through imported meat.

Significance: Our study contributes to the understanding of *Salmonella* dissemination through the poultry meat trade. Surveillance programs benefits from using WGS to understand the diversity of *Salmonella* contaminating meats.

P3-151 Genomic Analysis of *Vibrio cholerae* Strains Isolated from Cholera Patients in Mexico

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Introduction: ***Vibrio cholerae*** is a bacterial pathogen which naturally habit in aquatic environment and is a causal agent of cholera a sever diarrheal infection. Not all *V. cholerae* strains encoded the main virulence factors, and can cause sporadic episodes of gastrointestinal infections, due the absence of the main virulence factors. In addition, the study of antimicrobial susceptibility due to the antibiotic treatment is recommended to avoid the mortality of the cases. EnteroBase is a strain database, associated with metadata and genomic assemblies, as well as with deduced genotyping data.

Purpose: To performed a characterization of genomic diversity, virulence factors and antibiotic resistance among *V. cholera* assemblies isolated from cholera patients in Mexico available in EnteroBase.

Methods: Metadata and genomic assemblies were downloaded from EnteroBase (isolation year, cgST, and HC5 and HC10 hierarchical clustering). The minimum spanning trees from cgMLST data, using the web application version of GrapeTree with NINJA NJ algorithm, were constructed. Also, antimicrobial resistance and virulence factors were searched with ABRicate version 1.0.1 and Virulence Factor Database respectively. The HC5 and HC10 were compared, and the genomic diversity of strains among the cgST and collection year were evaluated, and categorized based on the antibiotic resistant profile gene and virulotype.

Results: One-hundred and fifty-three of *V. cholerae* strains assemblies were used. Different cgSTs (114) were found being cgST 606 (29/153) the most frequent. Eight HC10 and 24 HC5 clusters were identified in Mexico, respectively. The three main HC5 clusters found in Mexico are associated with a temporal pattern. Seven antibiotic resistant profiles were found, and three of them were multidrug resistant. Regarding virulence, 122 genes were found, and classified in 13 groups according similarities in physiological function.

Significance: The use of Enterobase might help in the better understanding of molecular characterization of *V. cholerae* and their use in epidemiology studies.

P3-152 Whole Genome Sequencing of *Cronobacter sakazakii* Strain, Sequence Type 40, Isolated from Fresh Produce

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Introduction: *Cronobacter sakazakii* is an emerging, opportunistic, Gram-negative, human-pathogenic foodborne bacterium that causes life-threatening meningitis and necrotizing enterocolitis, predominantly in neonates. It can survive in severe dry conditions, and has been linked predominantly to powdered infant formula (PIF) contamination worldwide. It has also been obtained from a wide varieties of foods. To date, the application of Whole Genome Sequencing (WGS) has facilitated bacterial typing and has been widely used for precise strain identification to understand the transmission of disease. In

our previous studies, we have identified several pathogenic *Cronobacter sakazakii* strains (CC4, ST21) from produce samples utilizing WGS technology. In this study, we describe the draft genome sequence of a *Cronobacter sakazakii* strain SRL-103, obtained from fresh produce.

Purpose: The major objective of this study was to identify the pathogenic strain of *Cronobacter sakazakii* isolated from produce samples by performing WGS analysis.

Methods: A *Cronobacter sakazakii*-like bacterial isolate was recovered from cucumber samples. Preliminary identification of this isolate was accomplished by employing VITEK 2 system, real-time PCR assay and MALDI-TOF MS analysis, following FDA's Bacteriological Analytical Manual and manufacturer's procedures. WGS was performed on an Illumina MiSeq system, using a Nextera XT DNA library preparation kit and a 250-bp paired-end read MiSeq Reagent v2 kit (500-cycle), following manufacturer's protocols.

Results: The VITEK MS system could provide species identification with high confidence value (>99%) to the *Cronobacter sakazakii* strain SRL-103. Genome sequence of this strain was 4,386,950 bp in length, and the draft genome was distributed in 40 contigs. The WGS data analysis ascertained to be the sequence type (ST) ST40 for the genome of *Cronobacter sakazakii* strain SRL-103.

Significance: The *Cronobacter sakazakii* ST40 strain has also been isolated from raw material, environment, and manufacturing premises of PIF and other foods. WGS technology can be employed for the strain identification of foodborne *Cronobacter sakazakii* known to contaminate PIF and cause acute disease in humans.

P3-153 Comparative Genomics of *Listeria monocytogenes* Strains Isolated from Listeriosis Cases in Ruminants from the Midwest U.S.

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❖ ***Developing Scientist Entrant***

Introduction: Ruminants are a well-known reservoir for *Listeria monocytogenes.* In addition to asymptomatic carriage of the pathogen, ruminants can also acquire listeriosis, with clinical manifestations in the form of bacteremia, central nervous system (CNS), or maternal-neonatal infections, similar to those occurring in humans. Genomic characterization of ruminant listeriosis cases in Europe identified Lineage 1 and 2 strains associated with infection, as well as clonal complexes that are commonly isolated from human cases of listeriosis. There is little information on the diversity of *L. monocytogenes* from ruminant listeriosis in the United States.

Purpose: To conduct comparative genomic analyses of *L. monocytogenes* from ruminant listeriosis in the U.S. and compare with common subtypes isolated from human illness.

Methods: This study was conducted using 73 *L. monocytogenes* genomes from ruminant listeriosis cases from the Midwest and upper Great Plains collected from 2015 to 2020. Genomes were assembled and the pangenome was identified. A core genome-based tree was generated to determine phylogenetic relationships. The Institute Pasteur Listeria database was used to classify genomes by sublineage and cgMLST type, and prophages were identified by PHASTER. Associations between clinical manifestation and Lineage were identified by the chi-square test. Pathogenicity islands and stress island (SS1) were identified with a custom BLAST database implemented in Geneious Prime.

Results: Among the 73 isolates, 23 were Lineage 1, 39 were Lineage 2, and 11 were Lineage 3. The isolates belonged to 13 clonal complexes, with CC1 (n=9), CC7 (n=8), and CC375 (n=8) most frequent. A total of 58 new cgMLST types were identified. Phages were identified in 13/73 genomes, none of which were in Lineage 3 genomes. Lineage 1 and 3 isolates were most commonly associated with CNS infections.

Significance: Extensive diversity was observed among ruminant listeriosis isolates from the U.S., including the hypervirulent CC1 clone, commonly associated with disease in humans.

P3-154 Survival Kinetics of *Salmonella* spp. in Low-Moisture Foods during Long Term Storage

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Introduction: Low water activity foods (Aw<0.70) including nuts, seeds and cereal grains, historically viewed as microbiologically safe, have been repeatedly implicated in outbreaks of salmonellosis in the last 20 years.

Purpose: The survival of *Salmonella* and a surrogate, *Enterococcus faecium NRLB-2354* in walnuts, hazelnuts, sunflower seeds and raw wheat kernels was assessed during a 26 week storage period.

Methods: Three biological replicates of walnuts, sunflower kernels, wheat kernels and hazelnuts were each inoculated with *S*. Muenchen, *S*. Cubana, or surrogate *E. faecium* NRRL B-2354 at log 9.12 CFU/g, 9.30 log CFU/g and 8.03 CFU/g. Inoculated batches were stored for 26 weeks post-inoculation at 20°C and 25% RH, and enumerated in triplicate at 2-week intervals. Enumeration was performed on TSA, as per FDA protocols for enumerating surrogates in almond process validation. Un-inoculated samples were held in parallel under the same conditions to monitor water activity.

Results: Collectively, *Salmonella* populations declined by 1.96 to 3.91log CFU/g in the tested matrices during the storage period. The type of food was found to have an effect on the survival of *Salmonella*. After 26 weeks of storage, *Salmonella* counts were significantly lower on hazelnuts irrespective of the inoculation strain ($P \leq 0.05$). No significant changes in water activity were observed during the storage period ($A_w = 0.50 \pm 0.09$).

Significance: Consistent with the scientific literature, *Salmonella* persisted under low water activity storage conditions for an extended period (≥26 weeks). The type of sample matrix had an influence on survival since a significant decrease in *Salmonella* counts was observed in hazelnuts compared to wheat kernels, sunflower seeds and walnuts. This effect may be due to differences in the structure and composition of the matrix, meriting further investigation. Future studies should also focus on investigating survival under varied storage conditions and low inoculum concentrations.

P3-155 Benchmarking Short-Read Assemblers for the Metagenomic Identification of Bacterial Pathogens Using Simulated Bacterial Communities

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Introduction: With the rapid evolvement of assembly algorithms for Illumina short reads, defining an optimal assembly approach based on their performance on the metagenomic identification of bacterial pathogens is warranted.

Purpose: The purpose of this study was to benchmark six short-read assemblers for the metagenomic identification of bacterial pathogens using simulated bacterial communities.

Methods: Bacterial communities on fresh spinach and in surface water were simulated according to the published data by generating paired-end short reads with different sequencing depths of Illumina HiSeq, MiSeq, and NovaSeq platforms. Multidrug-resistant *Salmonella* Indiana SI43 and *Pseudomonas aeruginosa* PAO1 were included in the simulated communities on fresh spinach and in surface water to represent enterobacteriaceae and pseudomonadales, respectively. Six assemblers, including ABySS, IDBA-UD, MaSuRCA, MEGAHIT, metaSPAdes, and Ray Meta, were benchmarked in terms of assembly quality, identifications of plasmids, virulence genes, *Salmonella* pathogenicity island (SPI), antimicrobial resistance genes (ARGs), and chromosomal point mutations, serotyping, multilocus sequence typing (MLST), and whole-genome phylogenetic analyses.

Results: Overall, IDBA-UD, MEGHIT, and metaSPAdes produced assemblies with greater numbers of contigs, total lengths, lengths of the largest contig, N50s, and L50s but less N's, mismatches, and indels compared to ABySS, MaSuRCA, and Ray Meta. IDBA-UD, MEGAHIT, and metaSPAdes assemblies showed plasmid, virulence gene, SPI, and ARG profiles congruent with the reference genome, whereas accurate serotyping and MLST was observed in all

assemblies. The reads classified as *Salmonella* or *P. aeruginosa* extracted from each assembly showed consistent phylogenetic topology with the reference genome when they were aligned with 30 *S.* Indiana or *P. aeruginosa* strains.

Significance: Our study demonstrated MEGHIT, metaSPAdes, and Ray Meta as the most robust assemblers to achieve accurate metagenomic identification of bacterial pathogens. As the underlying assembly algorithms advance and mature, evaluation of assemblers should be a continuous process.

P3-156 Evaluating the Relationship between Presence of Crystal Protein-Encoding Genes, Expression of Crystal Proteins and Cytotoxicity in *Bacillus cereus* s.s. Isolates

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Introduction: *B. cereus s.s.* biovar Thuringiensis (Bt) strains belong to the same species as foodborne pathogenic *B. cereus s.s.* and are identified based on the presence of genes (*cry, cyt* and *vip*) encoding for insecticidal crystal protein (Bt toxin).

Purpose: Due to the close relatedness of Bt and *B. cereus s.s.,* the safety of Bt is under scrutiny; hence the purpose of this study was to (i) carry out both genotypic and phenotypic assessment of the crystal proteins production and (ii) compare the *in vitro* cytotoxicity of *B. cereus s.s.* biovar Thuringiensis and *B. cereus s.s.* isolates.

Methods: A total of 67 *B. cereus s.s.* isolates were whole-genome sequenced (WGS). Sequences were *de novo* assembled using SPAdes (v3.15.2), and the crystal protein-encoding genes were detected using BTyper3 (v3.1.2). Isolates grown on T3 for 72 h at 30°C were screened for crystal protein using phase contrast microscopy. The sensitivity and specificity for predicting crystal protein production based on the presence of Bt toxin-encoding genes were calculated. The WST-1 assay was used to assess the cytotoxicity of isolate towards CaCo-2 cells. One-way ANOVA was used to evaluate whether the cytotoxicity of Bt isolates was significantly different compared to other *B. cereus s.s.*

Results: 33/67 isolates carried Bt toxin-encoding genes, and 32 of these also produced crystal proteins, resulting in 96.7% sensitivity and 97% specificity. 56/67 isolates (83.6%) were cytotoxic to CaCo-2 cells. There were no significant differences in cytotoxicity of Bt and other *B. cereus s.s*. isolates (87.5% vs. 78.4%; p = 0.212).

Significance: This study demonstrated that crystal protein production can be predicted with high sensitivity and specificity based on the presence of Bt toxin-encoding genes. Furthermore, the lack of significant differences in cytotoxicity of Bt and *B. cereus s.s*. isolates demonstrate the need for future assessment of risks associated with the application of Bt bioinsecticides.

P3-157 Prediction of *Salmonella* Contamination in Surface Water Samples Using Microbiome Data Analyzed with Machine Learning Classifiers

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Introduction: The use of agricultural water contaminated with *Salmonella* contributes to foodborne disease burden and there is a need for novel approaches for assessing pathogen status of agricultural waters.

Purpose: The purpose of this study was to investigate the utility of the water microbiome data for predicting *Salmonella* contamination of agricultural water using different machine learning methods.

Methods: One grab sample was collected from each of the 60 different streams in NY in 2018. Samples were tested for *Salmonella* using a PCR screen followed by culture-based isolation, and DNA was extracted from 100 mL sample aliquots using DNeasy PowerWater kit and used for Illumina shotgun metagenomic sequencing with 150 bp paired-end reads. Reads were trimmed using Trimmomatic (v 0.36) and used to assign taxonomy using KRAKEN2 (v 2.1.1), followed by analysis with BRACKEN (v 2.5.0). The conditional forest (cRF), regularized random forest (rRF), and support vector machines with the sigmoid kernel (SMV) were used to classify samples as *Salmonella*-positive or -negative. Model performance was validated using 10 times repeated 10 fold cross-validation to quantify the Area Under the Curve (AUC) and Kappa score. Variable Importance (VI) was calculated using a mean decrease in Gini index. The taxa identified as most informative for classification were compared with those identified with ALDEx2 as differentially abundant between *Salmonella*-positive and -negative samples.

Results: Conditional random forest outperformed the other two methods based on the AUC (0.82) and Kappa score (0.38). Both conditional random forest and differential abundance tests identified bacterial genera *Aeromonas* (VI= 0.32) and *Tabrizicola* (VI= 0.12) as the two most informative taxa for predicting *Salmonella* contamination in tested water samples.

Significance: The genera identified in this study warrant further investigation as putative indicators of *Salmonella* contamination in surface waters (e.g., in other agricultural regions and across multiple growing seasons).

P3-158 16S Microbiome Analysis of Microbial Communities in Food Distribution Centers

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❖ ***Developing Scientist Entrant***

Introduction: 16S amplicon sequencing presents a snapshot of the prokaryotic genomic composition of a sample and is increasingly leveraged as a tool to identify areas of food facilities with possible pathogen residence.

Purpose: The purpose of this study was to examine the microbiome within distribution center (DC) environments, specifically in those handling fresh produce, using 16S amplicon sequencing.

Methods: Environmental swabs (n=303) were collected on non-food contact surfaces in 18 DCs across the United States. Surfaces (40 cm x 40 cm) were swabbed using sterile sponge sticks pre-moistened with 10 mL of Dey-Engley broth. After collection, DNA was extracted from sponges using the DNeasy PowerSoil Pro kit and underwent 16S amplicon sequencing on the Illumina MiSeq platform. Raw reads were processed using *dada2* for taxonomic assignments and downstream analyses were completed using *phyloseq* on RStudio.

Results: Genera with the greatest relative abundance across all DCs were *Carnobacterium* (29.5%), *Psychrobacter* (28.6%), *Pseudomonas* (14.3%), and *Staphylococcus* (10.6%). Additionally, plant plastid DNA was also detected at a relatively high abundance (14.2%). Alpha diversity indices (observed, Shannon, Simpson) suggested significantly different ($P<0.01$) richness and evenness within microbiomes across individual DCs, sample site dryness, and US geographic region. ASVs identified as *Listeria* out of all reads had a relatively low prevalence (ca. 4%). In betadisper and PERMANOVA analyses, several variables (e.g., season, sample site dryness) demonstrated homologous dispersions or variances, but did not indicate homologous compositions among unweighted UniFrac distances.

Significance: Environmental conditions within DCs can possibly harbor a range of bacterial species, especially those that are cold tolerant, including potentially pathogenic organisms (e.g., *Pseudomonas, Staphylococcus*). Additionally, microbiome composition appears to vary significantly across groupings within variables (e.g., DC, season, general sampling location).

P3-159 Genetic Characterization of *Salmonella* Phage vB_SalS-KFSSE for the Construction of a Reporter Phage

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Introduction: Several easy and rapid methods have been developed to detect *Salmonella*, which is recognized as top three major foodborne pathogens. However, almost these methods couldn't differentiate live and dead bacteria. In differentiating live bacteria, phage has great advantage due to specific activities for live bacteria. Thus, in this study, lacZ gene, the reporter gene, was inserted into phage genome for engineering reporter phage for viable *Salmonella* detection.
Purpose: *Salmonella* phage vB_SalS-KFSSE was genetically analyzed to find out reporter gene insertion region for engineering reporter phage.
Methods: *Salmonella* phage vB_SalS-KFSSE was isolated and purified from the mixture of S*. Enteritidis* ATCC 13076 and chicken processing plant samples. A genomic DNA was extracted using phage DNA isolation kit for WGS analysis. Based on the NCBI database, the phage was analyzed for ensuing its novelty. Using Virulence Finder 2.0, CARD, and Allergen database, the phage was analyzed for ensuring its safety. To identify the reporter gene insertion region, open reading frames (ORFs) were annotated using NCBI BLASTP and RAST server, and visualized using CGView server.
Results: *Salmonella* phage vB_SalS-KFSSE was 59,715 bp in length with G+C content of 56.9% and showed 95.15% similarity to *S.* phage Chi, meaning a novel phage. In phage safety analysis, factors causing toxicity, mutations, or allergies were not identified in the phage. In ORFs prediction, the functions of 27 ORFs were confirmed by comparing with the data of BLASTP. As a result, downstream of major capsid protein coding region was selected as reporter gene insertion region. To insert reporter gene, homologous assembly was designed. In summary, *Salmonella* phage vB_SalS-KFSSE had its novelty and safety, and also it had reporter gene insertion region for engineering reporter phage.
Significance: This study demonstrated that *Salmonella* phage vB_SalS-KFSSE is appropriate candidate for phage engineering and it will contribute to develop "All-in-One" Rapid Kit for *Salmonella* detection.

P3-160 Microbial Diversity Analysis of Retail Poultry Meat via 16s Community Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* contamination in food products is typically detected through culture-based methods which eliminate the ability to examine the original microbial relationships present and often provide identification of pathogens simply to the genus level.
Purpose: This study sequenced the entire 16s ribosomal RNA gene, the internal transcribed spacer region, and a portion of the 23s ribosomal RNA gene in *Salmonella* positive retail meat samples to examine microbial relationships between *Salmonella* strains and other bacteria present in a non-enriched retail meat environment.
Methods: In the study, 129 retail poultry meat samples from Southern California and Hawaii were collected in accordance with the Food and Drug Administration's National Antimicrobial Resistance Monitoring System. Samples were removed from their retail packaging via aseptic technique and placed in 250 milliliters sterile 1x phosphate buffered saline. A 100 ml aliquot of the resultant rinsate from each sample was taken and centrifuged to generate a tissue and bacterial pellet. The pellet was then subject to DNA extraction and polymerase chain reaction amplifying the 16S-ITS-23S ribosomal RNA gene via the Shoreline Complete StrainID Kit. Resulting amplicons were run on the PacBio Sequel II. De-multiplexed data was mapped to Shoreline's Athena database15 for strain level identification.
Results: Relative abundance data was examined to generate measures of alpha diversity for each sample. Beta diversity was utilized to create pairwise comparisons of diversity between each positive sample. Principal component analysis of sequence data was utilized to demonstrate relative associations between *Salmonella* and other bacterial genera detected in the retail meat samples.
Significance: This study demonstrated the application of 16s community sequencing techniques employed in soil microbiome analysis as a novel detection method for *Salmonella* contamination in retail poultry meat. As well the greater taxonomic resolution this method provided allowed examination of *Salmonella* microbial relationships to the strain level in a non-enriched environment.

P3-161 *Vibiro parahaemolyticus* and *V. vulnificus* Profiles and Microbial Community Assessments of Blue Crabs (*Callinectes sapidus*) and Seawater Harvested from the Maryland Coastal Bays

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❖ ***Developing Scientist Entrant***

Introduction: Fluctuations in physicochemical parameters can affect the diversity and prevalence of microbial communities, including vibrios, in aquatic species and their surrounding environments.
Purpose: This study aimed to investigate the population dynamics of vibrios and the microbial community diversity of whole crab and seawater from the Maryland Coastal Bays (MCBs) using 16S rRNA sequencing.
Methods: During this study, three crabs and one liter of seawater were collected from two sites over three months ((3 crabs x 2 sites x 3 months = 18 crab samples) + (1L of seawater x 2 sites x 3 months = 6 seawater samples)). Hemolymph and crab tissue was extracted and pooled for each site. Extracted hemolymph, crab tissue, and seawater were analyzed for *Vibrio parahaemolyticus* (Vp) and *V. vulnificus* (Vv) using Most Probable Number (MPN) real-time PCR. Three different DNA extraction kits were evaluated to extract microbial DNA from individual crabs. Also, 500 ml of each seawater sample was filtered for DNA extraction. The V4 and V5 hypervariable regions of the16s rRNA gene from extracted DNA samples was amplified using universal primers and sequenced using a Illumina Miseq benchtop sequencer.
Results: Results indicated that sample types and sampling periods had a significant effect on the alpha diversity of the microbial community of crabs and seawater ($p < 0.05$). However, no statistical difference was found between DNA extraction kits and the microbiota of samples. Results indicated that Proteobacteria, Cyanobacteria, Actinobacteria, and Bacteroidetes bacteria were present in both crab and seawater samples. Vp and Vv were also detected in both crab and seawater samples, although crabs contained a higher concentration of 3. 68 logMPN g^{-1} (Vp) and 0.88 logMPN g^{-1} (Vv) and compared to the seawater samples.
Significance: Results from this study provide further insight on species diversity and phyla-genetic compositions of blue crabs and seawater from the MCBs. These approaches will help in risk assessments that are essential in the overall advancement of public health.

P3-162 Evolution and Diversity of Chaperone Usher Fimbriae Encoded by *Salmonella*

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Introduction: *Salmonella* encodes 36 different types of fimbriae (proteinaceous appendages that facilitate binding to surfaces, such as host cells), although the function of most remains uncharacterized.
Purpose: We characterized the sequence diversity of chaperone-usher [CU] type fimbrial ushers, representing the most common type of fimbriae in *Salmonella* spp. isolates (i) to determine the distribution of these fimbriae, and (ii) understand the evolutionary events shaping the resulting 'fimbriome' of *Salmonella* spp.

Methods: Assemblies representing all main phylogenetic groups of *Salmonella* spp. including *S. bongori* (13 isolates), and *S. enterica* subspecies *enterica* (242 isolates) - VII (5 isolates per subspecies), were downloaded from NCBI. Coding sequences were annotated with InterProscan 5.44-79.0, and amino acid sequences of the usher component of CU type fimbriae, were extracted. Phylogenies were inferred with IQ-TREE v. 2.0.7 for sequences aligned with MAFFT v.7.453.

Results: A total of 3,252 putative fimbrial ushers (used to represent presence of a fimbria) were identified, with members of the γ-fimbrial family representing the most numerous group (n = 2,083 ushers; 70% of all ushers detected). The number of fimbriae differed significantly by clade ($p < 2.2E-16$; Kruskal-Wallis test), with isolates in subsp. *arizonae* and *enterica* clade A1 having the lowest and highest (5 and 13 ushers per genome, respectively) median number of ushers per genome, respectively. With the exception of eight fimbrial ushers that were only detected in subsp. *enterica* isolates, most fimbrial ushers were found in non-subspecies *enterica* serovars suggesting that they were most likely acquired prior to the divergence of subspecies *enterica*.

Significance: Our study provides an updated, curated database of fimbriae encoded by *Salmonella*, to facilitate standardization of the detection and characterization of fimbriae. These data can be used to facilitate the identification of genomic features associated with likelihood to infect/colonize different agricultural hosts (e.g., poultry, cattle) for informing the development of targeted approaches to reducing *Salmonella* in the food supply.

P3-163 Context Matters: Environmental Microbiota of Ice Cream Processing Facilities Affects the Inhibitory Performance of Two Lactic Acid Bacteria Against *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Lactic acid bacteria which produce compounds inhibitory to *Listeria monocytogenes* may complement cleaning and sanitizing procedures in dairy processing facilities; however, it remains unknown whether the environmental microbiota of a dairy processing facility affects the antilisterial activity of biological control agents.

Purpose: The antilisterial activity of two lactic acid bacteria, previously tested in poultry processing facilities, was evaluated in the presence of environmental microbiota of three ice cream processing facilities (A, B, and C).

Methods: Lactic acid bacteria were co-cultured in polypropylene tubes containing BHI for 3 days at 15°C with and without an 8-strain cocktail of *L. monocytogenes*, in the presence of the environmental microbiota collected from ice cream processing facilities. After incubation, the concentration of attached *L. monocytogenes* was quantified using the MPN method, and the attached biomass was characterized by sequencing of the 16S rRNA V4 gene region.

Results: *L. monocytogenes* concentration increased compared to the positive control by 0.38±0.42 log MPN/ml in the treatments with the microbiota of Facility A, while it decreased by 0.99±0.59 and 2.54±0.45 log MPN/ml for all treatments when in the presence of the microbiota of facility B and C. The attached biomass had a high relative abundance of *Pseudomonas* and *Enterobacteriaceae* in facility A, of *Pseudomonas* in facility B, and of *Enterococcus* in facility C. Based on these results, we hypothesize that the presence of psychrotrophs, like *Pseudomonas*, may prevent adhesion of lactic acid bacteria to the surface reducing their inhibitory action against *L. monocytogenes*.

Significance: Our study indicated that the presence of certain bacteria may affect the attachment and inhibitory action of strains of lactic acid bacteria. Further work is being conducted to understand how *Pseudomonas* attach and may prevent inhibition of *L. monocytogenes* by lactic acid bacteria, and the optimal conditions for efficient inhibition of *L. monocytogenes* in dairy processing environments.

P3-164 Native Bacterial Communities Present in Romaine Lettuce and Their Interactions with *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Outbreaks of foodborne pathogens linked to Romaine lettuce (RL) continuously occurred in recent years, however the interaction between native RL microbiota and foodborne pathogens reminds largely unknown.

Purpose: This project aimed to characterize the interaction between RL microbiota and foodborne pathogen *Listeria monocytogenes* (*Lm*) and identify and isolate competitive exclusion (CE) bacteria with antagonist function over *Lm*.

Methods: Culturable total aerobic (APC) and anaerobic bacteria (AnPC) present in RL purchased from early and late seasons were evaluated by plating sample suspensions onto plate count and anaerobic agar during storage at 4 ℃ for 5 days. Part of the samples were also artificially inoculated with *Lm* at 3 or 6 Log CFU/g levels. The fate of *Lm* during 5 days of storage at 4 ℃ were studied with modified oxford agar. To investigate the interaction between RL native microbiota and *Lm*, DNA was extracted from both inoculated and un-inoculated samples and used for 16S rRNA gene sequencing. CE bacteria were isolated by using the double-layer agar method with trypticase soy agar and characterized using whole genome sequencing.

Results: No significant difference of APC (6.79 ± 0.26 Log CFU/g) and AnPC (4.24 ± 0.29 Log CFU/g) was detected in RL from two seasons between the storage. *Lm* decreased by 0.65 and 0.56 Log CFU/g in samples inoculated with low and high levels of *Lm* respectively in late season RL ($P < 0.05$). The 16S rRNA gene sequencing identified ninety-six genera across RL and *Pseudomonas* and *Pantoea* negatively correlated with *Lm* ($P < 0.05$). Late season RL comprised lower diversity of bacteria ($P < 0.05$). Forty-two CE bacteria inhibiting *Lm* were isolated from late season RL samples.

Significance: Native microbial populations present in RL impact the fate of *Lm* during storage; the presence of CE bacteria plays important roles in RL product safety.

P3-165 Microbiome Analysis of Raw Honey Reveals Important Factors Influencing the Bacterial and Fungal Communities

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Introduction: Raw honey contains diverse microbial communities. Previous studies focus on isolating bacteria and fungi that are culturable, while missing a large proportion of the microbial community. Using metagenomic methods to analyze the composition of microorganisms in raw honey can reveal the environmental and physicochemical variables that are associated with different microbial communities.

Purpose: The purpose of this study was to examine the microbial composition of raw honey and analyze the association between specific taxon abundance and the physicochemical properties of raw honey.

Methods: Four types of honey samples (monofloral, wildflower, manuka, and feral) were used for microbiome analysis. Genomic DNA was extracted directly from honey samples with PowerSoil Pro Kit. Illumina MiSeq with 2x250 bp paired-end reads was performed on amplified DNA sequences from 16S V3/V4 region and 5.8S/ITS2 region. Demultiplexing, quality filtering, OTU and taxonomy assignment was performed in QIIME 2. Decontamination, rarefaction and normalization, diversity analysis, and data visualization was performed in R. Four extraction controls and 2 PCR controls were included in this study. Physicochemical analysis of all honey samples, including pH, Brix, water activity, titratable acidity, and color, was performed in triplicate.

Results: Microbial community composition of 36 honey samples was visualized. Honey type was determined as a significant factor for both alpha and beta diversity based on ANOVA test, Kruskal-Wallis test, Wilcoxon rank sum test, and PERMANOVA test ($p < 0.05$). Other variables, including Brix, water activity, and titratable acidity, were weakly associated with bacterial and fungal community composition.

Significance: Results from this study provided important insights into the microbial composition of raw honey with culture-independent methods and revealed previously unidentified valuable probiotic organisms that were associated with specific types of honey.

P3-166 Impact of Chocolate Coating on the Survival of *Salmonella* on Dried Nuts and Fruits

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✦ ***Undergraduate Student Award Entrant***

Introduction: Most research on *Salmonella* survival in low-moisture foods has focused on single-ingredient products. Less is known about *Salmonella* survival in multi-ingredient low-moisture foods, or whether single-ingredient-based research is applicable when incorporated into a multi-ingredient product.

Purpose: The objective of this study was to investigate *Salmonella* survival on low-moisture foods (nuts and dried fruits) that are subsequently chocolate coated.

Methods: Roasted almonds, dried cherries, and candy-coating chocolate were obtained from a commercial supplier. Almonds (water activity, aw = 0.43) and dried cherries (aw = 0.48) were spot inoculated with 50 µl of a 5-serovar cocktail of *Salmonella*, and air-dried for 1 h at ~20°C. Half of the samples were immersed in the chocolate coating (tempered/maintained at 37°C prior to and during use) and allowed to set on wax paper. Control and chocolate-covered samples were then stored (~20°C up to 25 d), homogenized using a stomacher, serially diluted, plated on differential media (modified Tryptic Soy Agar), and survivors enumerated after incubation (48 h, 37°C). The experiment was performed in triplicate, and *Salmonella* survivor data were analyzed via analyses of variance.

Results: Almond (4.9 g) and cherry (4.1 g) samples were coated in 1.5-2.3 times as much chocolate (by mass), yielding average sample sizes of 12.5 and 13.6 g, respectively. *Salmonella* populations on almonds and chocolate-coated almonds did not decrease from initial population levels of 8.4 and 7.8 log CFU/g ($p > 0.05$), respectively. *Salmonella* populations on cherries and chocolate-coated cherries, initially 7.3 log CFU/g, decreased at different rates ($p < 0.05$), resulting in day-25 *Salmonella* levels below the limit-of-detection and 5.9 log CFU/g, respectively.

Significance: The persistence of *Salmonella* on products subsequently coated in chocolate depended on the initial product. This complicates the application of existing *Salmonella* survivor research to multi-component products.

P3-167 Bacterial Diversity of Different Melon Types Grown in Different Regions

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✦ ***Undergraduate Student Award Entrant***

Introduction: In the United States, 90% of commercial melons are grown in California and Arizona. Consumerism has other states expanding growing operations for healthier and more types of melons.

Purpose: The goal of this study was to determine the variation of bacterial diversity on different types of melons grown in the United States.

Methods: Different melon varieties (n = 438) grown in seven locations around the United States over a two-year period were used for microbiome analysis. Melon rinds were swabbed with a detergent solution to displace microbes on the surface, and then swabs were DNA extracted with the Qiagen PowerSoil Pro Kit. Samples were barcoded, chloroplast blocked, and the V3-V4 region of the 16S rRNA gene amplified, and then the barcoded samples were then sequenced on an Illumina MiSeq. Finally, the alpha and beta diversity and the taxonomic profile of the samples were determined with QIIME2 (v2020.2).

Results: Kruskal-Wallis one-way analysis of variance of the alpha diversity of the different samples found highly statistically significant differences in bacterial diversity based on the type of melon and location growth ($p < 0.01\%$). Yet, principal coordinate analysis using the Bray-Curtis distance matrix found melon samples clustering more by region of growth than type of melon. For example, taxonomic analysis found high levels of *Leuconostocaceae* in California grown melons that was not present on similar melon types from other states. Whereas Indiana and North Carolina grown melons had *Microbacteriaceae* in higher levels compared to other states.

Significance: This study provides the foundation of the bacterial diversity present on different melon varieties and the contribution the region of growth has in the diversity. Understanding this diversity could help to define the roles antagonistic and protagonistic bacteria have against foodborne pathogens to improve melon safety, but also can improve shelf life and plant health.

P3-168 Treated Soil Organic Amendments Alter Produce Phyllosphere Microbiome but Do Not Increase the Risk of Contamination with Foodborne Pathogens

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Introduction: Organic soil amendments are applied to soils to improve plant growth and maintain soil quality and health. However, the improper handling and application of organic soil amendments, especially in their raw or undertreated form or applying manure at the wrong time, may result in contamination of fresh produce with foodborne pathogens (FBP).

Purpose: The objective of this study was to assess the response of phyllosphere microbiome of collard greens to organic soil amendments.

Methods: Randomized field trials were set up with a plot size of 3 × 3 m^2. Soil was amended with composted poultry manure, composted dairy manure or treated sewage sludge (milogranite) at four levels (control: 0 kg/ha, low: 168 kg/ha, med: 336 kg/ha, high: 672 kg/ha) right before planting. Collard greens were grown in the plots. Each treatment was replicated three times resulting in a total of 36 plots. Six leaf samples were collected from each plot at the time of harvest. DNA was extracted from leaf washes, and V4 region of bacterial 16S rRNA gene was sequenced and analyzed.

Results: A significant increase in alpha-diversity was observed with produce grown in the amended soils compared to the control based on the observed OTUs ($p \leq 0.05$). Principal coordinates analysis (PCoA) of the weighted UniFrac distance metric showed a clear separation between the amendments. Gammaproteobacteria populations significantly increased in phyllosphere of collard greens grown in the treated soils ($p \leq 0.05$) but potential foodborne pathogenic genera and species were not enriched.

Significance: Application of properly treated organic amendments alter produce phyllosphere bacterial communities but do not increase potential FBP on fresh produce.

P3-169 Identification of the Genes of *Salmonella* Enteritidis That Contributes to Attachment and Biofilm Formation

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❖ ***Developing Scientist Entrant***

Introduction: In the United States and Canada, outbreaks of *Salmonella* Enteritidis infection have been linked to various types of low moisture food, including tree nuts and ground nuts. It has been proposed that biofilm formation is one of the strategies for *Salmonella* to survive in/on low moisture food. However, little is known about the molecular mechanisms underlying *S*. Enteritidis biofilm formation in/on low moisture food.

Poster

Purpose: The purpose of this study is to identify the genes that play a significant role in attachment and biofilm formation by *S*. Enteritidis.

Methods: To identify biofilm formation-associated genes of *S*. Enteritidis, mini-Tn*10* transposon mutagenesis was used to create a mutant library by randomly inserting the mini-Tn*10* transposon into the genome of *S*. Enteritidis almond outbreak strain. To determine the biofilm-forming ability of acquired mutants, biofilm mass accumulated by the mutants and parent strain on polystyrene tissue culture plates was compared using the crystal violet binding assay. Mutants that formed significantly less biofilms in comparison to their parent strain were selected. Subsequently, their genomic DNA was extracted for deep DNA sequencing. Specific gene in each mutant interrupted by the transposon insertion was identified by comparing the obtained DNA sequencing data with those deposited in the Genbank using the BLAST search

Results: A total of 64 mutant colonies of *S*. Enteritidis were obtained by the transposon mutagenesis, and 5 mutants that formed significantly less biofilms than the parent strain were selected for further study. Results of BLAST search revealed that the interrupted genes in the mutants are responsible for the biosynthesis of aldehyde dehydrogenase (EutE), cysteine desulfurase (SufS or SufE), a transporter protein, porin OmpL, and a ribbon-helix-helix protein from the CopG family.

Significance: Products of these identified genes could be promising targets for control of *S*. Enteritidis colonization on low moisture foods and their production environment.

P3-170 Role of SNRNAS in Biofilm Formation in *Salmonella enterica* Serovars Typhimurium and Enteritidis

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Introduction: The ability to form biofilms of *Salmonella* contributes to its persistence and resistance in both host and food processing environments. Recent evidence suggests a regulatory network of non-coding small RNAs (sRNAs) may play a crucial role in bacterial biofilm formation. However, the role of sRNAs in biofilm formation in *Salmonella* is not elucidated so far.

Purpose: To evaluate the distribution and function of sRNAs in biofilm formation in 12 clinically important strains of *S. enterica* serovars Typhimurium and Enteritidis.

Methods: A total of 26 sRNAs were selected based on the literature survey. The sequences of the orthologs of these sRNA genes were obtained from KEGG database. The genome BLAST was performed, and the sRNA sequences that returned the hit were used to search for targets within *S.* Typhimurium and *S.* Enteritidis genomes using TargetRNA2. The sRNA targets were then manually curated for understanding biofilm formation pathways.

Results: We have identified 21 sRNAs and their contagious mode of distribution in all 12 genomes of *S. enterica* through the genome BLAST approach. Additionally, 456 target mRNAs were identified using TargetRNA2. These targets were associated with different processes related to biofilm formation, including carbohydrate polymer biosynthesis and transport. However, these 21 sRNAs may be divided into two mechanistic pathways, viz. BarA/SirA/CsrA pathway and Environmental signaling pathway. In the first pathway, only two sRNAs (viz. csrB and csrC) were found to play a pivotal role by regulating mRNA of CsrA. The rest of the sRNAs are somehow associated with the Environmental signaling pathway.

Significance: This research will provide new insights into biofilm biology. The environmental signaling pathway of sRNAs may aid in the indirect molecular monitoring of *Salmonella* biofilm formation in food processing environments. sRNAs may even be potential targets to develop new generation therapeutics to counteract *Salmonella* infection.

P3-171 Identification and Characterization of Toxin-Producing Genes in *Bacillus cereus* Group Genome Assemblies

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Introduction: The *Bacillus cereus* group includes many closely related *Bacillus* species that carry toxin-producing genes, essential to emetic and diarrheal foodborne illness.

Purpose: This study intends to pinpoint the toxin-producing genes present in various *B. cereus* group organisms that may contribute to foodborne illness and provide a risk assessment of virulence potential.

Methods: Illumina kits and MiSeq sequencer were used to perform whole genome sequencing for 33 *B. cereus* group isolates acquired from food and environmental sources, including *B. thuringiensis*, *B. cereus*, *B. toyonensis*, and *B. mycoides.* Biochemical analysis of tyrosine decomposition, beta hemolytic activity, protein toxin crystal formation, plus ribotyping was also performed for these isolates. BTyper virulence gene-based typing, multilocus sequence typing, *panC* clade typing, and *rpoB* allelic typing were applied to the genome assemblies.

Results: Six toxin-producing virulence genes, including *bceT*, *entA*, *entFM*, *nheA*, *nheB* and *nheC*, were identified in 15 *B. cereus* assemblies, as expected. These six targeted genes were also detected in *B. mycoides* and *B. toyonensis.* The *bceT*, *entA*, *entFM*, *nheA*, and *nheB* virulence genes were detected in nine *B. thuringiensis* assemblies, while *nheC* was only partially detected. The strain assemblies were classified into four *panC* clades, including 29 strains that clustered into *panC* clade 4, in which all six toxin-producing genes were identified. Two assemblies clustered into *panC* clade 3, in which all targeted genes, except *bceT,* were detected.

Significance: Toxin-producing genes that contribute to foodborne illness were detected in several non-*B. cereus* assemblies, such as *B. thuringiensis*, *B. toyonensis*, and *B. mycoides*, making this a public health concern in agriculture as many *B. cereus* group organisms are ubiquitous to soil and utilized in biofertilizers. Accurate identification and classification is pertinent to preventing foodborne illness.

P3-172 Genomic Surveillance of *Bacillus cereus* Group Strains Isolated from Meat and Poultry Products in South Africa

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Introduction: Members of the *Bacillus cereus* group vary in their ability to cause illness, but are frequently isolated from foods; however, food safety surveillance efforts that employ whole-genome sequencing (WGS) often neglect these potential pathogens.

Purpose: Using *B. cereus* group strains collected via South Africa's national meat surveillance program, the utility of WGS for *B. cereus* group surveillance and source tracking in foodstuffs was evaluated.

Methods: *B. cereus* group strains (*n* = 25) isolated during routine surveillance of domestic and imported meat and poultry products in South Africa underwent WGS (Illumina HiSeq). Following quality control and assembly, genomes sequenced here were supplemented with publicly available *B. cereus* group genomes (*n* = 2,862) and underwent *in silico* taxonomic assignment, multi-locus sequence typing (MLST), *panC* group assignment, and virulence factor detection using BTyper3 v3.1.1. Taxonomic classification was additionally performed using the Genome Taxonomy Database (GTDB) Release 05-RS95 and GTDB-Tk v1.3.0. Core single nucleotide polymorphism (SNP) distances and average nucleotide identity (ANI) values were calculated within resulting lineages using Snippy v4.6.0 and FastANI v1.31, respectively.

Results: The 25 strains sequenced here were assigned to *panC* Groups IV, III, II, and V (*n* = 18, 5, 1, and 1, respectively). One strain possessed cereulide (emetic) synthetase-encoding genes. No strains harbored anthrax toxin- or capsule-encoding genes, including two strains assigned to GTDB's *B. anthracis* genomospecies. The 25 strains represented 15 lineages via MLST, six of which contained multiple strains sequenced in this study, which were identical or nearly identical at the whole-genome scale. Five lineages contained nearly identical genomes collected from two or three South African provinces; one lineage contained identical genomes from two countries (South Africa and the Netherlands).

Significance: Results presented here provide strong evidence that *B. cereus* group strains can be disseminated intra- and inter-nationally via the food supply chain.

P3-173 Application of Time-Temperature Indicator (TTI) Based on Maillard Reaction for Visual Monitoring of the Quality of Frozen Shrimp and Chicken Under Dynamic Temperature Conditions

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Introduction: To monitor temperature history during frozen food distribution in cold chain, time-temperature indicator (TTI), which alerts the temperature abuse by color change, is effective. The TTI based on Maillard reaction is highly flexible due to its wider reaction temperature range than those of other mechanism TTIs. The Maillard-reaction based TTI could allow to monitor the temperature history of frozen foods and also to predict the quality of frozen chicken and shrimp, whose quality deterioration is difficult to confirm by its appearance.

Purpose: The purpose of this study was to examine the applicability of Maillard-reaction based TTI as one of the temperature monitoring and quality prediction methods during distribution of frozen foods.

Methods: Maillard-reaction based TTI (D-xylose and glycine) and shrimp or chicken portions were stored under dynamic temperature conditions from -18°C to 10°C. We examined the changes in total viable counts (TVC) and total volatile basic nitrogen (TVBN) of chicken and shrimp (n=3) and the color difference of the TTI to investigate the correspondence between the TTI and the quality deterioration of the food samples.

Results: When the TVC and TVBN of the shrimp and chicken considering those standard errors reached or just before reached the safety standard values (TVC: < 7 log CFU/g; TVBN: <20-30 mg%), the TTI finished its reaction and changed from clear through blue to black. The color change kinetics of the TTI successfully agreed with the quality deterioration kinetics of the samples under frozen to chilled temperature. The difference in activation energy between the TTI and the quality deterioration was within the acceptable range of ±40 kJ/mol. The results suggest the applicability of the TTI to notify the deterioration of frozen food quality.

Significance: This study demonstrates the applicability of Maillard-reaction based TTI in frozen food distribution and contributes to the implementation of appropriate temperature monitoring in cold chain.

P3-174 Use of Fish Gelatin Coating and Moisture Scavenging Packaging to Extend the Shelf Life of Fresh Never Frozen and Frozen/Thawed Catfish Fillets

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Introduction: Fish gelatin can be used as an edible coating to act as a barrier protecting the product from losing moisture, and exposure to oxygen, and CO_2. Furthermore, the use of moisture scavenging packaging (MS) could assist extending product shelf life by reducing water activity.

Purpose: The purpose of this study was to determine the effect of fish gelatin coating and MS in the shelf life of fresh never frozen and frozen/thawed catfish fillets.

Methods: Catfish fillets were randomly divided into eight groups. Four groups were untreated, two of these were packed in nylon bags (C), and two in MS trays (CM). The other four groups were coated with gelatin, two of these packed in nylon bags (G), and two in MS trays (GM). Half of the samples were stored at refrigeration temperature for 16 days, tested every four days starting on day zero. The remaining half was stored frozen for seven weeks then thawed and tested as the first half (FC, FG, FCM, FGM). Two independent replicates for each treatment were analyzed. Samples were tested for physical/chemical and microbial stability.

Results: For microbial stability, gelatin coating did not show a benefit for fresh never frozen (F) and frozen/thawed (FT) catfish fillets packed in nylon and in MS trays. On the other hand, MS trays did not show a benefit for F samples, however a benefit was observed in FT samples. At day eight, FT mesophilic aerobic count (Log CFU/g) was 5.45±0.01 (FC), 5.45±0.01 (FG), 3.95±0.06 (FCM), and 3.59±0.25 (FGM). Gelatin coating and MS slowed down the rate of fat oxidation in F and in FT samples up to day eight ($P < 0.05$). No synergistic effect was observed between gelatin and MS trays.

Significance: Moisture scavenging packaging showed a benefit extending quality of frozen/thawed catfish fillets.

P3-175 Development and Evaluation of UV-Activated Oxygen Scavenging System Based on Natural Rubber Latex for Food Safety and Quality

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Introduction: Global food loss statistics are raising concerns. Food safety and security are one of the burning issues of the modern world. Sustainable development of food packaging materials that promote a circular economy is the hour of need.

Purpose: In the presence of oxygen, foods are highly susceptible to oxidative degradation. It affects the food quality and shelf life.
This research was aimed to explore the possibilities of Natural rubber latex along with a photocatalytic system consisting of acetophenone as a photoinitiator and manganese chloride as a catalyst as a biobased oxygen scavenging material, and named NRL/PCS1%, NRL/PCS3%, and NRL/PCS5%, respectively.

Methods: The developed oxygen scavenging system was studied by using Fourier transform infrared spectroscopy (FTIR), Colorimetry, and zeta potential. Further, the Rheological properties were evaluated to understand the futuristic coating applications. Later the OSS was subjected to its oxygen scavenging capacity evaluation.

Results: Colorimetry and Fourier transform infrared spectroscopy (FTIR) were performed before and after oxygen scavenging. The rheology along with particle size (399.6 nm) and zeta (-23.30 mV) was done to know viscosity and stability for the viability of NRL/PCS3 as active coatings in packaging applications. The prepared oxygen scavenging system showed an oxygen absorption capacity of 1045 ml O_2/g (at 25 ℃) and a rate of 17.42, 52.25 ml O_2/g. day at 25 ℃ and 45 ℃ respectively. The rate constant value was increased to 0.4761 h^{-1} from 0.2119 h^{-1} when the temperature was raised from 25 ℃ to 45 ℃. The sample size for all the measurements was taken as n=3 and performed one-way statistical analysis of variance with Tukey's multiple significant difference level of 0.05

Significance: This research significantly supports and improves food safety and quality in terms of packaging.

P3-176 Development of Gallic Acid Coated Label as a pH-Sensitive Oxygen Indicator for Smart Food Packaging Applications

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Introduction: Smart packaging techniques enhace the functionalities of a passive packaging. A better communication of the state of a packed product can be achieved using visual indicators. Oxygen indicators has a significant role to play in vacuum packed foods and post harvest produce packaging

Purpose: The objective of this work is to use gallic acid as a potential oxygen indicator which is catalyzed by the pH of the product.

Methods: Coating solutions were formulated using cellulose-acetate, ethyl acetate/ethanol, and different concentrations of GA. It was bar coated onto a paper substrate using a layer by layer technique and dried at ambient conditions

Results: Upon exposure to varying pH at atmospheric oxygen, the coated paper exhibited a color change from green to yellow for a pH range 8-12 as evident from UV-Visible spectra at 400-800 nm. AFM was performed for surface roughness of the coating. Rheological studies showed GA20% has better viscosity for coating application, FE-SEM, AFM images confirmed GA 20% has smoother surface. ΔE values has confirmed that GA30% has highest color

difference at all pH. A time dependant color change of gallic acid in pH 12 has been performed at different oxygen concentrations to understand its oxygen sensitivity.The sample size for all samples were taken as 3, and one way ANOVA was performed using tukey's multiple significance level of 0.05.

Significance: This work adds to the knowledge of using gallic acid as an oxygen indicator system which is novel of its kind.

P3-177 Evaluation of Invisishield™ Technology to Reduce *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica* on Blueberries and Tomatoes Using the Antimicrobial Chlorine Dioxide

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Introduction: Chlorine dioxide (ClO_2) is a promising antimicrobial with various food applications, one of those being antimicrobial packaging systems. The ability of controlled release ClO_2 to inactivate pathogenic foodborne bacteria has not been extensively studied.

Purpose: To evaluate a novel ClO_2 – based antimicrobial packaging system (InvisiShield™) for its efficacy against *E. coli, L. monocytogenes,* and *S. enterica* on blueberries and grape tomatoes.

Methods: Blueberries or grape tomatoes were placed in polypropylene trays and selectively inoculated with 10µl of *E. coli, L. monocytogenes,* and *S. enterica* overnight cultures. Trays were heat sealed with a three-phase polymer film consisting of a base, channeling agent, and the ClO_2 active (treatment); or control (no active) film and stored at 7°C for 0, 24, 48 hrs, and 7 days. At each time point, the fruit was collected and processed for enumeration of viable bacteria.

Results: Log_{10} reductions (LR) in *E. coli* CFU on blueberries was 4.69±0.61 at 24 hours and reached limit of detection (LOD; 5.23 LR) at 48 hours. LR in *L. monocytogenes* CFU on blueberries was 4.02±1.20 and 4.40±0.63 at 24 and 48 hrs, respectively, and reached LOD (4.72 LR) at 7 days. LR in *S. enterica* CFU on blueberries was 3.08±0.64 and 4.73±0.35 at 24 and 48 hrs, respectively, and reached LOD (4.74 LR) at 7 days. LR in *E. coli, L. monocytogenes,* and *S. enterica* CFU on tomatoes reached LOD (5.19, 4.40, and 4.61 LR, respectively) for all exposure times.

Significance: This novel ClO_2-based antimicrobial packaging system effectively reduced concentrations of *E. coli, L. monocytogenes,* and *S. enterica* on blueberries and grape tomatoes after 24 hrs, with efficacy reaching LOD after 7 days in all cases. This technology shows promise in reducing pathogenic foodborne bacteria on fresh produce items.

P3-178 Effectiveness of Insect-Repellent Film Encapsulating Essential Oil Mixture Against the Larvae of *Tribolium castaneum*

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Introduction: *Tribolium castaneum* is one of the insects that penetrate packaging materials and degrade food quality. A previous study confirmed that *Origanum vulgare* oil (OV) and *Tanacetum cinerariifolium* oil (TC) had a potent insecticidal activity against *T. castaneum*. However, their insect-repellent activity was reduced, when employed, due to their volatile property. Thus, the encapsulation of these two oils will be required to carry and release their repellent activity for the development of packaging materials.

Purpose: The purpose of this study was to compare the insect-repellent efficacies of celite- and silica-based films containing the mixture of OV and TC against larvae of *T. castaneum*.

Methods: The oil mixture was prepared in different ratios (OV+TC, EO1; 1.5%+0.5%, EO2; 1.0%+1.0%, and EO3; 0.5%+1.5%). The insect-repellent efficacy of each mixture was investigated by placing twenty *T. castaneum* inside of cylinder trap designed for calculating the repellency index (%). Celite- and silica-based film containing the oil mixture were prepared by mixing with LLDPE, PE wax, Zn stearate as well as the selected oil mixture. The surface of these two films was observed using SEM. Their insect-repellent efficacy was finally compared using the cylinder trap for the calculation of repellency index.

Results: EO1 exhibited the most effective insect-repellent activity against larvae of *T. castaneum* [(63.38±2.49)%] (P<0.05). The porous circular particles (33.22±7.50 µm) for encapsulating oil mixture were observed in celite-based film. However, the SEM images of silica-based one showed aggregated circular particles (38.80±12.27 nm) without any porosity. The insect-repellent efficacy of celite- and silica-based films were found to be (37.5±23.2)% and (-73.6±23.6)%, respectively, indicating that the celite-based one exhibited more effective insect-repellent film for carrying the oil mixture against larvae of *T. castane*um (P<0.05).

Significance: This study has proved that the celite-based film for carrying oil mixture was more effective one for encapsulating OV and TC against larvae of *T. castaneum*.

P3-179 Chitosan and Epigallocatechin Gallate Grafted Chitosan Based Composite Films: Antioxidant and Antimicrobial Activities, and Its Application for Shelf Life Extension of Refrigerated Asian Seabass (*Lates calcarifer*) Slices

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❖ ***Developing Scientist Entrant***

Introduction: Chitosan (CS) has been considered as an excellent candidate for the preparation of bioactive films or packaging, however, it is soluble in aqueous acid, which results in a strong smell and flavor in foods. To avoid this, CS-epigallocatechin gallate (EGCG) conjugate (CEC) possesses enhanced water solubility, and bioactivities were incorporated in CS film solution to develop CS/CEC composite (CS/CEC-COMP) film.

Purpose: CS/CEC-COMP film was prepared to enhance the bioactivities of CS-based films and its application for shelf-life extension of Asian seabass slices (ASBS) stored in air or under vacuum packaging at 4 ºC.

Methods: CS (1%, w/v) and CEC (1%, w/v) were dissolved in acetic acid (1%, v/v) and water, respectively. Both solutions were mixed in a ratio of 8:2 (v/v) to prepare CS/CEC-COMP film. CS film (without CEC) acted as the control. Antioxidant (AO) and antimicrobial (AM) activities of resulting CS and CS/CEC-COMP films were determined. ASBS were packed in CS and CS/CEC-COMP films for shelf-life extension, in which microbiological, chemical and sensory properties were monitored during storage at 4 ºC for 15 days.

Results: CS/CEC-COMP film had higher total phenolic content (18.69 mg EGCG equivalent/g sample) and AO activities than CS film (p<0.05). CS/CEC-COMP film showed enhanced growth zone inhibition toward *Pseudomonas aeruginosa* (18.3 mm) and *Listeria monocytogenes* (21.3 mm) (p<0.05). The SEM images of tested bacteria confirmed the antimicrobial activity of CS/CEC-COMP film. ASBS wrapped with CS/CEC-COMP film and packed under vacuum (ASBS-COMP-VP) conditions resulted in a lower total viable count (5.1 log CFU/g sample) than other samples (p<0.05) up to 15 days. ASBS-COMP-VP possessed the lower oxidation of polyunsaturated fatty acids, which was mainly caused by free radical scavenging and antimicrobial activities of CS/CEC-COMP film.

Significance: CS/CEC-COMP film had higher bioactivities than CS film. Furthermore, the shelf-life of ASBS with sensory acceptability was prolonged for at least 12 days at 4 °C.

P3-180 Assessment of Traditional Red Swamp Crayfish Boils in Reducing the Prevalence of *Vibrio* spp.

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❖ ***Developing Scientist Entrant***

Introduction: The presence of zoonotic pathogens, specifically *Vibrio* spp., in commercially farmed crayfish populations has been documented both within and outside the United States, however the effectiveness of traditional crayfish (*Procambarus clarkii*) boiling to reduce the prevalence of this foodborne pathogen has not been evaluated within these U.S.A, populations.

Purpose: Examine the effectiveness of traditional crayfish batch preparation techniques in reducing the prevalence of *Vibrio spp*.

Methods: A 4.54 kg sack of crayfish (206 total) was obtained from a commercial Louisiana farm. One sample consisted of swabbing the exterior surface of five individual crayfish due to their communal nature upon arrival. Ten samples were collected from live pre-boiled crayfish, ten samples came from boiled unseasoned crayfish and five samples came from boiled seasoned crayfish. Crayfish were added to boiling water and cooked for five minutes once the water retuned to a rolling boil (97.7°C-98.8°C). After boiling, crayfish remained in the cooking water for an additional fifteen minutes with the heat source removed. The same process was repeated with traditional Louisiana seasoning added. Microbial analysis followed recommendations in the Food and Drug Administration's Bacteriological Analytical Manual and a Fischer's Exact Test was performed.

Results: Of the live pre-boiled crayfish samples, 100% (10/10) returned characteristic growth for *Vibrio* on TCBS agar. In contrast, 10% (1/10) of samples of crayfish boiled in unseasoned water returned growth for *Vibrio* ($p<0.0001$). Of the samples from crayfish boiled in seasoned water, 40% (2/5) returned growth characteristic of *Vibrio*.

Significance: This data suggests that using the bare minimum cooking time for crayfish in the traditional batch like process is effective in reducing presence of *Vibrio*. The growth of colonies from cooked seasoned crayfish samples (40%) warrants further investigation into time-temperature combinations to ensure pathogen reduction.

P3-181 Determination of Okadaic Acid, Dynophysistoxin-1, Dynophysistoxin-2 and Dynophysistoxin-3 in South Korean Seafood by Liquid Chromatography-Tandem Spectrometry

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✦ ***Undergraduate Student Award Entrant***

Introduction: The major marine toxins causing diarrheic shellfish poisoning (DSP) are okadaic acid (OA), dynophysistoxin-1 (DTX1), dynophysistoxin-2 (DTX2), and dynophysistoxin-3 (DTX3). DTX3 is their acylated form and converted to OA, DTX1 and DTX2 by hydrolysis in the gastrointestinal tract. However, studies on the detection of DTX2 and DTX3 in South Korean seafood have not yet been reported.

Purpose: The concentration of OA, DTX1, DTX2, and DTX3 in South Korean sea food and their compositions are determined by liquid chromatography-tandem spectrometry.

Methods: Among the seafood samples in South Korean coast, a total of 221 samples consisted of 15 shellfishes (*Mytilus coruscus, Mytilus galloprovincialis, Ruditapes philippinarum, Mizuhopecten yessoensis*) and 7 non-shellfishes (*Paralichthys olivaceus, Portunus trituberculatus, Apostichopus japonicus*). The procedure involved conversion of DTX3 to OA, DTX1 and DTX2 by alkaline hydrolysis and cleanup by solid phase extraction prior to liquid chromatography-tandem spectrometric analysis. To quantify DTX3, two procedures with hydrolysis and without hydrolysis were compared.

Results: OA, DTX1 and DTX3 were detected in 25 samples (*Mytilus coruscus*, *Mytilus galloprovincialis*, *Ruditapes philippinarum*, *Mizuhopecten yessoensis*, *Argopecten iradians*, *Anadara broughtonii*, *Mercenaria mercenaria*, *Atrina pectinate*) during the monitoring period from April to October. DTX2 was not detected in all samples. In *Mytilus coruscus,* OA (7.49–67.27 μg/kg), DTX1 (8.46–131.22 μg/kg) and DTX3 (12.85 – 280.7 μg/kg) were detected. DTX3 was the most prevalent toxin (77.7%) with a positive mean concentration of 172.60 μg/kg, followed by DTX1 (17.6%) with a positive mean concentration of 17.41 μg/kg.

Significance: This is the first research of quantifying DTX3 in South Korean seafood. The two samples with the highest detection rate and concentration of OA, DTX1 and DTX3 were *Mytilus coruscus* and *Mizuhopecten yessoensis*.

P3-182 Fitness and Transcriptomic Analysis of Pathogenic *Vibrio parahaemolyticus* in Seawaters at Different Oyster Harvesting Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: *Vibrio parahaemolyticus (Vp)* is an important foodborne pathogen worldwide and has been the leading cause of seafood-associated human gastroenteritis in the United States. However, knowledge gap exists about the fitness and survival mechanisms of *Vp* in pre-harvest environment.

Purpose: This study aimed to evaluate the survival of *Vp* in seawater at 10 or 30 °C and investigate its survival mechanisms at the transcriptional level.

Methods: Two strains of pathogenic *Vp* (*tdh*+ VP43996 and *trh*+ VP17802) were separately inoculated into autoclaved seawaters. Inoculated samples were stored at 10 or 30 °C for 10 days and 120 hours, respectively. The survival of inoculated *Vp* was evaluated every day at 10 °C and every 2-hour for the first 12 h, then at 24, 48, 72, and 120 h at 30 °C. RNA was extracted from *Vp* and the cDNA library was prepared and sequenced on the Illumina Hiseq 2500 v4 system. Differential expression tests were conducted using DESeq2. Gene Ontology (GO) of *Vp* was annotated using EGGnog-mapper. Downstream GO-Gene Set Enrichment Analysis (GSEA) and Kyoto Encyclopedia of Genes and Genomes (KEGG)-GSEA tests were conducted using clusterProfiler 4.0. Proteomap of *Vp* transcriptomic profiles was established based on KEGG orthology database.

Results: Culturable *Vp* cells gradually decreased at 10 °C while increased at 30 °C. While *trh*+ VP17802 had higher survival rates at 10 °C, *tdh*+ VP43996 showed higher growth rates at 30 °C. More differentially expressed genes were detected at 30 than 10 °C for both strains. The virulence of *Vp* was temperature-dependent as illustrated by different upregulated virulence-associated genes at 10 or 30 °C. Moreover, biofilm formation pathway was significantly downregulated at 10 °C, indicating the potential effectiveness of 10 °C seawater to inhibit biofilm formation of *Vp*.

Significance: Pre-harvesting temperatures have impacts on the survival and virulence of *Vp*. Information generated from this study may of use for the design and optimization of post-harvest handling practices for oysters.

P3-183 Withdrawn

P3-184 Microbiome Signatures to Determine Oysters' Geographical Region of Origin

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Introduction: Oyster industry, a significant component of the United States aquaculture, is vulnerable to various food frauds, including species substitution and the mislabeling of oysters' geographical origin for economic gains. The geographical origin misrepresentations are performed to claim a famed region of origin, known for its unique flavor profile. DNA barcoding is the gold-standard method for identifying seafood species, but the method has limited resolution till species level and is not appropriate for geographical origin misrepresentations.
Purpose: To characterize the microbiome profiles of oysters harvested from two distinct geographical regions and evaluate if oysters' geographical origin could be identified based on their microbiome signatures.
Methods: Raw east coast oysters (*Crassostrea virginica*) were collected from two distinct geographical locations [Maryland (n = 48) and Mississippi (n= 48)]. High-quality genomic DNA was isolated from the samples, whereafter the microbiome composition was measured by high-throughput 16S rRNA gene sequencing and analyzed using QIIME bioinformatics package in accordance with the Earth Microbiome Project benchmarked protocol.
Results: The analysis of beta-diversity revealed significantly distinct microbiome clusters among the two oyster groups. Further downstream analysis revealed several distinct microbial signatures among the two oyster groups. The Mississippi group has a higher proportion of bacterial taxa, including *Shewanella, Arcobacter, Campylobacter, Leptonema, Psychromonas, Flabovacterium, Desulfotomaculum, Pseudomonas, Mycobacterium, Desulfobacetrium, Aeromonas,* and *Sulfurospirillum*. In contrast, the Maryland group has a higher abundance of genera, including *Pseudoalteromonas, Vibrio, Pilibacter, Photobacterium, Enterococcus, Spiroplasma, Enterovibrio*, and *Oceanispira*
Significance: Our findings demonstrate the applicability of microbiome analysis as an emerging concept and tool for identifying geographical origin misrepresentations. The study suggests that the microbiome signatures could be exploited as a tool to identify the seafood region of origin.

P3-185 *Aeromonas hydrophila* Biofilm Formation in Aquaponic Water

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❖ ***Developing Scientist Entrant***

Introduction: *Aeromonas hydrophila* is a zoonotic pathogen causing illness in freshwater fish and susceptible humans. This foodborne pathogen has been confirmed within aquaponics systems and could pose a risk for people consuming aquaponic produce.
Purpose: This study determined whether *Aeromonas* isolates from an aquaponics system could form biofilms in and on aquaponic water and materials.
Methods: Aquaponic water from 4 locations within three recirculating aquaponics systems was filter sterilized: (a) fish tank, (b) beginning of deep-water culture (DWC), (c) middle of DWC, and (d) end of DWC. Water was aliquoted (180 µl) into a 96-well plate, inoculated with individual strains of *Aeromonas* (4 aquaponic isolates) at 5.0 log CFU/ml, then incubated at 25°C for 72 h. Biofilm mass was measured via crystal violet stain and *Aeromonas* populations quantified. Test tubes containing 4.5 ml fish tank water and nylon, PVC, liner, bio-bead, or foam were inoculated with 500 µl *Aeromonas* at 5.0 log CFU/ml, and incubated at 25°C for 72 h with orbital shaking (100 rpm). *Aeromonas* were enumerated from recovered biofilm. Data analysis used ANOVA and Tukey-Kramer HSD ($p<0.05$) procedure of JMP Pro (v16.0.0).
Results: *Aeromonas* biofilm formation in aquaponic water varied significantly by water collection location. *Aeromonas* formed the most biofilm with fish tank water (OD_{570} of 0.166±0.039) and the least with end of DWC water (OD_{570} of 0.146±0.038). Enumerated *Aeromonas* from the biofilm did not significantly vary among water sources and ranged from 7.25±1.21 log CFU/ml (end of DWC) to 7.74±0.48 log CFU/ml (fish tank). *Aeromonas* biofilm formation varied depending on aquaponic material. The liner had the greatest biofilm population (2.17±0.09 log CFU/cm^2), while the bio-bead had the least (0.61±0.02 log CFU/cm^2).
Significance: Aquaponics production requires a flourishing biofilm, but frequent monitoring of the system is critical to ensure pathogenic *Aeromonas* are not endemic.

P3-186 Determination of Spoilage Microbiota of Atlantic White Shrimp (AWS) Using Next Generation Sequencing (NSG) as an Alternative Method to the Standard Quality Evaluation during the Cold Chain

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Introduction: This work utilizes NGS to determine and identify spoilage bacteria during cold storage of Atlantic Whiteleg Shrimp. Psychrotrophic count at various refrigeration temperatures was conducted simultaneously for the duration of the experiment and monitoring of quality changes.
Most bacterial contamination occurs during the processing and handling of those aquatic products. As psychotropic bacteria can survive at low temperatures, those strains, i.e., *Shewanella* spp. and *Pseudomonas* spp., contribute to the spoilage of the aquatic products. Some methods involve isolating bacteria from selective and non-selective media, DNA extraction, or using the 16S rRNA gene. This work attempts to describe if the NSG method is sufficient for monitoring SSO.
Purpose: To evaluate the safety of fresh Atlantic White shrimp and identify spoilage bacteria at low refrigeration temperatures at 4°C.
Methods: The Psychrotrophic count of shrimp stored at 4°C was determined for 10 days by incubating at 4°C in triplicate. Physicochemical (changes in pH, TVB-N and TMA, and Lab color values) of AWS were investigated. Selected clusters of bacterial isolates were characterized, identified, and analyzed using SDS-PAGE electrophoresis, polymerase chain reaction (PCR), and 16S rRNA gene sequencing.
Results: However, we found an inconsistent growth pattern at 4oC incubation by using selective media colonies of *Pseudomonas* spp. *Shewanella* spp. were detected. TVB-N content increased from ≤ 25 mg/100 g N to 31 mg/100 g N. NGS provides an overview of the AWS's entire microbiota as it varies with storage conditions. The dominant shrimp microbiota stored at 4 °C were *Acinetobacter* (>70%), while *Psychrobacter, Shewanella, Pseudomonas,* and *Vibrio* represented 30% prevalence.
Significance: Provide more comprehensive information about the dominant spoilage microbiota in Atlantic White Shrimp under cold storage, which may remain unnoticed with culture-dependent methods.

P3-187 Using Functional Ice to Reduce Seafood Waste and Its Effect on the Value Chain in Honduras

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❖ ***Developing Scientist Entrant***

Introduction: Highly perishable nature of seafood leads to post-harvest food waste. Functional Ice (FICE) is an innovation over regular ice that can be used to actively suppress microorganisms during seafood storage and transportation.
Purpose: Validating the antimicrobial efficacy of peracetic acid (PAA) FICE on reducing bacteria on shrimp and tilapia surface and study its effects on value chain in Honduras.
Methods: Whole, eviscerated tilapia (*Oreochromis niloticus)* and fresh shell-on shrimps (*Litopeneus vannamei)* was treated with FICE made with different concentrations of PAA (100, 200 and 300 ppm). The controls were potable water for ice preparation and rising of tilapia with a chlorine solution of 20

Poster

ppm. The product was stored in coolers placed at room temperature using ratios of 1.5:1 (ice:tilapia) and 2:1 (ice:shrimp). Microbiological sampling was conducted every 8 hours (n=96) over 24 h to determine the Aerobic Plate Count (APC), total coliforms (TC), *Escherichia coli* (EC) and lactic acid bacteria (LAB). A Hedonic test (scale 0 – 9) with untrained panelist was developed to assess organoleptic attributes. Data were analyzed using ANOVA with LSMeans ($P \leq 0.05$). Cost-benefit analysis of using FICE by the wet market fish sellers was conducted

Results: Compared to control, FICE at 200 and 300 ppm PAA demonstrated significantly low aerobic mesophilic bacteria counts ($p<0.05$) on tilapia and shrimp after 24 h of storage. FICE treated shrimp and tilapia were "acceptable" to the sensory panelists. Cost-benefit analysis indicated that the shrimp sellers would save $300 per month if they used FICE instead of regular ice in the wet markets.

Significance: Functional ice, can actively suppress spoilage microorganisms in shrimp and tilapia during post-harvest storage, reduce food waste and improve the socioeconomic status of the value chain actors in Honduras.

P3-188 Metagenomic Assessment of Human and Animal RNA Viruses in Sanaga Clams, Cameroon

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❖ ***Developing Scientist Entrant***

Introduction: Many recent pandemics have been recognized as viral and the foods play an important role in their emergence and transmission. Being able to describe the viral diversity in foods samples could be helpful in preservation of consumers health contribute to the understanding of the mechanisms of emergence and transmission. Shellfish by their capacity to filter small particles can concentrate a wide viral diversity.

Purpose: This work describes the use of a metagenomic approach to assess the diversity of RNA viruses in clams and to identify human or potentially zoonotic virus sequences.

Methods: Sanaga river clams were collected over two years from 2 sampling points, dissected, and digestive tissues analyzed by metagenomics. After cDNA synthesis, libraries were prepared including an enrichment step (VirCapSeq-VERT) before Illumina sequencing. A bioinformatic analysis including elimination of bacterial and hosts sequences, deduplication, *de novo* assembly has been performed.

Results: The viral fraction represented 0.8 to 15% of reads, with 68 to 87% unassigned. After assembly, contigs belonging to *Astroviridae* were the most abundant, with nearly complete genomes of bastrovirus identified. *Picobirnaviridae* were linked to strains infecting bats, humans, or other hosts. *Hepeviridae* were mainly linked to strains detected in sponge but also in pigs. As for *Caliciviridae* and *Picornaviridae*, most of the sequences were linked to strains infecting bats, with some sequences close to human norovirus, picornavirus, and simian hepatitis A virus.

Significance: Despite a need to improve the sensitivity, this study allowed the description of a great diversity of RNA virus sequences and shows the contamination of Sanaga clams by both humans and animal virus. Describing all the viral contaminants in this type of sample and being able to identify the original host can help to understand certain zoonotic transmission events and alert the health authorities of possible emergence risks.

P3-189 Survival of Inoculated *Vibrio* spp., *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. on Sugar Kelp during Refrigerated and Ambient Storage

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial pathogens such as *Vibrio* spp. persist in coastal waters and can contaminate edible seaweeds. Pathogens such as *Listeria monocytogenes*, STEC and *Salmonella* spp. occasionally contaminate diverse foods including sugar kelp. Moreover, storage period and temperature are important factors that can affect the survival of these pathogens.

Purpose: To investigate the influence of storage temperature on survival of *Vibrio*, STEC, *L. monocytogenes* and *Salmonella* inoculated onto shredded- and whole blade- sugar kelp.

Methods: Sugar kelp (whole blade and shredded slaw) was inoculated with 7.0 log CFU/g of two-strain mixtures of *L. monocytogenes* and STEC, and two-species mixtures of *Vibrio* and *Salmonella*. Cultures were grown and applied in salt-containing media to simulate pre-harvest contamination for *STEC* and *Vibrio*, whereas *L. monocytogenes* and *Salmonella* inocula were prepared to simulate post-harvest contamination. Samples were sealed and stored at 4°C, 10°C and 22 °C for up to 7 days. Microbiological analyses were performed periodically (1, 4, 8, 24 hr, etc.) to evaluate the effects of storage temperature on pathogen survival. One-way ANOVA ($p\leq0.05$) and linear regression model were used to determine significant differences.

Results: The product form of kelp (whole blade, shredded slaw) had no significant impact on pathogen survival. Pathogen populations decreased under all storage conditions, but survival was greatest for all species at 22°C, with STEC and *Salmonella* exhibiting significantly lower reductions (1.2 and 1.7 logCFU/g, respectively) than *L. monocytogenes* and *Vibrio* (2.5 and 2.4 logCFU/g, respectively). Populations of *Vibrio* and STEC decreased with storage temperature, whereas *L. monocytogenes* survived better at 4 than 10°C. Regardless of temperature all pathogens remained detectable at the end of the study duration.

Significance: Pathogen survival suggests supplemental measures during processing should be considered to enhance product safety. Results emphasize need for strict adherence to temperature control for kelp. Temperature abuse may support pathogen survival, especially STEC, during storage.

P3-190 Seasonal Effect on Indicator Organisms in Catfish Parts and Catfish Processing

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Introduction: In the United States, Mississippi, Louisiana, Arkansas, and Alabama are the leading hybrid catfish producers. In 2017, USDA-FSIS compliance guidelines for Siluriformes, implemented the testing of generic *Escherichia coli* to ensure product wholesomeness and safety.

Purpose: The current study was carried out to determine the seasonal effect on generic *Escherichia coli* and other indicator organisms, while also identifying possible contamination sources in catfish processing environments.

Methods: Environmental, fish, and liquid samples were collected in 3M™ Swab-Sampler (with 10 ml of Buffered Peptone Water) and Whirl-pak® sterilized stomacher bags respectively from two different catfish processing plants. (site) located in the state of Mississippi, USA. Replications were defined by the number of visits. Each two visits represented a season and for each visit, 25 samples were collected. All samples were plated on the following corresponding media: aerobic plate (APC), psychotropic plate (PPC), and indicator organisms: generic E. coli, total coliforms (TCC), and Enterobacter following FDA-BAM and AOAC methods.

Results: *Escherichia coli* counts were similar ($P \leq 0.05$) for all sampling points with slightly higher incidence in the Late Summer than the rest of the seasons and these were higher than ($P \leq 0.05$) recommended limits of 1 CFU/g. Within the types of sampling, liquid and fish part sample had higher counts than ($p\leq0.05$) environmental samples. Liquid samples consisting of truck water (5.3 log CFU/ml) and chilled water (5.1 log CFU/ml) resulted in higher than ($p\leq0.05$) offal water and injector solution (4.4,4.7 log CFU/g, respectively).

Significance: Prior to 2017, detection of generic *E. coli* was not identified as a target organism in food safety testing for catfish processors. It is possible that the presence of indicator organisms evaluated varies according to the sampling season, considering that such microorganisms have been found to have a relationship with pathogens, *E. coli* could be present in the natural microflora of the catfish ponds where processing plants collect the hybrid catfishes.

Author and Presenter Index

Developing Scientist Competitors

Abdallah-Ruiz, Angelica, *Mississippi State University* (P2-41)
Acheamfour, Chanelle, *University of Maryland Eastern Shore* (T3-12)
Aditya, Arpita, *University of Maryland* (T10-08)
Ahuja, Manveen Kaur, *North Carolina State University* (P2-05)
Akomea-Frempong, Samuel, *University of Maine* (P3-189)
Alvarado-Martinez, Zabdiel, *University of Maryland* (P2-46)
Antaal, Shivani, *University of Central Oklahoma* (P2-50)
Archila-Godínez, Juan C., *Purdue University* (T9-11)
Arvaniti, Marianna, *Agricultural University of Athens* (P3-37)
Arya, Richa, *University of Maine* (P2-158)
Aryal, Jyoti, *Louisiana State University* (P1-120)
Ayuk Etaka, Cyril Nsom, *Virginia Tech* (P1-138)
Bai, Jimeng, *Kansas State University* (P2-141)
Bakin, Charles Bashiru, *The Ohio State University, Center for Foodborne Illness Research and Prevention* (T8-01)
Balamurugan, Sampathkumar, *Agriculture & Agri-Food Canada* (T11-11)
Berger, Bryan, *University of Virginia* (T6-10)
Berglund, Zachary, *Purdue University* (P2-54)
Bernier, Charlie, *Institute of Nutrition and Functional Foods (INAF), Université Laval* (P1-24)
Betancourt-Barszcz, Gabriela K., *Texas Tech University* (P2-138)
Black, Micah T., *Auburn University* (P1-54)
Bolten, Samantha, *Cornell University* (T5-09)
Bonny, Patrice, *Ifremer, Laboratoire de Microbiologie* (P3-188)
Bouchard, Simon, *Institut sur la nutrition et les aliments fonctionnels, Université Laval* (P1-162)
Browning, Alik, *West Virginia University* (P1-37)
Byun, Kye Hwan, *CHUNG-ANG Univ.* (P2-40)
Cain, Sarah, *Rutgers University* (P1-165)
Caliskan-Aydogan, Oznur, *Michigan State University* (P3-61)
Chaggar, Gurpreet K., *Purdue University* (T5-06)
Chattopadhyay, Suhana, *University of Maryland* (T5-01)
Chen, Han, *Purdue University* (P3-91)
Chen, Hanyu, *Cornell University* (P3-31)
Chen, Pengyu, *Virginia Tech* (P1-100)
Chen, Ruixi, *Cornell University* (T2-06)
Chenggeer, Fnu, *University of Missouri* (P2-107)
Cheong, Sejin, *UC Davis School of Veterinary Medicine* (P1-70)
Choi, Donghyun, *Korea University* (P2-09)
Choi, Yunseo, *Korea University* (P2-20)
Chuang, Shihyu, *University of Massachusetts* (T3-01)
Cook, Camryn, *Virginia Tech* (P1-61)
D'Amico, Dennis, *University of Connecticut* (P2-105)
Dai, Yaxi, *The University of Georgia* (P2-173)
Dev Kumar, Govindaraj, *University of Georgia* (P2-43, P2-42, P1-55, P3-30)
DeVillena, Juan, *Texas Tech University* (P1-35)
Dewi, Grace, *University of Minnesota* (P1-80)
Dhital, Rajiv, *University of Missouri* (P3-83)
Dhulappanavar, Gayatri Rajashekhar, *University of Arkansas* (P2-108)
Dorick, Jennifer, *University of Georgia* (P3-185)
Dudley, Aaron, *Alabama A&M University* (P2-162)
Eshun, Ama Adadzewa, *Alabama A&M University* (P2-84)
Everhart, Emily, *University of Connecticut* (T11-12)
Feng, Yaohua (Betty), *Purdue University* (P2-68, T9-05)
Fukuba, Julia, *University of Massachusetts Amherst* (P3-139)
Gao, Zhujun, *University of Maryland* (P3-35)
Garsow, Ariel, *The Ohio State University, Center for Foodborne Illness Research and Prevention* (T11-04)
Gathman, Rachel, *University of Illinois at Urbana Champaign* (P1-73)
Ghoshal, Mrinalini, *University of Massachusetts* (P2-45)
Godínez-Oviedo, Angélica, *Universidad Autonoma de Queretaro* (P3-120)
Goodwyn, Brian, *University of Maryland Eastern Shore* (P1-75)
Grace, Delia, *Natural Resource Institute, University of Greenwich* (T1-07)
Gragg, Sara, *Kansas State University* (P1-83, P1-82)
Gunathilaka, Gayathri, *Michigan State University* (P3-29)
Ha, Sang-Do, *Advanced Food Safety Research Group, Chung-Ang University, Chung-Ang University* (P2-103, P2-104)
Haley, Olivia, *Kansas State University* (P1-182)
Hamilton, Alexis, *Virginia Tech* (P3-138)
Haque, Manirul, *University of Nebraska-Lincoln* (P3-123)
Hassan, Jouman, *University of Georgia* (P2-39)
Herron, Charles, *Auburn University* (P2-163)
Heydenreich, Rosa, *ETH Zurich, Institute of Food, Nutrition and Health, Sustainable Food Processing Laboratory* (T3-10)
Hildebrandt, Ian, *Michigan State University* (P1-38)
Hong, Haknyeong, *University of Massachusetts* (P1-26)
Hong, Hyunhee, *Oregon State University* (T3-06)
Horn, Connor M., *Purdue University* (P1-47)
Howell, Allison, *The Ohio State University* (P2-166)
Hua, Zi, *Washington State University* (P2-177)
Humphrey, Jessica, *University of Nebraska-Lincoln* (P3-10)
Hur, Minji, *University of Georgia, Center for Food Safety* (P1-101)
Irakoze, Zilfa, *Kansas State University* (P1-184)
Jiang, Xingyi, *Florida State University* (T3-03)
Johnson, Tahirah, *University of Maryland Eastern Shore* (T7-12)
Jones, Amy, *University of Florida* (T12-05)
Joshi, Shivam, *University of Central Oklahoma* (P1-108)
Jung, Hyeju, *Kyungpook National University* (T13-07)
Kamarasu, Pragathi, *University of Massachusetts Amherst* (T8-09)
Karanth, Shraddha, *University of Maryland* (T1-04)
Karolenko, Caitlin, *Oklahoma State University* (P3-92)
Kavanaugh, Melissa, *Drexel University* (T9-12)
Kharel, Karuna, *Louisiana State University* (P2-01)
Khasapane, Ntelekwane George, *Central University of Technology* (P1-23)
Khouja, Bashayer, *Illinois Institute of Technology, Institute for Food Safety and Health* (P1-53)
Kim, Minho, *University of Illinois Urbana-Champaign* (T1-09)
Kim, Minji, *University of Massachusetts Amherst* (P3-53)
Kingston, Emily, *North Carolina State University* (P2-63)
Koo, Andrea, *National University of Singapore* (P3-03)
Kwon, Hee Jin, *University of Maryland* (P3-45)
Larsen, Katalin, *The University of Vermont* (T4-04)
Lawal, Opeyemi, *University of Guelph* (T2-07)
Lee, Seulgi, *The University of Georgia* (P3-169)
Leopard, Jacinda, *Mississippi State University* (P1-14)
Li, KaWang, *Kansas State University* (P3-149)

Li, Tengfei, *University of Nebraska-Lincoln* (P2-161)
Li, Xiran, *University of California, Davis* (P1-134)
Liao, Chao, *University of California, Davis* (P3-164)
Lightbown, Ashlyn, *University of California, Davis* (T8-05)
Lin, Yawei, *Michigan State University* (P3-99, P3-98)
Lituma, Ivannova, *Louisiana State University* (P1-91)
Liu, Shuxiang, *Sichuan Agricultural University* (P3-104)
Liu, Xingchen, *University of Maryland, College Park* (T8-04, P1-87)
Liu, Zhuosheng, *University of California, Davis* (P3-182)
Lopez Gachuzo, Alexia Joana, *Universidad Autonoma de Queretaro* (T13-08)
Louvau, Hanna, *University of California, Davis* (P3-102)
Low, Megan Mei Yee, *Purdue University* (P2-61, P3-89, T9-04)
Manes, Makayla, *The Ohio State University* (P2-14)
Manjunatha, Vishal, *Clemson University* (T4-06)
Manville, Erin, *Kansas State University* (P1-64, T12-04)
Mao, Liang, *University of Missouri* (P2-109)
Martinez-Soto, Carlos, *University of Guelph* (P1-42)
Mate, Madhuri, *Illinois Institute of Technology, Institute for Food Safety and Health* (P1-122)
McCaughan, Kyle, *University of Delaware* (P1-169, P1-30)
McGovern, Chloe, *The Ohio State University, Center for Foodborne Illness Research and Prevention* (P1-119)
Mendez, Ellen, *Kansas State University* (P1-71)
Mendez Acevedo, Marysabel, *Penn State University* (P2-175)
Mendoza, Janny, *Lousiana State University* (P1-97)
Mensah, Abigail Aba, *Ohio State University* (P1-95)
Miderho, Christus, *Pedagogical Institute of Bukavu(ISP-Bukavu)* (T11-05)
Mittal, Ajay, *International Center of Excellence in Seafood Science and Innovation, Prince of Songkla University* (P3-179)
Moodispaw, Margaret, *The Ohio State University* (P1-86)
Moreira, Juan, *Louisiana State University* (T8-08)
Morris, De'Anthony, *Michigan State University* (P1-98)
Muchaamba, Francis, *Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich* (T13-02)
Murphy, Claire M., *Virginia Tech* (P1-63)
Mydosh, Jennifer, *The University of Arizona* (P3-34)
Nabwiire, Lillian, *Iowa State University* (T9-08)
Nehls, Jordan, *University of Wisconsin - Madison* (P1-39)
Nickodem, Colette, *Texas A&M University-CVMBS* (T4-11)
Oh, Hyemin, *Sookmyung Women's University* (P1-51)
Ohman, Erik, *Oregon State University* (P1-118)
Omar, Alexis, *University of Delaware* (P1-79)
Pageau, Gabrielle, *Institut sur la nutrition et les aliments fonctionnels, Université Laval* (P1-166)
Pahariya, Prachi, *School of Agricultural Sciences, Southern Illinois University Carbondale* (P3-131)
Palillo, Jack, *The Ohio State University College of Public Health* (P3-180, T7-10)
Park, Hyeon Woo, *The Ohio State University* (P2-101)
Park, So-Hui, *Kyungpook National University* (T13-06)
Park, Ye-Rim, *Kyungpook National University* (P1-136)
Péloquin, Laurence, *Institut sur la nutrition et les aliments fonctionnels, Université Laval* (P1-151)
Prabha, Krishna, *University of Georgia* (P1-132)
Punchihewage Don, Anuradha, *University of Maryland Eastern Shore* (T4-03)
Qiu, Yan, *Sichuan Agricultural University* (T12-06)
Quach, Emily, *University of Maryland* (P1-68)
Quintanilla Portillo, Jorge, *University of Illinois at Urbana Champaign* (P1-69)
Raad, Rawane, *The University of Georgia* (P1-94)
Rana, Yadwinder Singh, *Cornell University* (P2-186)
Randriamiarintsoa, Narindra, *Michigan State University* (P1-40)
Resendiz-Moctezuma, Cristina, *University of Illinois at Urbana-Champaign* (P2-36)
Reuben, Rine, *German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig* (T11-08)
Reyes, Gustavo, *University of Illinois at Urbana-Champaign* (T1-10)
Rodriguez, Karla M., *Texas Tech University* (P2-89)
Rolon, M. Laura, *Penn State University* (P3-163)
Roth, Katerina, *Cornell University* (P3-105)
Sajewski, Elizabeth, *Emory University* (P3-63)
Sanchez, Angelica, *Texas Tech University* (P2-10)
Schlange, Sara, *University of Nebraska-Lincoln* (P3-12)
Sekhon, Amninder Singh, *Washington State University* (P3-95)
Selladurai, Manivannan, *Kansas State University* (P1-137)
Shah, Urvi, *North Carolina State University* (P2-83)
Sharief, Saad Asadullah, *Michigan State University* (P3-86)
Sharma, Dimple, *Michigan State University* (P1-183)
Shen, Xiaoye, *Washington State University* (P1-142, P1-141)
Siceloff, Amy, *University of Georgia* (T3-07)
Siddique, Aftab, *Auburn University* (T1-02)
Singh, Amandeep, *University of Texas Rio Grande Valley* (P2-88)
Singh, Dharamdeo, *University of Guelph* (P3-154)
Sloniker, Natasha, *Michigan State University* (T8-07)
Smalls, Jasmine, *Uni. of Maryland Eastern Shore* (P3-161)
Sobolik, Julia, *Emory University* (T3-05)
Solis Salazar, Karla, *Texas A&M University* (P1-88)
Soorneedi, Anand, *University of Massachusetts, Amherst* (T3-08)
Stearns, Rebecca, *West Virginia University* (P1-147, P1-102)
Stoufer, Sloane, *University of Massachusetts, Amherst* (P3-54)
Strickland, Ali, *University of Minnesota* (T1-08)
Su, Yuan, *Washington State University* (P1-139)
Sudagar, Varalakshmi, *PhD Scholar, Institute for food safety and health (IFSH), Illinois institute of technology* (T7-03)
Suganthan, Bavithithira, *Nano Electrochemistry Laboratory, School of Chemical, Materials and Biomedical Engineering, University of Georgia* (T6-12, P3-59)
Swinehart, Maeve, *Purdue University* (P2-59, T9-02, P2-60)
Tabashsum, Zajeba, *University of Maryland* (T3-04)
Tembo, Geraldine, *Purdue University* (T5-12)
Tobar, Zachary, *University of California, Davis* (P3-160)
Townsend, Anna, *University of Georgia* (P3-158)
Trigueros, Sylvain, *University Grenoble Alpes, CEA, LETI* (P2-160, T6-08, T3-09)
Trudel-Ferland, Mathilde, *Institut sur la nutrition et les aliments fonctionnels, Université Laval* (T10-09)
Tung, Chuan Wei, *University of Maryland* (T13-05)
Unger, Phoebe, *Washington State University* (P1-140)
Vargas, David A., *Texas Tech University* (P2-143)
Vega-Iturbe, Manuel Alejandro, *Universidad Autonoma de Queretaro* (P1-121)
Velez, Frank, *Florida State University* (P2-133)
Vice, Zachariah, *Texas A&M University* (P2-120)
Wambui, Joseph, *Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich* (P2-24)
Wang, Chao, *Ghent University* (T12-12)
Wang, Kaidi, *McGill University* (T6-07)

Wang, Rory, *Washington State University* (P1-111)
Wason, Surabhi, *University of Arkansas* (T12-09)
Wong, Catherine, *Food, Nutrition and Health, University of British Columbia* (P1-110)
Wormald, Christina, *University of Massachusetts Amherst* (T9-07)
Wu, Bet, *Auburn University* (P3-187)
Wu, Biyu, *University of Hawaii at Manoa* (P2-21, P2-35)
Wu, Jiaying, *University of Illinois at Urbana-Champaign* (P1-148)
Wu, Weifan, *University of Georgia* (P2-27)
Xiong, Zirui Ray, *Cornell University* (P3-165)
Xu, Xinran, *University of Georgia* (T2-09, T1-05)
Yao, Lang, *Canadian Food Inspection Agency* (T3-11)
Yi, Jiyoon, *University of California, Davis* (T3-02)
Zeng, Hui, *Michigan State University* (P3-153)
Zhang, Liyun, *University of Nebraska-Lincoln* (P3-09)
Zhao, Xingchen, *Ghent University* (T2-12, P3-32)

Undergraduate Student Award Competitors

Bollinger, Chris, *University of Maryland* (P1-89)
Deaton, Ashley, *Michigan State University* (P3-90)
Elnaham, Asma, *University of West Alabama* (P2-04)
Goforth, Madison, *The University of Arizona* (P3-167)
Jung, YeonJin, *Cornell University* (P3-136)
Kearney, Andrew, *Michigan State University* (P3-166)
Kim, Erica, *The Ohio State University* (P1-28)
Klug, Ian, *Michigan State University* (P2-189)
Nelson, Kasey, *Michigan State University* (P1-52)
Park, Jong Bin, *Chung-Ang University* (P3-181)
Tanaka, Saki, *Hokkaido University* (P3-173)