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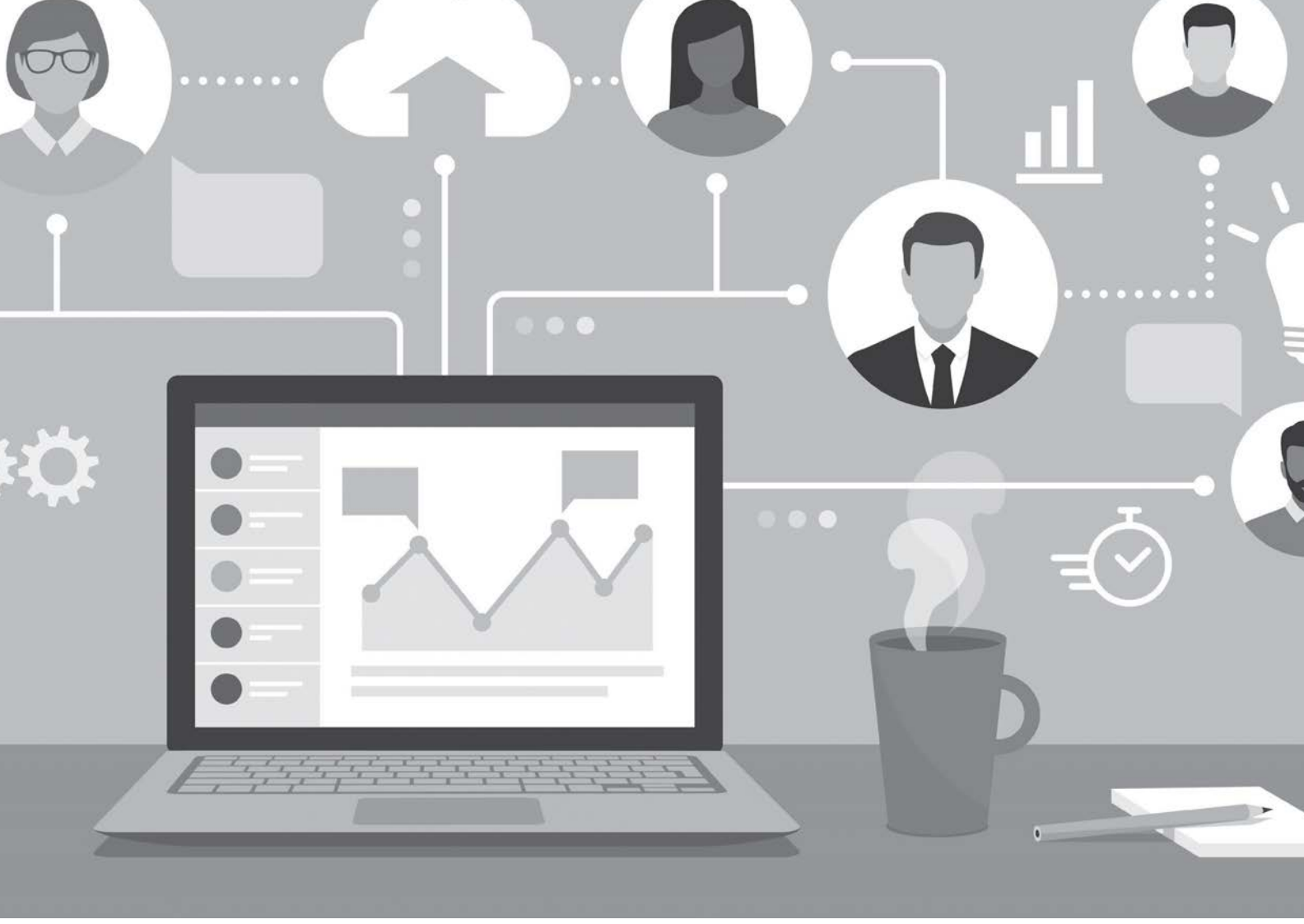

IAFP 2020

A VIRTUAL ANNUAL MEETING
OCTOBER 26–28

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ABSTRACTS

This is a collection
of the abstracts
from IAFP 2020,
A Virtual Annual Meeting.

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Scope of the Journal. The *Journal of Food Protection*® (*JFP*) is an international monthly scientific journal in the English language published by the International Association for Food Protection (IAFP). *JFP* is intended for publication of research and review articles on all aspects of food protection and safety. Major emphases of *JFP* are placed on studies dealing with (1) Tracking, detecting (including traditional, molecular, and real-time), inactivating, and controlling food-related hazards including microorganisms (including antibiotic resistance), microbial (mycotoxins, seafood toxins) and non-microbial toxins (heavy metals, pesticides, veterinary drug residues, migrants from food packaging, and processing contaminants), allergens and pests (insects, rodents) in human food, pet food and animal feed throughout the food chain; (2) Microbiological food quality and traditional/novel methods to assay microbiological food quality; (3) Prevention of food-related hazards and food spoilage through food preservatives and thermal/non-thermal processes, including process validation; (4) Food fermentations and food-related probiotics; (5) Safe food handling practices during pre-harvest, harvest, post-harvest, distribution and consumption, including food safety education for retailers, foodservice, and consumers; (6) Risk assessments for food-related hazards; (7) Economic impact of food-related hazards, foodborne illness, food loss, food spoilage, and adulterated foods; (8) Food fraud, food authentication, food defense, and foodborne disease outbreak investigations.

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IAFP2020
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IVAN PARKIN LECTURE

AUDACIOUS INNOVATION: CRITICAL TOOLS FOR THE 21ST CENTURY

MONDAY, OCTOBER 26
9:00 A.M. – 10:00 A.M.

CAROLINE SMITH DEWAAL
Deputy Director of EatSafe
Global Alliance for
Improved Nutrition (GAIN)
Washington, D.C.

The future is upon us. While Al Gore warned us of a future shaped by emerging climate change and crisis, he may have overestimated the time we have to respond. The evidence of a changing world is all around us, from extreme weather to collapsing glaciers to uncontrolled forest fires. We need scientific out-of-the-box thinking now, as well as personal behavior changes, to address these challenges.

But do we have the tools? In the last 20 years, we have completed the mapping of the human genome, and built computers that fit in our pocket. In the food safety area, we have made great strides, but with each repeated outbreak, evidence is mounting that we are not changing fast enough.

Audacious Innovation is a critical tool for the 21st century. While innovation is a natural part of the scientific process, we need to push it further and faster. Although good scientific practice will be essential to our success in addressing these challenges, we need to set goals that are audacious to stimulate innovation, and set the stage through effective communication and advocacy to meet those goals.

Audacious Innovation takes both the ability to see around corners and to manage difficult conversations. In my professional life, I developed the first comprehensive food-attribution outbreak database which started with fewer than 500 outbreaks. Working with the government on the development of HACCP, I couldn't fathom how the food industry would conduct an accurate Hazard Analysis without using real-world outbreak data. This led to CSPI's first efforts to gather data from the CDC. The objective: Using real-world data, CSPI would develop a comprehensive source for identifying food/hazard combinations to assist the development of more accurate hazard analysis.

Using real-world evidence was touted by the FDA Commissioner in a recent speech about the need to unleash the power of data. But in 1998, before the era of big data, aggregating data collected from public health departments in 50 states was not done, especially by those outside of the Centers for Disease Control and Prevention. Pushing the envelope is part of the process of Audacious Innovation.

One Health calls on scientists to work across disciplines, with the understanding that public health encompasses environmental health and diseases both in wildlife and domesticated animals. The repeated outbreaks linked to leafy greens illustrates a One Health problem requiring an interdisciplinary solution.

One Health also illustrates Audacious Innovation. Last fall, at a Salzburg Global Seminar in Salzburg, Austria, surveillance experts from all over the world elaborated the concept of integrated surveillance, encompassing environ- mental health, zoonotic diseases and human health, to track, predict and prevent emerging disease outbreaks.

There are many examples of Audacious Innovation in the food industry as well. Walmart has developed a traceability system to track certain produce from the store to the farm in seconds rather than days or weeks. And Perdue spent a decade developing a system for raising chickens without the use of antibiotics.

While examples of Audacious Innovation abound, it is important for young professionals to understand and embrace their role to push boundaries in order to tackle the challenges ahead. It takes the willingness to ask hard questions, seek solutions that may not be apparent and push ideas that may not be popular with others, including those with more experience.

One final example is the United Nations' Agenda for Sustainable Development calling for eliminating poverty and hunger by 2030. Those are audacious goals! And they will require innovation to match. So let's think big when it comes to tackling the challenges of the 21st century.

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JOHN H. SILLIKER LECTURE

AN INTERVIEW WITH PETER BEN EMBAREK
WEDNESDAY, OCTOBER 28
9:00 A.M. – 10:00 A.M.

PETER K. BEN EMBAREK, PH.D.
Manager, International Food Safety Authorities Network (INFOSAN)
World Health Organization
Geneva, Switzerland

Join Dr. Peter K. Ben Embarek in an in-depth discussion and question-and-answer session, moderated by Dr. Leon Gorris, Food Safety Expert in Nijmegen, The Netherlands.

Dr. Ben Embarek will provide updates and information on how the World Health Organization (WHO), together with partners, is responding to the COVID-19 pandemic, providing nutrition and food safety guidance and advice for governments, food businesses, health workers, and the general public, to maintain good health and prevent malnutrition in all its forms.

Symposium Abstracts

SS1 COVID Session

MOEZ SANAA: *Department of Risk Assessment, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Maisons-Alfort, France, France*
LEEN BAERT: *Nestle, Vers-ches-les Blanc, Switzerland, Switzerland*
KRISTEN GIBSON: *University of Arkansas, Fayetteville, AR, USA*
ALBERT BOSCH: *University of Barcelona, Barcelona, Spain, Spain*

S1 Food Omics: Is Food Safety Missing out?

PUSHPINDER KAUR LITT: *University of Delaware, Newark, DE, USA*
BRANDON KOCUREK: *U.S. Food and Drug Administration, CFSAN, Laurel, MD, USA*
FARHANA PINU: *Plant and Food Research, Auckland, New Zealand, New Zealand*

The rapid development of omics-based approaches (metagenomics and metabolomics) have demonstrated their potential to face future food safety challenges. Metagenomics studies have described the influence of food microbiomes potential associations between pathogen proliferation and transfer in production environments, on food safety and quality. Metabolomic research characterizes the entire small metabolite composition of a particular system or organism as metabolites may be considered final downstream products of the genome and its interaction with the environment. These characteristics might reveal interesting relationships between foods and natural food microflora.

However, little is discussed about the role of metabolomics and metagenomics in pathogen persistence in food and food environment. Combining information could provide a different perspective to metabolomics data with potential unique pathogen signatures and may answer critical data gaps. The goal of this symposium is to provide a framework of utilizing omics in predicting foodborne pathogen signatures concerning food in a different processing environment at the metabolite level.

Presenters will discuss the past, present, and future of omics technology. The symposium begins with the application of food microbiome on pathogen persistence and prevalence in the food production environment, continues with an analysis of food metabolomes, profiling foods in different production systems and food adulteration, and finishes with insights on using complex omics data (metagenomics and metabolomics) to generate unique food profiles for food safety assessment. Food safety specialists must become more familiar with omics technology that is swiftly becoming an essential tool in food science.

S2 Food Safety Challenges and Benefits of Capturing and Reusing Water in Food Processing Facilities

ALLEN SAYLER: *EAS Consulting Group, Alexandria, VA, USA*
MELANIE ABLEY: *U.S. Department of Agriculture–FSIS, Springfield, VA, USA*
YULIE MENESES: *University of Nebraska-Lincoln, Lincoln, NE, USA*
RICK MOLONGOSKI: *CDM Smith, Inc., Latham, NY, USA*

The food manufacturing/processing industry makes use of significant volumes of water which are then discharged and require treatment. The primary processing of food animals requires the largest use of water. However, due to environmental, governmental and social pressure, food manufacturers are facing increasing cost, and in some cases more difficult access, to safe water sources. The regulatory framework for water usage in the U.S. stipulates that water must comply with standards similar to National Primary Drinking Water regulations, while internationally, many countries follow the World Health Organization "Guidelines for Drinking Water Quality." Although the technology to recondition and reuse food processing water has the capability to return this water to drinking water standards, the regulatory requirements and public perceptions to allow for its reuse are more complex. Continued advancements in treating food processing water have resulted in the capture, reconditioning and reuse of this water stream within a food processing facility with some food processing facilities achieving a "zero discharge" of water. However, reuse of processing water carries with it the risk of contamination.

We will explore treatment technologies to restore the safety of food processing water, and discuss if the benefits offset the potential food safety risk from a practical and operational perspective. Sharing insights on data, current thinking and the efforts to update government regulations; the latest scientific approaches for water reconditioning and reuse, with an emphasis on the latest terminology and most promising technological developments; as well as insight into current processing water reconditioning technology, we will review practical examples of current on-site treatments used to restore the biological and chemical safety of food processing water and share the regulatory and financial hurdles encountered in installing and operating water reconditioning technology.

This symposium complements proposal #6208, which focus primarily on microbial risk assessment and microbial quantification methods for water reuse in food processing.

S3 Frozen Food Fallout: Food Safety Challenges Faced by Manufacturers in the Frozen Food Arena

ELLEN SHUMAKER: *RTI International, Research Triangle Park, NC, USA*
SEAN LEIGHTON: *Cargill, Inc., Wayzata, MN, USA*
DONNA GARREN: *American Frozen Food Institute, Arlington, VA, USA*

Frozen food products encompass a variety of foods including fruits, vegetables, meats, poultry, and even grains. Some frozen foods are classified as Ready-to-Eat (RTE) meaning they do not require a lethality step by the consumer and some as Not-Ready-to-Eat (NRTE), meaning that a lethality step is required. Due to the large array of product types and processing requirements, frozen foods production touches multiple sectors of the food industry and manufacturing them safely presents challenges. There have been several outbreaks and recalls related to a range of frozen food products in recent years. *Listeria monocytogenes*, along with other pathogens like human norovirus and hepatitis A, pose a threat to the safety of some frozen foods because they can survive the freezing process. Industry and government entities have taken steps to detect and limit these hazards, but some argue for a zero-tolerance rule or a classification of all frozen products as RTE. Additionally, for NRTE frozen foods, some consumers do not always follow the

manufacturer's cooking instructions for a lethality step to ensure a safe final product. To address the long-term safety of frozen foods, we need to better understand the logistics involved in their production and the complex food safety issues associated with them. The objective of this session is to discuss the unique food safety challenges inherent to manufacturing frozen food products, examine the steps and initiatives taken to combat food safety issues in these products, and provide insight on how consumer handling affects the safety of certain frozen food products.

S4 Recent Advancements in Beverage Processing: Considerations and Outcomes

ANKIT PATRAS: *Tennessee State University, Nashville, TN, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
ANKIT PATRAS: *Tennessee State University, Nashville, TN, USA*
BALA BALASUBRAMANIAM: *The Ohio State University, Columbus, OH, USA*

Among the biological, physical and harmful chemical agents associated with foods, biological infectious pathogens are the most significant, resulting in the majority of foodborne illness. The production of beverages must include dedicated bacterial and bacterial spore inactivation steps to minimize the risk of spoilage or illness. Typically beverages are pasteurized or sterilized based on pH and storage conditions. Heat can inactivate vegetative cells and bacterial spores, but heat treatments also destroy vitamins and other small molecules and can adversely change the flavor and aroma of beverages (affecting quality). Heat resistant food-borne pathogens are high risk contaminants of beverages, and inadequate processing has led to numerous outbreaks of foodborne diseases. To address these important food safety issues, novel non-thermal technologies are needed to control microbial growth (pathogenic and spoilage) and mycotoxins, ensuring compliance with regulatory requirements while retaining the flavor and aroma of the beverage. These technologies employ agents such as high pressure, electric field, UV, or ultrasound with or without heat to ensure microbiological safety of the product with relatively minimal impact on product quality. This symposium aims to discuss current non-thermal technologies and their efficacy in inactivating vegetative cells, endospores and enteric viruses. It will also discuss regulatory requirements for novel non-thermal technologies for both high- and low-pH beverages. Since the challenges of implementing non-thermal technologies are both theoretical and practical, this symposium has been designed as collaboration between academic, government research, and industry experts. This symposium will briefly introduce the fundamental principles and present approaches for evaluation of product and process parameters in applications of selected non-thermal technologies for liquid foods.

S5 Complementary Approaches to Quantitative Microbial Risk Assessment: Emerging Computational and Modeling Approaches for Risk Analysis

DANIEL MUNTHER: *Cleveland State University, Cleveland, OH, USA*
DANIEL MUNTHER: *Cleveland State University, Cleveland, OH, USA*
ASHRAFUR RAHMAN: *Oakland Universtiy, Rochester, MI, USA*
ZACHARY MCCARTHY: *York University, Toronto, ON, Canada, Canada*

While the quantitative microbial risk assessment (QMRA) framework has played an important role in foodborne pathogen risk analysis, other tools are perhaps better suited to filling in key knowledge gaps that exist, for example, during processing and at consumption. Processing techniques involving water contact for fresh produce and meat products have been identified as points of concern – here mathematical approaches utilizing dynamical systems may provide insight towards mechanisms of cross-contamination, leading to better control strategies. In terms of the dose-response assessment phase of QMRA, characterizing the infection risk due to ingesting low pathogen doses is still an important problem – here agent-based modeling describing key physiological processes could play a main role towards a solution. The aim of this symposium is to highlight computation and modeling tools to address these and other problems and in doing so discuss how such perspectives may be applied to various steps in the farm-to-fork continuum to improve food safety.

S6 Safe (Smart Affordable Fresh Efficient) Farming Version 2.0

PUSHPINDER KAUR LITT: *University of Delaware, Newark, DE, USA*
MANAN SHARMA: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
ANGELA MARIE C. FERELLI: *University of Maryland, College Park, MD, USA*
MICHELLE DANYLUK: *University of Florida CREC, Lake Alfred, FL, USA*

Controlled environment farming, including vertical, hydroponics, aquaponics, a combination of aquaculture with hydroponics is growing every year. Public health and food safety risks associated with traditional farming are well defined and used to develop policies and regulations. However, potential risks associated with new age or urban farming are yet to be defined and understood. Previous studies have suggested while hydroponics does have a lower risk of foodborne illness contamination because the plants are not in contact with soil or at risk of flooding or of being invaded by animals or pests, but do not necessarily exclude contamination with foodborne pathogens. A study conducted to determine food safety risks of hydroponically cultivated greens found 14% and 5% prevalence of *E. coli* on leafy vegetables and herbs, respectively thus indicating a variable potential risk of hydroponically grown vegetables. More importantly, they are not explicitly addressed in the FSMA-PSR rule. If these trends continue, it is imperative to address these issues. The goal of this symposium is to describe the challenges faced by both traditional and new-age farming practices with attention to PSR rule. Presenters will discuss the risks associated with traditional farming in light of FSMA-PSR rule, followed by data obtained from growers survey on the perspective form of controlled environment agriculture and the role of current policy to address food safety challenges in both agriculture systems. Together these talks will provide information about the risks associated with both types of farming and policy development with these farming practices.

S7 Who Will Win the Race to Zero? Analytical Challenges in the Food Industry

PAUL HANLON: *Abbott Nutrition, Columbus, OH, USA*
JENNIFER MCENTIRE: *United Fresh Produce Association, Washington, DC, USA*
MELANIE DOWNS: *University of Nebraska-Lincoln, Lincoln, NE, USA*
ANDREW PEARSON: *Ministry for Primary Industries, Wellington, New Zealand, New Zealand*

Availability of appropriate analytical methods is important for the control and monitoring of food safety hazards including chemical contaminants, microbiological contamination, and allergens. While each of these hazards have unique risk assessment and risk management considerations, the "absence" of these hazards is defined by the limitations of analytical methods. The definition of a safe amount of these hazards, that present no appreciable risk of adverse effects, informs the limits of quantitation for analytical methods in the development of ideal risk management strategies.

Unfortunately, continuous advancement of analytical method technology in many cases results in an uncoupling of the risk assessment for these hazards, which should define the target for analytical methods, and the development of analytical methods. The race to continually drive detection limits towards zero has real-world consequences for risk management and risk communication. This session will examine the challenges associated with each of these hazard types, highlighting unique challenges as well as areas where learnings could be applied across all aspects of food safety.

S8 Challenges in Developing Alternative Pre- and Post-harvest Water Treatments Used in Fruit and Vegetable Production

DONNA CLEMENTS: *Produce Safety Alliance, Riverside, CA, USA*
DONNA BISHEL: *Biosafe Systems, East Hartford, CT, USA*
ALISON LACOMBE: *USDA, ARS, Western Regional Research Center, Albany, CA, USA*
FAITH CRITZER: *Washington State University, School of Food Science, Pullman, WA, USA*

In the wake of recent produce outbreaks attributed to the use of contaminated water, the produce industry has emphasized treating pre- and post-harvest water to reduce contamination risk. The most common methods for treating agricultural water are based on chlorine chemistries, which have limitations in the agricultural environment including the need for monitoring several variables to ensure treatment efficacy. Chlorine-based treatments can negatively affect worker health, soil fertility, and the environment, through the release of byproducts such as trichloramines. The lack of alternative options is a barrier to widespread implementation of pre- and post-harvest water treatments. This symposium highlights innovations in water treatment technologies, while introducing regulatory and practical considerations in implementing alternatives in the farm and processing environment.

Several considerations are made when selecting an alternative water treatment, including efficacy against pathogens and fecal indicator organisms, regulatory requirements, and ease of use. This full symposium will begin with discussing the distribution of enteric pathogens in U.S. surface water sources to highlight the need for effective treatments against a range of microorganisms, followed by challenges to registering antimicrobial pesticides (sanitizers) with the U.S. Environmental Protection Agency. An overview of innovative treatment technologies will follow, including limitations to each technology and obstacles that industry faces when validating new economically feasible methods. The final talks will include the extension perspective on water treatment education and implementation barriers, and a case study describing Dole Food Company's ongoing efforts to implement alternative pre-harvest treatment methods in Hawaii.

S9 2019 State and Local Outbreak Investigations

STEVEN MANDERNACH: *Association of Food and Drug Officials, York, PA, USA*
MARK BUXTON: *Missouri Department of Health and Senior Services, Jefferson City, MO, USA*
DANNY RIPLEY: *Tennessee Department of Health, Nashville, TN, USA*
COLBY BROWN: *Georgia Department of Agriculture, Atlanta, GA, USA*
LAURIE KIDWELL: *Indiana State Department of Health, Indianapolis, IN, USA*
SHERI MORRIS: *Pennsylvania Department of Agriculture, Harrisburg, PA, USA*
D'ANN WILLIAMS: *Maryland Department of Health, Baltimore, MD, 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 US Food and Drug Administration funded Rapid Response Teams (RRTs). The session will focus upon the use of techniques such as environment assessments, environmental sampling, and whole genome sequencing to solve outbreaks at the state and local level. The session will also discuss lessons learned and contributing factors identified during the investigations.

S10 Emerging Biological and Computational Methods for Rapid, High-throughput Monitoring of Food and Water Safety: Role of DARPA-funded Research

ISABEL WALLS: *USDA, Washington, DC, USA*
PAUL SHEEHAN: *DARPA, Arlington, VA, USA*
MICHAEL SPRINGER: *Harvard University, Cambridge, MA, USA*
KIRSTY MCFARLAND: *Draper Laboratories, Cambridge, MA, USA*
GIRIJA GOYAL: *Wyss Institute - Harvard, Cambridge, MA, USA*
MIKE FERRY: *Quantitative BioSciences, Inc., San Diego, CA, USA*
JIM SAMUEL: *Texas A&M University, College Station, TX, USA*

The last decade has seen major advances in sequencing, synthetic biology, and microfluidics. Individually and in combination these technologies are impacting many areas of science and commerce. Several projects initiated by the Defense Advanced Research Projects Agency (DARPA) in biosecurity have high overlap with food safety, and many of the cutting-edge technologies being developed as part of these DARPA projects will be directly relevant for food safety.

DARPA Friend or Foe program aims to deliver technology for isolation and phenotype-based identification of possible pathogenic microbes from environmental samples without the need for culturing. Researchers are developing novel microfluidic technologies to isolate bacteria, and using "organ on a chip" technology to determine pathogenicity of isolated organisms. To date, researchers have been working on soil or other environmental samples, but are planning to test food and diarrheal samples to try to identify unknown pathogens. This is highly relevant to the food safety community, as most foodborne disease outbreaks are of unknown etiology. Being able to detect unknown pathogens will make it quicker and easier to respond to outbreaks.

Many DARPA-funded researchers are not members of or largely do not interact with the food safety community. The purpose of this symposium is to introduce the IAFP community to these projects and to introduce the teams developing these technologies to the IAFP community. We believe this session will help to serve as a bridge between these communities and help to facilitate the rapid transfer of technologies as these develop and potentially even spur the develop of technologies or devices specifically suited to food safety and defense.

S11 May the Force(meat) be with You, but without Pathogens

BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
TED MCCALL: *Johnston and Wales, Providence, RI, USA*
WILLIAM SHAW: *U.S. Department of Agriculture-FSIS-OPPD, Washington, DC, USA*
ANNA PORTO-FETT: *U.S. Department of Agriculture-ARS, Wyndmoor, PA, USA*

Forcemeat is a culinary term for dishes that are made through the uniform mixing of specialty meats (or garde manger) by grinding, sieving, or puréeing. Forcemeats from various animal species are used to make many foods including, pâtés, terrines, roulades, and galantines. One type of force meat, specifically, chicken liver pate has been linked to multiple outbreaks of *Salmonella* and *Campylobacter* over the past decade. Although forcemeats should be cooked to safe internal endpoint, recipes from culinary professionals and consumers commonly require a rare or seared degree of doneness for reducing the negative impact on the quality attributes of a finished dish. While foodborne illnesses have largely been connected to restaurant service due to undercooking forcemeats in preparation step, addressing the food safety risks using a farm-to-fork approach is needed. Thus, this symposium will provide a better understanding of the potential risks of foodborne illnesses associated with consumption of dishes made from force meats from various viewpoints. Speakers will frame the problem from each of their perspectives, and collectively, will provide science-based information for helping lower the risks associated with these inputs and finished dishes. The symposium will include a culinary perspective on various common processes of making forcemeat dishes in a food service setting. In addition, the session will include the current regulatory landscape of sourcing, storing, and producing the inputs. Speakers will also explore the recovery rate, levels, and control of pathogens in this class of foods.

S12 An Update on the Integration of "Omics" into Risk Assessment

HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands, Netherlands*
EDUARDO TABOADA: *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada, Canada*
MARIEM ELLOUZE: *Nestlé Research Centre, Lausanne, Switzerland, Switzerland*
KALLIOPI RANTSIOU: *University of Torino-DISAFA, Grugliasco, Italy, Italy*

Omics approaches can serve to reduce the uncertainty in the different steps of risk assessment - hazard identification, exposure assessment, hazard characterization, and risk characterization. Understanding the molecular mechanisms of growth, survival, adaptation throughout the food chain and how such phenotypes vary within pathogenic species, is a necessary step in the process of fine-tuning exposure assessment. Similarly, studying the virulence mechanism of foodborne pathogens, how they interact with the human host to cause disease, and identifying key molecular events that determine such virulence and their distribution among members of the same species, is fundamental for an upgraded hazard characterization. This symposium has the goal of providing an update regarding the integration of "omics" into risk assessment as a following-up of the IAFP meeting in 2017 and speakers from industry, academia and government will share recent advances in this field.

S13 One Health: Its Implication in Food Safety

PRATIK BANERJEE: *University of Illinois at Urbana-Champaign, Urbana, IL, USA*
PRATIK BANERJEE: *University of Illinois at Urbana-Champaign, Urbana, IL, USA*
KALMIA KNIEL: *University of Delaware, Newark, DE, USA*
SIDDHARTHA THAKUR: *Department of Population Health and Pathobiology, CVM, NCSU, Raleigh, NC, USA*

The One Health paradigm adopts a multi-disciplinary approach consisting of human medicine, veterinary medicine, public health and the environmental health professionals working locally, nationally, and globally to attain optimal health for people, animals, and our environment. Recently, this approach has gained considerable attention to address food safety issues. The extensive globalization of the food supply has created conditions conducive for emergence, reemergence, and the spread of foodborne pathogens and has significantly increased the challenge of predicting, detecting, and responding to foodborne illness. It is well known that effectiveness of any food safety program depends on our success in handling several factors and their complex interactions throughout the entire food production and supply chain, from pre- and post-harvest food safety to consumer education. The systems approach of One Health has huge potential to improve our understanding of several critical components of food safety systems and their interactions. Important issues such as attribution and transmission of foodborne pathogens and the spread of antimicrobial-resistant bacteria in food production and distribution chains can have a better resolution with One Health approach. This symposium will cover the basics of the One Health concept and its application to food safety, environmental attribution of foodborne pathogens, One Health in deciphering complex interactions among human, pathogen, and foods (from molecular to macro-levels). Application of One Health concept will also be discussed in terms of some recent foodborne disease outbreaks with global public health impact.

S14 Simulating Leafy Green Production to Improve Food Safety System Performance

MATTHEW J. STASIEWICZ: *University of Illinois at Urbana-Champaign, Urbana, IL, USA*
MATTHEW J. STASIEWICZ: *University of Illinois at Urbana-Champaign, Urbana, IL, USA*
CLAIRE ZOELLNER: *iFoodDecisionSciences, Inc., Seattle, WA, USA*
ERIC WILHELMSEN: *FREMONTA, Fremont, CA, USA*

Modern leafy green production systems rarely test positive for foodborne pathogens. Yet, recurrent foodborne disease outbreaks associated with these commodities suggest that risk assessment is needed to evaluate all potential sources of contamination. One challenge to evaluating and reducing contamination risks is that real-world experiments on production systems with an expected (and desired) low-prevalence of pathogens are difficult and expensive to design for powerful results, and practically nearly impossible to execute. One solution for such an experimentally intractable problem is computer simulation, where appropriate models allow scientists and food safety practitioners the ability to study contamination events and test out food safety interventions at a scale impossible in the real world. This symposium will describe current work to simulate in-field production, harvest, and packing of leafy greens aimed at identifying where food safety system verification, via such as environmental or product sampling, could drive improvements in overall safety metrics. We bring an academic researcher, decision software provider, and consultant for a large leafy green grower to provide diverse perspectives on the use of these new simulation tools to tackle specific food safety issues facing the leafy green supply chain.

S15 A Highwire Act: Balancing Sustainable Agricultural Irrigation Approaches with Food Safety Priorities in the Face of Water Shortages

EWEN TODD: *Ewen Todd Consulting, Okemos, MI, USA*
OLFA MAHJOUB: *National Research Institute for Rural Engineering, Water, and Forestry (INRGREF), Tunis, Tunisia, Tunisia*
MANAN SHARMA: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
DIMA FAOUR-KLINGBEIL: *School of Biological and Marine Sciences, University of Plymouth, Devon, United Kingdom, United Kingdom*

Surface waters in different regions of the world provide different hazards, including microbiological pathogens and chemical contaminants that may affect human health through dissemination on fruits and vegetables after irrigation. Hazards in different water types (ponds, rivers, creeks, reclaimed waters) are affected by season, location, and time. Reuse of wastewaters, including those from polluted urban sources, is recognized worldwide as an alternative resource to cope with water shortage in arid and semi-arid regions where irrigated agriculture and food security are threatened; however, contaminants in wastewater must also be mitigated. Therefore, irrigation practices for agricultural use of surface and wastewaters have to be strengthened. Cost-effective mitigation practices, like sand and zero-valent iron filtration, need to be developed and customized for small-scale growers. Microbiological water standards for irrigation of produce have been implemented by industry (California and Arizona Leafy Greens Marketing Agreement) and proposed by the U.S. Food and Drug Administration. Compliance with these standards requires new practices and testing regimes in the field, and also new research approaches which identify and assess environmental and agricultural risk factors which can affect the persistence or introduction of these pathogens to irrigation waters. This symposium brings together speakers who are currently investigating wastewater reuse and also researchers collecting data on pathogens in irrigation water and compliance with emerging standards. Characterization of these risks can lead to better management and mitigation of contaminant presence in irrigation water intended for produce, not only for the U.S., but for countries with arid regions.

S16 Impact of U.S. Food Safety Regulations on Compliance of Manufacturing Facilities in India

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The recent Food Safety Modernization Act (FSMA) by the FDA requires that foreign suppliers in India are producing food using processes and procedures that offer same level of public health protection as the preventive control (PC) requirements and current good manufacturing practices (GMPs) rules for human and animal food. The suppliers require guidance and training on PC provisions and current GMP requirements for foreign supplier verification and to be eligible for food export to the U.S. The Food Safety and Standards Authority of India (FSSAI) regulates manufacture, processing, distribution and sale of food for human consumption in India. It sets science-based standards for safety and quality of food and food products. The FDA Office of International Programs (OIP) and the USDA Foreign Agriculture Service (FAS) have conducted several training courses in India to develop a cadre of food industry professionals knowledgeable about FSMA regulations. This symposium will review the FSMA Preventive Control for Human Food (PCHF) and FSSAI standards in India, and gaps in PC and GMP requirements for food supplier verification. It will elucidate the role of FDA inspections to compare existing GMP programs and corrective actions necessary to meet the FDA requirements. The symposium would be of interest to importers, certified trainers, and food industry personnel in India and other foreign countries.

S17 Perspectives on the Current State of Food Fraud Prevention: Regulatory Investigations, Harmonization of Standards, and Supply Chain Management

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FERNANDO ANTUNES LOPES: *Ministry of Agriculture, Livestock, and Food Supply - Brazil, Brazil, Brazil, Brazil*
GINA CLAPPER: *USP, Rockville, MD, USA*
CLARE MENEZES: *McCormick & Company, Haddenham, United Kingdom, United Kingdom*

This session will explore the current state of food fraud prevention from three diverse perspectives. First, an inspector with the Brazilian Ministry of Agriculture, Livestock, and Food will describe his recent experience applying simple but effective forensic techniques to gather evidence of food fraud in Brazil. The results of this work demonstrate that fraud was uncovered in every beverage sector inspected. This presentation will summarize the methods applied and the results of these targeted inspections. The lessons learned about food fraud opportunities and vulnerabilities were remarkable. Second, a speaker from the Food Chemicals Codex will describe their work to develop, validate, and recommend new analytical tests and specifications for honey. This presentation will provide an update on a project aimed at defining honey authenticity and quality through the creation of an identity standard. Honey is a particularly challenging commodity for standards development due to the natural variation of the product and the wide variety of stakeholders involved in global trade. The speaker will also use honey as an example to provide attendees with an interesting discussion of the challenges inherent in food standards development for many products prone to fraud. Finally, a global industry leader in spice production and food integrity will walk through the leading practices embedded within their business to deliver integrity in products, including an overview of how supply chain controls, long term alliances, and global standards drive food fraud prevention. The three elements of their approach are strategic vendor alliances, source material control, and manufacturing control. These elements form the cornerstone of supply chain management, from the plants growing in the field to manufacturing facilities, ultimately ensuring that customers and consumers only receive adulterant-free products.

S18 Microbiomes and Plastispheres – Effects of Plastic Pollution on Food Safety

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Microplastics are particles (smaller than 5 mm) which have become ubiquitous environmental pollutants and consequently are now found in food and beverages. Microplastics are either made that size for use in personal care and industrial products or are formed when larger plastic items slowly break into increasingly smaller pieces from exposure to wind, waves, and/or sunlight. Record concentrations of microplastics have been discovered in Arctic sea ice, and plastic bags have been sighted in the Mariana Trench, the deepest part of earth. Predominant plastic marine debris includes grocery bags, food wrappers, bottles and straws.

The topic of microplastics was introduced at the 2019 IAFP Annual Meeting with two symposia: one focused on impact on seafood species, while the other described contamination of agricultural soil from application of biosolids as fertilizer and use of weed block or mulching film. However,

microplastics research is rapidly emerging with new findings continually published. In addition to microplastics' abundance in diverse ecosystems, new studies suggest its presence can alter the structure of microbial communities, as well as the function of individuals within an ecosystem or community. Scientists have discovered that microbes such as *Vibrio* can colonize the surface of microplastics in marine environments, developing unique communities (plastispheres) and enabling pathogen transport to different areas. Microplastics can also alter microflora in soil used for agricultural production.

Because plastic production continues to exponentially increase, stressing global waste management, it is likely that microplastics ingestion from contaminated food and beverages will also increase. The goals of this symposium are to: 1) foster understanding of this threat to water and soil quality to better evaluate the impact of microplastics on human health, 2) learn how microbes/pathogens adapt to or use plastic pollution, and 3) motivate the food and beverage industry to reduce their use of plastic to help protect the food supply.

S19 Effective Approaches to Measure Food Safety Behavior Change

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BRITA BALL: *Brita Ball & Associates, Guelph, ON, Canada, Canada*
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Self-reported behavior used to assess food safety practices is fraught with bias. Still, food safety researchers and businesses need to be able to effectively assess existing food safety knowledge, skills, attitudes and behavior, and measure changes that follow education, training and culture change initiatives. Questionnaires are commonly used assessment tools for surveys partly because of their perceived ease of use. Yet questionnaires and survey research have been poorly designed and implemented leading to questions about the reliability and validity of results. Qualitative data collection and analysis techniques commonly used in social sciences provide rich information unavailable from questionnaires but are not as simple as many people think. Furthermore, triangulation of techniques strengthens results but requires additional resources and may not be seen as a valuable use of time and money. This symposium will answer the questions: What are realistic options to assess various needs, demonstrate behavior and/or culture change, and show that the change has had an impact on food safety? What are the advantages and pitfalls of relevant social science research approaches, and how can the issues be addressed? How have advances in technology affected research for food safety training and culture? The session will include examples from the field and consider options for small and medium sized businesses to that want to conduct their own assessments and impact studies related to food safety behavior. It brings together and provides context for key considerations for effective use of social science research methods to advance food safety practices in the food industry.

S20 Quantitative Microbiological Risk Management for Safe Water Re-use in Food Processing

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LEON GORRIS: *Food Safety Expert, Nijmegen, Netherlands, Netherlands*
SUCHART CHAVEN: *PepsiCo, New York, NY, USA*
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For over fifty years, The International Commission on Microbiological Specifications for Foods (ICMSF) has developed risk-based concepts and approaches in the field of food microbiology to continuously evolve food safety and stability best-practices and standards.

Recognizing the global challenges around the globe today, and even more so in the future, regarding access to safe and sufficient water for food processing and handling, the ICMSF has started to develop science-based quantitative standards for water re-use in food processing operations that help to verify that consumer risks are adequately addressed.

Among the possible sources of water available to the food industry could be water that is recovered from food or from particular operations in a food processing/handling facility. Such water could be re-used in different ways and for different purposes, but the possible occurrence of microbiological and other hazards needs to be dealt with.

As increasingly more industries experience the need to re-use water from within their operations, governments recognize that (harmonized) standards would add particular benefit for local and cross-border food safety management. There is especially a need for quantitative guidance on microbiological limits as reference points for best-practice food safety management. However, given the very variable microbiological status of water sources from within food processing operations, establishing such quantitative standards is not straightforward since many aspects of consumer risk and science need to be considered by operators.

The Commission is building on the work of Codex and other international organizations that are progressing conceptual frameworks that guide the establishment of sound sustainable water (re-)use practices. The latest thinking and case examples will be presented and discussed in the proposed symposium.

S21 How Do We Measure the Effectiveness of Food Safety Systems?

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CAROLINE SMITH DEWAAL: *Global Alliance for Improved Nutrition, College Park, MD, USA*
JAN BAELE: *Directorate-General Health & Food Safety, European Commission, European Union, Brussels, Belgium, Belgium*
SHERRI MCGARRY: *Centers for Disease Control and Prevention, Washington, DC, USA*

This session will examine the tools for measuring the performance of national food safety systems. Presenters will describe international Codex guidance on the framework for national food control systems, together with guidance on how to evaluate their performance. It will also examine tools used in both Europe and the United States to gather information on outbreaks, recalls and other adverse events and their use in evaluating national performance. Issues to be discussed include the framework for a national food system, performance monitoring, uniformity of reporting, the probability that foodborne illness cases will be detected, the transparency of the database for use by the public, and the implicit economic incentives to produce safer food.

S22 Safety Considerations for Hemp-derived CBD

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NORBERT KAMINSKI: *Michigan State University, East Lansing, MI, USA*
SCOTT COATES: *Association of Official Analytical Chemists Research Institute, Rockville, MD, USA*
MARTIN HAHN: *Hogan Lovells, Washington, DC, USA*

Recent years have seen a rapid rise in the widespread use of cannabidiol (CBD) in foods and dietary supplements. In its pure form, CBD possesses no psychoactive properties. The 2018 U.S. Farm Bill legalized the production of hemp (*Cannabis sativa*), a strain of cannabis that is rich in CBD but produces

very low levels of THC, the principal psychoactive constituent of cannabis. The increased use of CBD presents unique complexities for its regulation due to unanswered questions related to short- and long-term effects on health and safety. This session will summarize current knowledge of biological and toxicological effects of CBD. The session will also identify research and analytical gaps related to hemp-derived CBD, as well as strategies for addressing these gaps to inform regulatory and public health decisions. Last, the session will address regulatory considerations related to ensuring safety of hemp-derived CBD products.

S23 How to Talk to People That Don't Know What You are Talking About: Effectively Communicating Food Safety Information

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SERGIO NIETO-MONTENEGRO: *Food Safety Consulting & Training Solutions, LLC, El Paso, TX, USA*
WENQING (WENNIE) XU: *Louisiana State University AgCenter, Baton Rouge, LA, USA*
SHELLEY FEIST: *Partnership for Food Safety Education, Arlington, VA, USA*

As food safety knowledge continues to advance, content experts need to be equipped with the tools to effectively communicate meaningful concepts that will limit risk in the food industry. This symposium will focus on the most effective methods to convey information to persons with limited knowledge on food safety practices.

The first speaker will consider the multifactorial dimensions of wellbeing that can influence consumer food safety cognition, behavior and engagement with food safety education and how such dimensions can be utilized to inform the design and development of future food safety interventions.

The second speaker will share her journey of building a consumer food safety program from scratch in her state. The technique of scaffolding has been adopted in communicating food safety knowledge with Extension field agents, educators and frequent clients. The speaker will also showcase the multi-faceted communication strategies she has chosen during the scaffolding process.

Lastly, success stories on methods and past experiences will be highlighted as case studies to help audience members tailor their communication strategies to improve food safety education. This symposium will focus on the most recent approaches to effectively communicate food safety knowledge, while also providing relevant strategies that IAFP members can implement in all sectors.

S24 Passport to Food Safety in Low- and Middle-income Countries: Rationale and Reflections for Recent Research Initiatives

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KRISTEN MACNAUGHTAN: *Bill and Melinda Gates Foundation, Seattle, WA, USA*
HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
ASHAGRIE ZEWDU: *Addis Ababa University, Addis Ababa, Ethiopia, Ethiopia*

In 2015, the World Health Organization released the first-ever global burden of disease estimates for foodborne illness. The estimates — 600 million illnesses and 420,000 deaths annually — demonstrate that, despite the disparate investments, the burden of foodborne illness is similar to the burden of malaria and tuberculosis. As a result, key inter-governmental agencies — including the Food and Agriculture Organization of the United Nations, World Health Organization, World Trade Organization and African Union — held the First International Food Safety Conference and the International Forum on Food Safety and Trade to identify key actions and strategies to address current and future challenges to food safety globally. At the same time, governmental and non-governmental organizations have begun to fund major international food safety projects, especially in low- and middle-income countries which bear the greatest burden. There is clearly a mutual and increasing interest on the part of multinational organizations and national governments to fund and support food safety initiatives.

The purpose of this proposed symposium is to provide an overview of several active research projects in low- and middle-income countries from a variety of perspectives involved. Specifically, representatives from funding agencies will present the rationale for current and proposed research while discussing why support of international food safety research in low- and middle-income countries is a key priority. Subsequent speakers representing four different ongoing projects in low- and middle-income settings will discuss initial results and reflect upon experiences designing and implementing international food safety projects to inform future investment and research activities. This symposium is one of three proposed complementary sessions on improving food safety in Africa. The other two sessions focus on Creating Awareness and Creating Engagement.

S25 Best Practices to Manage Produce Risks from Farm to Retail

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ANNA STAROBIN: *Ecolab Inc., Greensboro, NC, USA*
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Fresh produce risk controls, such as those detailed in the FSMA Produce Safety Rule, FDA Guidance Documents and industry guides, have enhanced the preventive controls during growing, harvesting, packing, holding, and processing. However, since there is typically no kill step for pathogens on whole, raw produce, it can still be contaminated when entering commerce.

Different methods are used to wash produce, including submersion, spray, or both. Rinsing with water, rather than full submersion, may be less likely to cross-contaminate produce. However, this method may not be practical for large quantities of produce.

Currently, submersion is a common method for washing produce in retail. Under certain conditions, pathogens washed from the surface of produce may cross-contaminate via water or be internalized via water infiltration.

The Conference for Food Protection (CFP) created a committee charged with developing a Produce Washing and Crisping Guidance document for Retail Food Establishments. The purpose is to provide retail food establishments washing whole, raw produce, with risk management steps for the most commonly used methods.

Manufacturing experience in produce washing operations shows that cross-contamination via wash water must be prevented, typically using antimicrobials in the wash water. How does this translate to the process of washing or crisping fresh produce at retail? What is the risk of a similar type of cross-contamination at retail, and how do the available mitigation differ from those in production environments?

Retailers are seeking the safest, most practical and sustainable methods aimed at reducing cross-contamination and food waste, and enhancing produce quality for their operations.

The outcomes of the CFP committee work and the need for further research, including academic and industry perspectives, will be discussed. These talks will give a balanced overview of an issue that is more complex than it may seem.

S26 Food Safety Risk from *Clostridium perfringens*, *Clostridium botulinum*, and *Bacillus cereus* in Cooked Meat and Poultry Products

SUBASH SHRESTHA: *Cargill, Inc., Wichita, KS, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
ABANI PRADHAN: *University of Maryland, Department of Nutrition and Food Science, College Park, MD, USA*
SCOTT UPDIKE: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*

The primary food safety risk in cooked meat and poultry products for not undergoing a rapid chilling is poised by the growth and/or toxin formation by the spore forming bacteria, namely, *Clostridium perfringens*, *Clostridium botulinum*, and *Bacillus cereus*. The USDA FSIS identifies that the lower and upper growth limits for *C. perfringens*, *C. botulinum* (proteolytic), and *B. cereus* are 6°C and 52°C, 10.0°C and 50°C, and 4°C and 55°C, respectively. Likewise, the minimum growth limit for non-proteolytic *C. botulinum* is 3.3°C.

The proposed USDA FSIS Compliance Guideline for Cooling (Revised Appendix B, 2017), especially the option 2 for cooling, has posed challenges to the meat industry. The recommended cooling time and temperature is based on the predictive microbiology and not necessarily based on the actual growth data for these heat resistant spore formers. Understanding the multiplications of these pathogens in various conditions is important to know the actual food safety risk. Recently, the USDA and the North America Meat Institute have collaborated to determine the comprehensive growth potential of these pathogens.

The USDA FSIS suggests ≤ 1.0-log growth of *C. perfringens,* ≤ 3.0-log growth of *B. cereus* and no multiplication of *C. botulinum* growth (mean net growth ≤0.30 log) as the food safety criteria. The lack of proper prevalence and concentration data of these spores in raw meat and poultry has presented many questions against the proposed USDA guidelines. Moreover, the risk is present not only in the slow cooling but potentially also in properly chilled products that are stored refrigerated for a long term.

The purpose of this session is to provide the most recent research information on the growth and quantitative risk assessment. Also, speaker from the USDA will provide updates on the proposed USDA Appendix B guidelines.

S27 What Should I Eat? Integrating Food Safety Risks and Nutritional Health Outcomes in Multi-risk and Risk-benefit Assessment Frameworks

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SOFIA SANTILLANA FARAKOS: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
MAARTEN NAUTA: *National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark, Denmark*
JEANNE-MARIE MEMBRÉ: *Secalim, INRAE, Oniris, Nantes, France, France*

Consumers need to integrate information on food safety, nutrition, and health when making dietary choices. Multi-risk (risk-risk) and risk-benefit assessments focused on dietary choices can provide critical information for all stakeholders by integrating data on health risks and benefits. Research institutions, industries and public health regulatory bodies are moving towards a systems approach to food safety and health when labelling food and making dietary recommendations. In this symposium, an overview of multi-risk and risk-benefit assessments associated with dietary choices or shifts will be presented by examples of using multi-risk and risk-benefit assessments to evaluate and inform public health.

S28 Validation of New and Emerging Molecular Technologies for Pathogen Characterization

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MORGAN WALLACE: *Rheonix, Ithaca, NY, USA*
RUTH TIMME: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
NUR HASAN: *EzBiome, Rockville, MD, USA*

New molecular-based technologies that aim to analyze food samples for relatedness or molecular patterns of pathogens are becoming more common in the food safety field. Having the ability to determine the relatedness of one sample to the next provides greater insight into the "microbiome" of a food plant, including the identity of both resident and transient pathogens and spoilage organisms. This insight can lead to changes in sanitation programs, changes in the traffic flow throughout a manufacturing facility to reduce the spread of contaminants from one part of the plant to another, or other operational changes. Pattern-based systems can allow improvements in food plant management without creating data that might link to regulatory investigations of outbreaks of foodborne illness. In contrast, whole genome sequencing (WGS) is used for pathogen identification and outbreak investigation by both the FDA and CDC. FDA has even launched GenomeTrakr, a public database to collect and share genomic and geographic data from foodborne pathogens across the world. However, WGS or molecular pattern-based technologies do not neatly fit into established method validation schemes that were set up for a more traditional presence/absence of detection of an organism of interest. Material cost can be quite high for some technologies, especially WGS, making analyzing large volumes of validation samples extremely costly. Moreover, the "high-stakes" nature of the traceability provided by these platforms creates a need for great confidence in the reliability of the methods. Speakers in this symposium will provide guidelines and best practices for validating these sophisticated technologies, and indicate the limits of performance that can reasonably be expected.

S29 Current Best Practices for Extrusion Cooking Processes: A Holistic Approach to Controlling Pathogens in Low Water Activity Foods

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TIM HARTTER: *Wenger Corporate Project Services, Sabetha, KS, USA*
LISA LUCORE: *Shearer's Foods, Massillon, OH, USA*
PABLO CARRION: *Nestle Purina, St. Louis, MO, USA*

Outbreaks of salmonellosis have been associated with a number of extrusion cooked low water activity products including breakfast cereals, puffed snacks, pet food and pet treats. Some of these outbreaks resulted from post-process contamination by persistent strains harbored in the facilities over several years or transient strains recently introduced due to zoning failures. These outbreaks underscore the wide diversity of low water activity food and feed products that can be contaminated with *Salmonella* and the difficulty with managing *Salmonella* in the dry manufacturing process environment. Therefore, a holistic approach to controlling pathogens in extrusion cooking processing facilities is needed. Application of hygienic design principles to the building and grounds, zoning and equipment can mitigate potential *Salmonella* contamination risk factors. With appropriate validation, control during primary (extrusion cooking) processing will significantly reduce or eliminate *Salmonella*. With appropriate process controls for secondary processing (drying, cooling, coating, etc.) the potential for recontamination of product after the 'kill step' can be minimized. In this symposium, speakers will primarily utilize case studies to emphasize current and emerging best practices including the application of hygienic design principles, as well as

emphasize critical points in primary and secondary processing areas. Since the approaches for what is "best practice" for greenfield projects (i.e., new construction on not previously developed land) vs. legacy facilities (i.e., an existing facility constructed at an earlier time) are often uniquely different, the speakers will also discuss the challenging and complex issues surrounding implementation of best practices as *ideal* designs and practices that are not always feasible in legacy facilities. Still, improvements can be made in older plants, which highlights the need for continuous improvement of practices.

S30 Allergen Control – Challenges, Perspectives and Solutions

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STEVE L. TAYLOR: *University of Nebraska-Lincoln, Lincoln, NE, USA*
DEB SMITH: *Vikan (UK) Ltd., Swindon, United Kingdom, United Kingdom*
JOHN HOLAH: *Holchem Laboratories, Cardiff Metropolitan University and EHEDG, Bury, United Kingdom, United Kingdom*

In the U.S., food allergies affect about 3.5 – 4.0% of the population. The Food Allergen Labeling and Protection Act (FALCPA) mandated special labeling requirements for eight major food allergens when used in packaged foods. More recently, the Food Safety Modernization Act (FSMA) requires that food facilities implement allergen controls to prevent allergen cross-contact, the unintended introduction of allergens into food. Despite the enactment laws and regulations aimed at protecting consumers with food allergies, undeclared allergens remain one of the leading causes of recalls in the U.S. Whilst legislation and key allergens are slightly different in the EU, the same situation has arisen with respects to recalls.

Our first presentation will focus on consumer impacts (prevalence, severity, fatalities, quality of life); food industry impacts (primarily recalls); and international regulations (with focus on USA, EU and Canada). It will also introduce the approaches and guidance available regarding preventive control.

Our second will provide a greater understanding of the impact allergens can have on vulnerable individuals; a summary of GFSI-bench marked standard requirements, regarding allergen management; and practical advice on measures to assist in the reduction of allergen cross-contact through the appropriate use of area and equipment segregation; color-coding; cleaning practices; and sanitary design.

And finally, our third will provide guidance on allergen control though application of appropriate validation, verification and monitoring. If separate processing lines are not possible, cleaning becomes the major control of allergens on shared production lines. As a prerequisite controlling a hazard, and as required by best practice and GFSI auditing standards, such cleaning requires validation and subsequent monitoring and verification.

S31 Help! I Have a Presumptive Pathogen Detection. What are My Options?

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DANIEL DEMARCO: *Eurofins, Louisville, KY, USA*
PATRICK BIRD: *PMB BioTek Consulting, West Chester, OH, USA*
VICKIE LEWANDOWSKI: *Saputo Cheese, USA, Lincolnshire, IL, USA*
THOMAS HAMMACK: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
CATHARINE CARLIN: *Cornell University, Ithaca, NY, USA*
ROGER HOOI: *DFA Dairy Brands, Dallas, TX, USA*

Discussions at IAFP 2019 raised the issue of addressing presumptive results from rapid screening methods in three different Professional Development Groups: Applied Lab Methods; Advanced Molecular Methods and Dairy Quality & Safety. When these methods were first released approximately 25 years ago, they dramatically shortened the time needed to complete pathogen testing and reduced stress on food plant operations by providing data before it was needed. Today, it's not unusual for food companies to have trucks ready to load, or loaded and geared up to roll, the minute test results are reported, only an hour or two after the minimum time technically required for analysis. In these circumstances, a presumptive screening result can be disruptive and nerve-wracking. Anecdotal reports suggest that method sensitivity has increased leading to a higher incidence of presumptive results, but the "gold standard" approach to confirmation involves cultural methods that take several days to a final result. Six speakers will explore issues around presumptive results, including: incidence of non-confirming presumptives (NCPs), how to minimize the incidence of NCPs while still detecting pathogens if present, and how to respond to a presumptive result; as well as multiple aspects of confirmations including: what is required, "heroic" measures and when they are justified, and rapid confirmations.

S32 Foodborne Disease Outbreak Update

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CATHERINE CARILLO: *CFIA, Ottawa, ON, Canada, Canada*
GAMOLA FORTENBERRY: *USDA Food Safety & Inspection Service, Washington, DC, USA*
PETER BEN EMBAREK: *World Health Organization, Geneva, Switzerland, Switzerland*
ELISA ELLIOT: *U.S. Food and Drug Administration, College Park, MD, USA*
TYANN BLESSINGTON: *U.S. Food and Drug Administration, College Park, MD, USA*
KATHERINE VIERK: *U.S. Food and Drug Administration, College Park, MD, USA*

This symposium covers five different topics surrounding specific outbreak investigations and outbreak investigation mechanics. Additionally, a slot will be held open for a late-breaking outbreak highlighting a recent localized illness outbreak investigation. An outbreak of *Salmonella* Poona cases in infants and young children was identified in France, Belgium and Luxembourg in 2018 and 2019 by genome multi-locus sequence typing analysis. Rice-based infant formula products were implicated and recalled. Lessons learned from the outbreak and the impact of the INFOSAN alert system will be discussed. Canadian officials will discuss how whole genome sequencing (WGS) is used to detect and investigate foodborne illness outbreaks. Specifically, a Canadian outbreak linked to flour will serve as an example to highlight the investigational process and precision WGS brings to the process. Officials from CDC and FDA will present research findings on trends identified with STEC outbreaks caused by leafy greens from 2011 to present. Epidemiological factors, traceback findings, and contributing environmental conditions will be highlighted. Officials from New York and FDA will discuss a large outbreak of Scombrotoxin poisoning that occurred in 2019. Ill persons reported consumption of tuna that was traced to a common importer. Challenges with implementing a regulatory strategy to address the impacted product will be highlighted. FSIS will discuss a multistate *Salmonella* Infantis outbreak from May 2018 – February 2019. The outbreak strain of *Salmonella* Infantis was identified in 142 raw chicken samples collected by FSIS; 96% of the FSIS chicken isolates exhibited multidrug resistance (MDR), and whole genome sequencing analysis of clinical and non-clinical isolates predicted a similar multidrug resistance profile.

S33 The Future of the Poultry Gut Health Nexus: Improving Food Safety

KRISTINA FEYE: *University of Arkansas, Fayetteville, AR, USA*
NADIA YACOUBI: *Evonik Operations GmbH, Frankfurt, Germany, Germany*
KRISTINA FEYE: *University of Arkansas, Fayetteville, AR, USA*
STEVEN RICKE: *University of Arkansas, Fayetteville, AR, USA*

The nexus of the gut-microbiome axis and how it interplays with gut health and production is rapidly emerging as a keystone chapter of knowledge for the field of Poultry Science. Ultimately, by understanding the microbiome, the metabolome, the host, pathogen biology, nutrition, and the interactions thereof, an opportunity exists for poultry scientists to further improve food animal production and reduce foodborne disease. Emerging evidence highly suggests that the microbiota may be the root effector agent or potential canary for what can go right or wrong with poultry health. For instance, *Salmonella* can both create or suppress inflammatory responses, which in turn can augment the microbiota. Changing the inflammatory potential of the microbiota can then shift metabolomic output and in turn modulate the host immune system. Therefore, is it possible to potentially edit or change the microbiota and optimize the balance of that nexus to the exclusion of potential pathogens? Ultimately, this nexus may be exploitable by the poultry industry to further improve food animal production and health. This symposium aims to solidify some of that research and propose new and exciting ideas to our community.

S34 From Policy to Practices, Developing Environmental Monitoring Programs for Raw Agricultural Commodity (RAC) Packinghouses

FAITH CRITZER: *Washington State University, School of Food Science, Pullman, WA, USA*
JENNIFER MCENTIRE: *United Fresh Produce Association, Washington, DC, USA*
ALEXIS HAMILTON: *Washington State University, Richland, WA, USA*
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The importance of developing and implementing Environmental Monitoring Programs (EMPs) has increased dramatically for raw agricultural commodity (RAC; e.g., whole, intact, and minimally handled/processed fruits and vegetables) packers over the past five years. Moreover, outbreaks and recalls have been traced back to the packinghouse environment. It is well understood that fruits and vegetables are grown in environments where pathogens may be present; albeit sporadically and in low numbers. Thus, it is possible for pathogens to enter and reside in packinghouses, if proper sanitation and mitigations are not implemented to reduce the likelihood of harborage. Most RAC packinghouses have a constant exchange of equipment (e.g., forklifts, bins) from the outside environment, which may introduce or re-introduce microbial contaminants after cleaning and throughout operation. Additionally, they also operate with a highly variable operating and sanitation frequency driven by weather (e.g., rain events) and crop maturity. The industry is seeking to strike a balance in commodities that are considerd both raw and ready-to-eat. While a significant amount of work has been translated into best practices for fresh-cut produce, RAC packers have limited resources on developing data-driven programs to successfully monitor and reduce risk of contamination events in the packing environment. Therefore, this symposium will discuss the unique challenges faced by the RAC produce industry; specifically, (i) evaluating FDA guidance and buyer requirements related to EMPs, (ii) recent research highlighting harborage areas and control strategies for *Listeria* in packinghouses, and (iii) how industry is developing and implementing EMPs to control and reduce risk of contamination events while packing raw commodities.

S35 Navigating the Benefits and Barriers of Whole Genome Sequencing (WGS) for the Food Industry from the Food Industry

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PIERRE VENTER: *Fonterra, Palmerston North, New Zealand, New Zealand*
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Ever since whole genome sequencing of pathogens was introduced for foodborne outbreak investigations in the U.S. over five years ago, it is increasingly being adopted by the industry for several applications including for the root cause analysis of a pathogen contamination event. However, the level of adoption of WGS widely varies between companies. A survey was conducted by the industry by contacting 30 companies in order to understand the use or lack of use including the barriers and benefits of using WGS to improve food safety. Results that were obtained from 18 companies, representing major food industry players, indicated that though the benefits of using WGS is recognized, major obstacles prevent its use in routine applications. The results were further discussed with participating companies in an industry workshop in September 2019. The objective of this symposium is to present the results of the survey detailing the thoughts and status of WGS in the industry with an emphasis on the benefits noticed, limitations, and regulatory implications of using this technology. A summary of workshop outcomes and the ensuing actions to enable widespread adoption of WGS including by small and medium enterprises, through for example, third party laboratories will be outlined. In addition, an industry expert will present the short-term operational benefits of using WGS and finally a regulator will share examples of cases where WGS evidence has been used to exonerate foods that were suspected to be associated to an illness.

S36 Confirmatory Tests for Non-culturable Foodborne Pathogens in Produce for Regulatory Testing Purposes: Recent Advances and Challenges Ahead

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Foodborne viruses have been the cause the majority of foodborne outbreaks for many years. In recent years, Cyclospora cayetanensis has been increasingly implicated as a cause of large and complex foodborne outbreaks. Unlike bacterial pathogens, some of these viruses and parasites cannot be cultured or easily enriched from the implicated food. Thus, although PCR is the gold standard for detecting and identifying these pathogens, especially

viruses, unlike bacterial pathogens, positive PCR results cannot be confirmed by culture. Other methods such as additional molecular and/or genomic methods, are necessary to understand outbreaks and may help support regulatory actions. This symposium will provide information on the different types of methodologies that are or can be used and the associated challenges, to verify the presence of foodborne viruses and C. cayetanensis in produce, to support epidemiological investigations and regulatory actions.

S37 I Will Survive! Molecular Basis of Pathogen Survival in Low-moisture Foods

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VICTOR JAYEOLA: *North Carolina State University, Raleigh, NC, USA*
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YUAN FANG: *University of Alberta, Edmonton, AB, Canada, Canada*

Low-moisture foods (LMF) including nuts, dried fruits, cereal products, flour, and chocolate are often associated with recalls and outbreaks due to the long-term survival of bacterial pathogens under low-moisture conditions. The mechanisms underlying the persistence of pathogens such as *Salmonella*, pathogenic *E. coli*, and *Listeria monocytogenes* in LMF are not fully understood. This symposium will explore proposed mechanisms for survival including the detection of novel genes that regulate physiological changes in bacterial cells and additional genetic alterations in genomes that allow long term pathogen survival in LMF. Understanding the underlying mechanisms pathogens use to tolerate and survive desiccation stress is important to their control in LMF food production processes and facilities.

S38 Forecasting Hot Topics: Strategies That Signal the Occurrence of Emerging Chemical Threats

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Forecasting the weather can be a challenge to predict. Forecasting emerging chemical threats in food is an exercise in science. Future weather and food risks are determined by external factors that ultimately shape the predicted outcome. Meteorologists utilize Doppler Radar to forecast weather conditions for tomorrow and even an entire month. The food industry is ripe for a "tool" that would predict future chemical risks in food. According to the 2019 Food and Health Survey, chemicals in food are a growing concern for consumers (53%), set to surpass public concerns about foodborne illness (60%). Addressing chemicals in food before they become a public concern, could ultimately be measured in terms of purchase behavior, consumer confidence and trust in food. But where do we start? What data or research do we need? Most importantly, how is any science-based tool transparent to the public. How can we share information across companies without it being used by upstarts in the industry or global competitor? Lastly, can we combine ideas about forecasting into a core definition that transcends all food and beverage companies? This panel will bring together food safety experts and "futurists" to share perspectives and explore the development of a tool to identify future chemicals risks. These experts will share their expertise on futures (predictions for chemicals in food) and share examples of successful strategies. Special emphasis will be given to tools that are currently available to monitor emerging chemical issues and others that may (or should) be relevant to your business.

S39 Whole Microbial Community and Metagenomics Applications to Characterize Water Used in Food Production

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SHIRLEY A. MICALLEF: *University of Maryland, College Park, MD, USA*
NIKKI SHARIAT: *University of Georgia, Athens, GA, USA*

Technological and computational developments in genomics and metagenomics have made it possible to characterize microbial communities, as well as to detect target pathogens. It has been recognized that the composition of the whole microbial community can affect survival and phenotypical behavior of human pathogens in a number of systems. Community fingerprints also carry information that may be useful for trace-back and source tracking. However, data on community composition are still resource-intensive to obtain, and often collected only for research purposes. At the same time, there is a need to leverage whole-community information to develop cost-effective approaches to characterize microbial contamination sources, detect shifts that could be used as early-warning signals, and link community composition to pathogen presence, and potentially also behavior. Water used in food production, both preharvest and postharvest, is one area that could particularly benefit from whole-community characterization, leading to practical applications under current regulatory frameworks (e.g., shifting from reliance on one indicator to a broader system assessment). This symposium will bring together researches from different disciplines and regions to share method innovations and results in metagenomics and whole-community characterization, applied to water used for food production. Specific objectives of the symposium are to: (a) provide updates on the effectiveness of novel metagenomics methods, in a way that is accessible to both technical experts and risk managers that would use the information; (b) illustrate a range of metagenomics applications to answer different research questions; and (c) present new results on how whole-community data are providing insight into risk factors and may inform risk management options. Overall, the symposium tackles the overarching question of: how are whole-community characterization methods already being applied in food safety, and how close are we to bring them from research labs into routine applications in the food industry and public/environmental health?

S40 Consumer Animal Welfare Demands and Their Impact to Food Safety

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PRAFULLA REGMI: *North Carolina State University, Raleigh, NC, USA*
LUIZ DEMATTÊ: *Korin, Ipeúna, Brazil, Brazil*
ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*

Current and new marketplace food trends and consumer demands related to animal welfare are changing the way animals are raised for food production. Food industry stakeholders (producers, retailers, food service, etc.) are taking steps and developing policies to address consumer concerns about animal welfare. Due to this shift, the food marketplace is experiencing new food safety challenges caused by changes in farm management to address consumer perceptions of animal welfare. For example, in 2018 a large outbreak of *Salmonella* was linked to cage-free eggs. While farming practices continue adapting to satisfy consumer demands for animal welfare, it is imperative to develop science-based tools and share knowledge about

the potential consequences of implementing new animal welfare practices at farm production and strategies to address these challenges. During this symposium, participants will hear from experts on 1) Animal Welfare Trends in poultry production; 2) Food Safety issues that need to be addressed when changing farm practices; 3) Successful examples of farm practices that address consumer animal welfare perception issues while maintaining food safety (global perspective).

S41 Jumping into the Deep End: Lessons Learned from Water Treatment Implementation under New LGMA Metrics

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TERESSA LOPEZ: *Arizona LGMA, Phoenix, AZ, USA*
JAY SUGHROUE: *BioSafe Systems, La Quinta, CA, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*

New Leafy Greens Marketing Agreement (LGMA) metrics, adopted in spring of 2019, have set a never before seen precedent of mandating preharvest agricultural surface water treatment for 21 days prior to harvest for water that directly contacts the edible portion of the crop. This includes surface water which is likely to contain generic *E. coli* and is applied by overhead irrigation, such as that obtained from canal systems. This new approach to managing risks on farm, comes after two high-profile outbreaks of *E. coli* O157:H7 linked to romaine lettuce in 2018. While many growers sporadically treated irrigation lines to prevent clogging, this new mandate left many with far greater questions than answers when attempting to meet the new standards in subsequent growing seasons. To support the industry, there has been a great amount of effort in coordinating research projects and delivering outreach to the leafy greens growers in Arizona and California. This symposia will share insights to the new requirements adopted by the LGMA(s) and observations from those in charge of implementing the program; provide a comparison of common chemigation approaches and critical factors which should be considered when implementing any treatment in order to achieve the desired water quality metrics; and finally explore into the numerous knowledge gaps which are currently being investigated in order to assist growers with consistent and effective water treatment.

S42 Identifying Tools to Predict Food Safety Failures and Financial Costs

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DAVID HATCH: *Corvium, Reston, VA, USA*
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MEHRDAD TAJKARIMI: *EAS Consulting Group, Los Angeles, CA, USA*

The identification of risk and use of actuarial tools is the fundamental basis for the insurance industry and all premiums are based on the identified risk. Yet, in the food industry, there is no similar effort to utilize common indicators of strong or weak food safety programs such as environmental testing results, operational records, written food safety program implementation and effectiveness and the food sector and industry history to predict the likelihood of a food safety failure. Because of this shortcoming in quantifying and predicting food safety risk, the food industry has to make resource allocation decision based on best guesses with no real certainty that these guesses will actually reduce the food safety risk to their customers and financial risk to the company. This symposium is intended to expose the food safety professional to approaches from other industries including advanced actuarial and statistical methodology to crunch "big food safety data" and provide meaningful predictive risk profiling for use by the food safety industry, state and federal food safety regulators and propose various risk ranking systems that might be beneficial to consumers, making food purchase and consumption decisions.

S43 Microfluidic-based Sensing for Rapid Food and Water Safety

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CHUCK HENRY: *Colorado State, Fort Collins, CO, USA*
IRWIN QUINTELA: *U.S. Department of Agriculture-ARS, Western Regional Research Center, Albany, CA, USA*
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Microfluidic based sensors targeting food and waterborne pathogens overcome many of the ongoing challenges faced by industry, military, and inspectors, especially when acquiring rapid screening results in remote locations. These challenges include assemblages that can be costly, heavy, require refrigeration, and have long time requirements to obtain results. Paper-based platforms are applicable to a wide range of sensor complexities and designs, from simple 1D and 2D lateral flow tests to complex 3D microfluidic devices. These diagnostic devices can be adapted to many different targets while maintaining the low cost per test needed for screening technologies to process large numbers of food and water samples in austere environments. Despite these advantages, there are factors that limit the detectable concentration for pathogens in food samples required by many end-users. As a result, many paper microfluidic tests require an enrichment step of 4–16 hours to achieve the desired limit of detection. This limitation originates from the detection methods used and the complexity of food matrices that inhibit performance. By combining new methods of sample preparation and detection with existing paper microfluidic biosensor technology a rapid, sensitive test can be developed for low-cost detection of pathogens in food samples. This session will address strategies to overcome microfluidic-based sensing challenges from diverse government and academic research laboratories.

S44 Multidisciplinary Perspectives on Salmonella Reading Illnesses Linked to Turkey

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COLIN BASLER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
DOUG NOVEROSKE: *U.S. Department of Agriculture-FSIS, Washington, DC, USA*
OLUWAKEMI ONI: *Iowa Department of Public Health, Des Moines, IA, USA*
MICHELLE KROMM: *Jennie-O, Willmar, MN, USA*

In 2018, the Centers for Disease Control and Prevention (CDC), state and local health departments, and the US Department of Agriculture (USDA) began investigating an outbreak of multidrug resistant Salmonella Reading infections. This investigation ultimately linked these illnesses to handling and consumption of turkey products (both human and pet food) and demonstrated that Salmonella Reading was an emerging strain of concern in turkeys. In addition to the government response to the outbreak, this session includes presentations on the overall emergence of this Reading strain in turkeys (Dr. Johnson) and the industry response to control the strain (Dr. Kromm).

S45 What is Ready-to-Eat and How Safe is My Smoothie?

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LAURA GIERALTOWSKI: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*

Consumer eating habits have changed with more focus on healthy choices and quicker meal preparation. Foods that were historically eaten cooked, either alone, or as an ingredient, are being incorporated into novel recipes and consumed raw resulting in potential food safety risks and illness outbreaks. Fresh and frozen ingredients, such as frozen sweet peas and berries, may be added to smoothies. This muddies the waters as to what is considered "ready-to-eat" and how these foods are currently regulated. Recently, public health agencies have investigated some of these foods in illness outbreaks. In 2016, an outbreak of listeriosis was linked to frozen vegetables. There were nine illnesses reported in four states. All nine people were hospitalized and one person died of listeriosis.

This symposium will provide an overview of illness outbreaks linked to foods eaten in new ways as part of a healthy diet and the regulatory prevention framework for "ready-to-eat" foods, in addition to trends in healthy eating that may contributed to an increased food safety risk.

S46 Spoiler Alert! Food Spoilage is Eating Our Lunch!

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KATHLEEN GLASS: *Food Research Institute, University of Wisconsin-Madison, Madison, WI, USA*
EMILIA RICO-MUNOZ: *BCN Research Laboratories, Rockford, TN, USA*
NICOLE MARTIN: *Cornell University, Ithaca, NY, USA*

Food insecurity has led to massive human health impacts physically and psychologically. Globally, however, food spoilage as a major contributor to this problem does not receive a lot of attention. Crop losses prior to reaching the marketplace are largely the result of spoilage during the distribution chain. In the case of processed food, spoilage can be attributed to several factors including lack of proper transport and holding infrastructure, food formulation, processing and packaging failures. At the same time, consumer demand is increasing for minimally processed, clean label foods with long shelf life that also need to withstand challenging delivery conditions.

The symposium brings together experts who will highlight little known or appreciated aspects of the global spoilage problem, review case studies that exemplify the challenges and techniques available to the food industry. The discussion and knowledge shared should be relevant to both veteran and new food industry professionals and provide new insights into spoilage investigations to address this urgent global challenge.

S47 They Get by with a Little Help from Their Friends

CHIP MANUEL: *GOJO Industries, Akron, OH, USA*
DEB SMITH: *Vikan (UK) Ltd., Swindon, United Kingdom, United Kingdom*
DIANE WALKER: *MSU Center for Biofilm Engineering, Bozeman, MT, USA*
HENDRIK DEN-BAKKER: *University of Georgia, Center for Food Safety, Griffin, GA, USA*
KRISTIN WILLIS: *EPA, Washington, DC, USA*

The microorganisms that we are concerned with controlling in food and food facilities are rarely present as pure cultures. This session will discuss the varied and complex ecologies of the biofilms that harbor microorganisms and protect them from the tools and processes that we traditionally use to manage them. A better understanding of the complex ecology and interactions that occur in biofilms can optimize controls. This session will cover four topics;

1. First will be a brief review of how we are currently trying to control biofilms, what works (and does not), and what information we need to better manage such dynamic, complex communities of microorganisms.
2. The second talk will focus on laboratory methods to study biofilms. Traditionally, such controls are studied in pure culture. Even when biofilms are grown in a laboratory, they are often pure culture biofilms containing only a single kind of microorganism. Complex multispecies biofilms are much more complex to work within a laboratory setting, however they may be able to provide better models to predict the efficacy of the control strategies that we use in our facilities.
3. The third talk will relate the reality of these biofilm communities to public health risks.
4. Finally, a regulatory update will be given. Biofilms control claims are regulated, in the U.S., by the Environmental Protection Agency. A regulatory expert will speak to current EPA regulations regarding biofilm control claims. Topics covered include what data are needed to support such claims, the test methodologies to generate that data, performance standards, and a look at the agency thinking about biofilm claims for food contact surfaces.

S48 How to Protect Foods Delivered to Your Consumers' Doorstep

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TIA GLAVE: *Milk Bar, New York, NY, USA*
BRIAN EYINK: *Hogan Lovells U.S. LLP, Washington, DC, USA*
GLENDA LEWIS: *U.S. Food and Drug Administration, Washington, DC, USA*

In the fast-growing meal-kit industry and as more and more people rely on online/e-commerce delivery of their foods, delivering safe food to consumers' doorstep is critical. One of the main challenges in the food delivery system in e-commerce is keeping the cold chain intact throughout the process from the point in which the package leaves the cold facility to consumer's doorstep. This challenge is present for all online food delivery from retailers to meal kits. This symposium will be discussing the importance managing the cold chain aspect of the process and share best practices from industry leaders specifically for food that requires temperature control for safety. In addition, speakers will share strategies on how to mitigate the risk inherent to the products, packaging materials and configuration, and customized transportation methods. Attendees will hear whether a HACCP or Food Safety plan and risk assessment are required as well as how to educate the consumers that they play a role as part of the food chain.

S49 Novel Technologies for Extended Shelf Life

GENEVIEVE FLOCK: *U.S. Army Combat Capabilities Development Command Soldier Center, Natick, MA, USA*
SEZIN YIGIT: *Mori, Somerville, MA, USA*
BARRETT ANN: *U.S. Army CCDC – Soldier Center, Natick, MA, USA*
DOMINIQUE PACITTO: *U.S. Army CCDC – Soldier Center, Natick, MA, USA*
DAVID HAGEN: *Kuraray America, Inc., Pasadena, TX, USA*

Approximately half of all fresh food grown in the U.S. ends up as food waste. In order to increase shelf-life, traditional food preservation and sterilization methods have been used, however, processes such as retort have been shown to degrade food quality as well as be time-consuming and costly. Consumers' increasing preference for high-quality, shelf-stable foods with improved nutritional and organoleptic properties and the food processors quest to find more energy-efficient, high throughput, and cost-effective technologies have led to the development of alternative food processing and packaging technologies. This session will address new strategies being developed to preserve and extend a variety of products for increased and even multiyear shelf life.

S50 Creating Meaningful Quantitative Microbial Risk Assessments Using Imperfect Data

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MAARTEN NAUTA: *National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark, Denmark*
DONALD W. SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
LEON GORRIS: *Food Safety Expert, Nijmegen, Netherlands, Netherlands*
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Quantitative microbial risk assessment (QMRA) modeling aids in evaluating the impacts of food safety interventions on public health. Regulatory agencies and food processing firms use QMRA to base their decisions in a more systematic approach. QMRAs usually integrate experimental data, related to pathogen contamination prevalence and concentration, obtained along the lines of harvest/production, processing, storage, along with consumption data tied to a dose-response model, to produce quantified risk estimates for final product safety. All these steps require diverse, high-quality data to provide the most accurate model outputs to capture the real-world scenario. For instance, although there is frequent occurrence of foodborne disease outbreaks associated with fresh produce, production systems for fresh produce rarely test positive for foodborne pathogens. Therefore, it becomes difficult to conduct risk assessments needed to evaluate all potential sources of contamination and set risk-based standards to mitigate contamination. Potential solutions for such a data starved problem are either making assumptions based on scientific evidence or computer simulation of an outbreak scenario. Scenario simulation involves assuming a range for the unknown variable and evaluating the impact of the changes on risk outputs and can be a valuable tool in determining the impact of the individual variable on final risk estimates, and its overall importance in the QMRA. This symposium will describe how to overcome data challenges in estimating exposure, dose-response, or consumer behavior while building a meaningful QMRA for meat, produce and ready-to-eat (RTE) food products for which it is practically impossible to conduct risk assessments in real life. It will conclude with what tools are currently available to manage data deficit and how the software industry is approaching these challenges. We bring academic researchers and an industry professional to provide diverse perspectives on the use of imperfect data to create meaningful QMRAs.

S51 Inspire Future Consumers through Formal and Informal Food Safety Education

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YAOHUA (BETTY) FENG: *Purdue University, West Lafayette, IN, USA*
KALMIA KNIEL: *University of Delaware, Newark, DE, USA*
BARBARA CHAMBERLIN: *New Mexico State University, Las Cruces, NM, USA*
GREG MCCURDY: *Salem Community Schools (Retired), Salem, IN, USA*

The microbiological safety of our food supply has increased markedly in the last few decades. Unfortunately, consumers have taken this advancement in food safety for granted. Therefore, the overall goal of this symposium is to increase public recognition of potential risks associated with processed food consumption. Young generation are known to be future consumers. Improving their food safety knowledge and food handling practices is shaping the future of consumer food safety. Studies showed that young people often lack knowledge of and fail to practice safe food handling. Sustained efforts have been made by extension educators, school teachers, and researchers to develop engaging and effective food safety education programs for youth. Those programs range from in-classroom formal curricula to informal modules, including games, videos, and social media engagement. Nevertheless, program evaluation is the key to document the impact and assess the effectiveness. Some programs are evaluated by self-reported knowledge and behavior, others by mixed methods or observation. The discrepancies between self reported and the actual behaviors can be addressed by observation. This symposium will provide a discussion on challenges and high priority strategies to inspire youth with engaging and integrated programs. The session will also shed light on new ways to evaluate program effectiveness.

S52 "One Health" Syst-Omics Approach to Combat *Campylobacter* in Agri-Food Chain

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MICHAEL KONKEL: *Washington State University, Pullman, WA, USA*

Campylobacter has been recognized as a major foodborne pathogen in many parts of the world. Campylobacteriosis is the most frequently reported bacterial foodborne illness in North America, outnumbering the reported cases of *Listeria*, *Salmonella*, and *Escherichia coli* infections combined. *Campylobacter* contamination also has a significant economic impact on the poultry industry as different domesticated and wild birds are its natural reservoirs. Understanding this microbe in the context of North America's agro-ecosystem and poultry production chain is vital to monitor, prevent and control *Campylobacter*-associated food contamination. For this symposium in IAFP 2020, we aim to summarize and discuss the most recent studies of using multi-pronged approaches by developing and integrating novel characterization, detection and biocontrol tools to combat *Campylobacter* contamination in the agri-food chain. We will firstly introduce the development and application of whole genome sequencing and analysis to characterize the clinical and environmental *Campylobacter* isolates collected in both Canada and USA. We will then investigate two major protection models that *Campylobacter* can receive so as to be tolerant to the unfavorable environmental conditions, namely bacterial biofilms and bacterial dormancy state (i.e., viable-but-non-culturable and persisters). Furthermore, the recent advances in the studies of *Campylobacter* antimicrobial resistance and its control in the agri-food chain will be systematically introduced. Finally, novel vaccination strategies that have been developed during the past decade will be summarized.

Some of these vaccinations have been successfully validated to reduce the load of *Campylobacter* in the poultry gastrointestinal tract. The outcome of this symposium will provide significant social and economic benefits to poultry and meat industry by 1) offering increased efficiency, accuracy and affordability of *Campylobacter* surveillance and detection and 2) developing novel strategies to reduce *Campylobacter* in raw poultry products, which will enhance poultry and food safety.

S53 Communicating with Consumers about Outbreaks and Food Safety: Research-based Approaches

AARON LAVALLEE: *USDA Food Safety and Inspection Service, Washington, DC, USA*
AARON LAVALLEE: *USDA Food Safety and Inspection Service, Washington, DC, USA*
FANFAN WU: *Food and Drug Administration, Washington, DC, USA*
MISHA ROBYN: *Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*

As public health agencies, the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration and U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) have a responsibility to share information about foodborne illness outbreaks with the public. In partnership with state and local health departments and other public health advocates, these federal agencies seek to balance transparent information-sharing with the public and avoiding unnecessary panic when limited information may be available. There are advantages and disadvantages to consider when determining when and how these communications are shared. However, there is limited contemporary research available related to public communications on foodborne illness outbreaks and food safety alerts. To address this knowledge gap, CDC, FDA and FSIS conducted qualitative and quantitative research on foodborne illness outbreak and food safety alert communications. CDC's focus groups tested how consumers currently perceive outbreak communications and what improvements they would suggest. FDA tested a number of food safety alert and outbreak advisory communication templates with consumers to determine which was more successful in imparting desired information; FDA also developed population estimates on consumer behaviors that will inform food safety alert and outbreak advisory communications. USDA-FSIS fielded a nationally representative survey to estimate fatigue surrounding messages of foodborne illness outbreaks and food recalls, and to determine how, when, and through what platforms consumers prefer to receive this information. This session will discuss the findings, implications and potential uses of the communications research conducted by the respective public health agency.

S54 How to Meet the Food Safety Concerns Surrounding Meat Alternatives

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ANTHONY FLOOD: *IFIC, Washington, DC, USA*
STEPHEN GROVE: *Nestlé Development Centre – Solon, Solon, OH, USA*
JOSEPH BAUMERT: *University of Nebraska-Lincoln, Lincoln, NE, USA*

Consumers have become increasingly aware of their environmental/carbon footprint, the sustainability of the food system, and their health. To accommodate the rising number of consumers aiming to incorporate meatless products into their diets, alternative meat-like protein substitutes have become increasingly available in foodservice and retail. Meatless options include both plant-based alternatives that mimic traditional meat and cell-cultured meat products grown in a laboratory environment. Recent studies by Dupont and Mintel found that 52% of U.S. consumers and 65% of citizens worldwide, are adopting meatless alternatives. 33% of U.S. consumers have expressed interest in increasing plant-based protein consumption for 2020. As alternative meat-based proteins are inherently different from traditional meat products, safety and processing concerns are unique. Production practices, technologies, and regulations guiding alternative meat proteins may be different between plant- (e.g. legumes, nuts, produce, etc.) or fungi-based meat alternatives. Food safety issues associated with plant-based alternatives include the creation of new proteins that may have allergenic potential and/or contamination by stress-tolerant bacteria. Ingredient sourcing, manufacturing, and distribution must also be considered. This symposia will seek to identify and address safety, regulatory, and processing concerns of alternative meat products by: (a) reviewing market trends and current alternative meat products available; (b) identifying safety issues inherent to these products; (c) discussing the role of producers, academics/educators, and regulators in addressing and ensuring safe production; and (d) identifying knowledge gaps.

S55 Foodborne Parasites of Emerging Importance

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Parasites have been overlooked as foodborne pathogens when compared to viruses and bacteria. However, several parasites are now emerging as foodborne threats. This is partially due to the identification of some parasites in geographical regions where they were not previously found (e.g., *Angiostrongylus cantonensis*), unexpected increases in parasitic disease incidence in some areas (*Cyclospora cayetanensis*), and/or to the fact that some parasites are now linked to food consumption, which previously did not seem to have a main role in the transmission of the parasite (*Cryptosporidium parvum, Trypanosoma cruzi*). Parasites have complex cycles and are very well adapted to the environment for survival. Parasitic stages usually show high resistance to temperatures and disinfectants; some also have low infective doses, and therefore, constitute a major risk for consumers. The presence of parasites in ready-to-eat produce indicates that sanitation processes currently used for ready to eat produce do not guarantee a product free of parasites of fecal origin. In addition, the change in our dietary habits and the increased access to the global food market are increasing our contact with these emergent parasites which need to be considered as a major public health threat. This symposium will discuss the diagnosis and detection of specific foodborne parasites in food and water and the mitigation approaches to prevent contamination.

S56 Breeding Crops for Enhanced Food Safety

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The demand for nutritious and safe food will likely increase as the benefits of healthy diets are realized, and standards of living and changes in agricultural production reshape supply and consumption patterns. While a wide variety of fresh and minimally processed produce is becoming available year-round, the number of foodborne disease outbreaks related to its consumption has been increasing. Beyond the burden on public health, disease outbreaks negatively affect the industry and consumers (a single outbreak can cost >$74 million). In addition, health concerns exist due to the consumption of mycotoxins produced by fungi that can be found infecting seeds, grains and nuts. The health burdens caused to consumers and economic burdens caused to the farmers by the presence of these toxins can be severe.

On June 5-6, 2019, UC Davis hosted the first workshop on Breeding for Enhanced Food Safety to identify knowledge gaps and research priorities in this emerging field. Some genotypes of tomatoes and leafy greens were reported less likely to harbor pathogens than others, suggesting genetic basis for interactions between human pathogens and plants. Manipulations of plant microbiomes, production of secondary metabolites and leaf surface structures can make plant surfaces inhospitable to human pathogens. Potential targets for plant breeding, and strategies for capitalizing on the genetic diversity of crops were identified, which could be the basis of future research to reduce pathogens and mycotoxins in foods.

S57 A Global Perspective on New Generation of Food Processing/Preservation Techniques for Food Safety: Riding the Tides of Clean Labels

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Globalization makes food available to consumers across the world and drives the demand for novel food processing and preservation technologies to ensure safety and quality. Increased demand for healthy foods has led to many changes in the quality and safety of foods and in the current conformation of food ingredients. Processed foods with minimal food additives and either mild thermal or non-thermal treatments are an increasing trend among consumers. These consumer demands have led to the exploitation of alternative food processing and preservation techniques, especially those that could result in clean labels. Government agencies worldwide develop regulatory policies to help manage food labeling and food safety risks to protect public health and alleviate consumer concerns. Consumers have been made aware of the importance of appropriate labeling and microbial hazards associated with misbranding of foods. Severe consequences for violating regulations and policies have also made producers aware of how a product is perceived, binding the entire food industry as one unit. Thus, understanding clean labels and the impact of clean labels on food processing and preservation is essential. This symposium will facilitate a discussion of the challenges and opportunities faced by clean-label driven new technologies for food safety. A strategic plan to advise the adoption will be developed by the speakers and audiences.

S58 *Salmonella* and Ground Beef – Persistent, Recurring, or Emerging Risk?

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ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*
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During 2016–2019, CDC, USDA-FSIS, state and local public health partners investigated multiple outbreaks of *Salmonella* Newport infections linked to ground beef, including the largest ground beef outbreak ever reported in the United States. These recent outbreaks resulted in almost as many illnesses, and more hospitalizations, as all 22 *Salmonella* outbreaks linked to ground beef during the previous 36 year period.

During a 2016–2017 *Salmonella* Newport outbreak, investigators isolated the outbreak strain from sick people, leftover ground beef, and dairy cows. No recall occurred because traceback of case-patient ground beef purchases did not identify a single common supplier; dairy cows were hypothesized to be the source. In 2018–2019, a strain of *Salmonella* Newport that was closely related to the 2016–2017 outbreak by whole genome sequencing was investigated; ground beef was identified as the likely source. Traceback identified a single common supplier, leading to a recall of over 12 million pounds of ground beef products.

This symposium will focus on recent illnesses and outbreaks caused by *Salmonella* contamination of ground beef, including a review of outbreaks and investigational challenges, current *Salmonella* control standards and policies for slaughter and processing facilities, and the latest research on pre- and post-harvest interventions to reduce *Salmonella* contamination.

S59 Microbial Warfare: The Effect of Native Microbial Communities on the Survival, Growth, and Persistence of Foodborne Pathogens Along the Food Processing Continuum

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Ready-to-eat (RTE) foods are a special food safety risk due to the lack of a kill step before consumption. Many RTE foods have a complex microbiome which may inhibit (or enhance) the survival, growth, and/or persistence of contaminating pathogens. While much research has addressed the effect of physicochemical parameters (pH, Aw) on the survival and growth of foodborne pathogens, little effort has gone into understanding how competition with native microflora may affect these pathogenic bacteria.

The mechanisms of competitive exclusion by the native microflora may include competition for nutrients or attachment sites, or the production of antibacterial substances (e.g., bacteriocins). Competitive exclusion of pathogens in foods has been previously reported, however, the mechanisms were not fully uncovered. With the use of high-throughput sequencing, researchers can now probe pathogen-microbiome interactions at a greater depth.

This symposium seeks to explore recent research into how the microbiome may affect the survival, growth and/or persistence of foodborne pathogens along the RTE food processing continuum. Specific topics will include:

1. The impact of the cow udder microbiome on the safety of raw milk used for cheese production,
2. The influence of the plant microbiome on the occurrence and persistence of foodborne pathogens on fresh fruits and vegetables, and
3. The relationship(s) between the house microbiome and the occurrence/persistence of foodborne pathogens in a food processing facility.

These topics will provide new insight to the members on how advances in microbiome research can help identify high-risk environments and understand ecological factors that drive occurrence and persistence of foodborne pathogens.

This symposium is intended for food safety professionals in academia, government, and industry who are interested in how the microbiome influences foodborne pathogens in food commodities. Current microbiome research, as well as a regulatory perspective on the role of the microbiome in food safety, will be discussed.

S60 Linking Predictive Analytics with Artificial Intelligence, Machine Learning, and Other Innovative Technologies to Enhance Risk-based Food Safety Approaches

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HAO PANG: *U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA*
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BOCE ZHANG: *University of Massachusetts, Lowell, Lowell, MA, USA*

Emerging technologies such as Artificial Intelligence (AI) and Machine Learning (ML) have been rapidly expanding in the last few years due to increasing data availability, rapid advancement in analytical algorithms, and improved processing power. As more food safety data becomes rapidly available due to advancements in modern food safety approaches, enhanced monitoring and tracing capabilities, and progress in multidisciplinary research, there is a lot of excitement in leveraging AI and ML in the field of food safety. The overall goal of this symposium is to discuss how to realize the full potential of predictive analytics, AI, and ML approaches across a variety of disciplines to enhance food safety risk-based approaches. Specifically, this session will provide case studies integrating diverse applications of AI and ML in Quantitative Risk Assessment (QRA) and predictive microbiology and an examples of opportunities and challenges of AI and ML for food safety surveillance and monitoring.

S61 Regulatory Testing for Viruses and Parasites: The Crossroads between Public Health and Industry

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RACHEL CHALMERS: *Public Health Wales, Microbiology and Health Protection, Singleton Hospital, Swansea, United Kingdom, United Kingdom*
JAN VINJÉ: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Parasites and viruses have emerged as significant causes of foodborne outbreaks in recent years. This trend has generated increased efforts in the development of new sensitive and specific commercial clinical diagnostic methods, as well as improved regulatory testing methods for detection of viruses and parasites. Thus, molecular methods have emerged as the best choice for that since foodborne viruses and parasites such as norovirus and *Cyclospora cayetanensis* are non-culturable and very challenging to enrich from the food sample. These newly developed methods have been impacting both surveillance and epidemiological investigations, rapidly identifying foods implicated in large and complex outbreaks; e.g., 2018 quick-service related cyclosporiasis outbreaks. However, these methods could also be extremely useful for industry to provide baseline data for pathogen prevalence and for the implementation of mitigation strategies. This symposium will discuss the evolution of regulatory methods for viruses and parasites and how that has impacted epidemiologic investigations and industry.

S62 Alternative Protein Sources for Future Foods: Food Safety Challenges

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CARRIE MCMAHON: *U.S. Food and Drug Administration, Office of Food Additive Safety, College Park, MD, USA*
PAUL MOZDZIAK: *North Carolina State University, Raleigh, NC, USA*
RICHARD GOODMAN: *University of Nebraska, Lincoln, NE, USA*

A growing number of people are turning to alternative protein sources, including plant-based proteins, cell-cultured meats and fish, and other novel protein sources, as a means to satisfy health, environmental or animal welfare concerns. This behavioral change in consumers has led to the rise of alternative protein products on the market. There remain questions related to the impact of these significant trends on our food safety systems and public health. Therefore, this symposium brings together a unique cohort of presenters to discuss potential food safety risks and challenges that need to be addressed. The speakers will provide an overview on how various alternative proteins are derived, identify challenges facing regulatory science, share chemical and microbiological safety challenges, and outline risks around known and novel allergens related to new sources of proteins. After this session, attendees will have a better understanding of potential food safety challenges for alternative protein sources to ensure that the industry continues to provide safe and wholesome food to consumers.

S63 Climate Change: Impacts on Food Safety and What Food Safety Professionals Can Do to Prepare and Respond

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PETER BEN EMBAREK: *World Health Organization, Geneva, Switzerland, Switzerland*
EWEN TODD: *Ewen Todd Consulting, Okemos, MI, USA*
ERIKA AUSTHOF: *University of Arizona, Tucson, AZ, USA*

The impact of global climate change on food safety will be profound. Changes in weather patterns, increased incidence of extreme weather events, and the movement of population as a result of these changes will exacerbate current challenges to food safety and possibly present new ones. Current events, like the devastating hurricanes of 2018 and 2019, and the associated flooding of agricultural lands in the United States present a prescient example of these impacts. In this session, speakers from academia and international working groups will present research identifying and describing some of these challenges, as well as the efforts that are being taken to identify the impacts, prepare for them, and respond to them. In one talk, the overall burden of climate change as a cause of food safety issues in the future and what food safety professionals can do to prepare for and adapt to these challenges. Another talk will present the impact climate change on agriculture and fisheries. Additionally, research and ideas from the WHO-FAO will be presented to contextualize the problem, and the potential impacts of climate change, from a holistic perspective.

S64 Process Validation – Challenges and Best Practices

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ROY BETTS: *Campden BRI, Chipping Campden, United Kingdom, United Kingdom*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands, Netherlands*
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Based on HACCP principles process steps that are used to control hazards need to be validated. Before undergoing a validation there are many points that have to be discussed and agreed upon. That starts with hazard analysis, going further to determine suitable process step(s) for control of the specified hazard, evaluating in detail process conditions and product(s), define adequate performance criteria (e.g., determine required log reductions for specific biological hazards), and agree on the validation approach.

Where some of these points are easier to address and answer - others pose challenges for the industry. Where validation studies are "outsourced," i.e., an external service being used for the validation study, it is still the responsibility of the manufacturer to ensure it fulfills its purpose. Therefore, manufacturers play an active role in validations – and need to have the knowledge to determine the framework of the validation in order to make the right decisions. That is much easier being said than done; although there are many invaluable guidelines/publications out there, they are often either too specific or too general to provide a practical answer to other validations.

This symposium will focus on practical aspects like: How many different products would need to be validated on a line and if/how can they be grouped? How are variabilities in process accounted for? Is there an acceptability threshold? For specific biological hazards, how many log reductions are enough? How to account for biological (strain) variability? Is there a way to determine and calculate adequate log reductions? Is so, how could that be done? Furthermore, a case study will be presented going through challenges which needed to be addressed to ensure successful validations.

S65 Vive La Résistance: Biocide Resistance Strategies among Foodborne Pathogens

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MARIA HOFFMANN: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
BYRON BREHM-STECHER: *Iowa State University, Ames, IA, USA*
YEN-CON HUNG: *University of Georgia, Griffin, GA, USA*

Foodborne pathogens have been implicated in illnesses, outbreaks and product recalls with remarkable consistency despite widespread use of chemical biocides. Biocides commonly used in the food industry include oxidizers, iodophors, organic acids and quaternary ammonium compounds. These chemicals are used individually or in combinations as sprays, washes, foams and mists. The efficacy of chemical biocides against target organisms dictates if they are categorized as sanitizers or disinfectants. Sanitizers are required to result in a 3-log reduction on non-food contact surfaces within 5 minutes or 5-log reduction on food contact surfaces within 30 seconds and disinfectants are required to inactivate all microorganisms within 10 minutes.

Several foodborne pathogens have been reported to transition into a non-culturable state when exposed to biocides and resuscitate upon cessation of the antimicrobial stressor. This phenomenon might result in misinterpretation or overestimation of the antimicrobial efficacy of a biocide. Further, organisms that survive biocide treatment often manifest an increased virulence and have the potential to repopulate and establish environmental persistence. The proposed session will consist of original presentations addressing bacterial resistance strategies that may result in the misinterpretation of biocide efficacy. Discussions will include how the use of biocides at sub-inhibitory concentrations can lead to the development of resistance in foodborne pathogens, even in strains that do not carry mutations or genes that confer resistance to specific biocides. Examples of physiological adaptations in bacteria contributing to persistence including slower growth rates, altered morphotype, including multicellularity and filamentation will also be discussed. A greater understanding of bacterial adaptation to or evasion of biocide action may lead to improved outcomes for the efficacy and interpretation of biocide treatment regimes.

Intended Audience: Food industry personnel, Food safety professionals, academics, regulatory personnel, industrial microbiologists, healthcare professionals

S66 Stay out of the Weeds: Three Simpler Things That Accomplish Produce Safety

MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
CRISTINA MCLAUGHLIN: *U.S. Food and Drug Administration, College Park, MD, USA*
DON STOECKEL: *Cornell University, Geneva, NY, USA*
JEFFREY LEJEUNE: *The Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, Italy*

In November, 2015, FDA published the Produce Safety Rule. Enforcement began in 2019. The produce farming community does not have experience with FDA regulations on par with the food manufacturing industry. As such, FDA chose an integrated approach: in some instances, incorporating measures that are universal and well recognized; in others establishing easily assessed numerical requirements; and elsewhere, establishing mechanistic requirements to inspect and monitor on-farm routes of contamination and take appropriate actions, as necessary. In October, 2018, FDA published draft guidance to provide information about how farms can meet rule requirements. Comments submitted in response indicate that questions remain. What exactly does the term "dropped covered produce" mean? Which crops can or cannot be grow in manure-amended soil? Is fecal material from invertebrates really a Biological Soil Amendment of Animal Origin hazard?

As produce safety professionals engage with the language of the rule, our dialog becomes more complicated and academic. This can obscure important risk-reduction concepts and take energy away from activities and operational decisions that most impact the safety of produce. This symposium focuses on three key factors that are simple to accomplish and provide recognized benefits toward produce safety: 1) reducing contact of ill workers with fresh produce, 2) understanding risks to the quality of agricultural water sources through observation and 3) avoiding direct contamination of produce with animal feces. Our three professionals in risk/benefit analysis, public health epidemiology, sanitary surveys and other processes understand the hazards posed by people, water, and animal populations. Their talks will discuss the benefits of these three simple concepts for produce safety and help emphasize the value of relatively simple, proven practices as a counterbalance to other uncertainties. As understanding of the Produce Safety Rule language continues to evolve, their insights can be applied to further practical recommendations for farms.

S67 Deep Sequencing and Deep Learning: What Can Combining High-throughput Sequencing and Machine Learning Offer the Food Industry?

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XUWEN WIENEKE: *Mérieux NutriSciences, Crete, IL, USA*
XIANGYU DENG: *University of Georgia, Center for Food Safety, Griffin, GA, USA*
JASNA KOVAC: *The Pennsylvania State University, University Park, PA, USA*

Metagenomics and whole genome sequencing (WGS) are becoming valuable and useful tools for food safety and quality research, through the widespread adoption of high-throughput sequencing. Metagenomics can be used to survey the microbiome of a complex sample, such as a food or an environmental sample, and to identify functional genes and metabolic pathways within the microbiome, whereas WGS determines the entire DNA content of a bacterial isolate. Current bioinformatics tools enable researchers to analyze large amounts of DNA sequence data generated by metagenomics and WGS. However, to further enhance food protection, there is a need for more advanced analyses of and deeper learning from those data. Machine learning, a sub-discipline of artificial intelligence, can provide this more advanced analysis and understanding. When combined with metagenomics or WGS data, it is possible to use machine learning to find hidden patterns in those data, which can be used to predict issues, such as spoilage and

pathogen contamination, or to predict attributes, such as the source of an ingredient. For example, machine learning applied to microbiome analysis of wine grapes and their surrounding soil environment predicted the geographical origin and cultivar of grapes with high accuracy. There is also discussion around machine learning approaches being used to accelerate foodborne disease outbreak investigation and to improve source identification and accuracy during outbreaks, but limitations do exist with this approach.

This symposium will introduce machine learning concepts and applications and explore how machine learning applied to metagenomics and WGS can benefit the food industry. The session will address practical considerations when using machine learning, metagenomics, and WGS, separately and together. Use cases on machine learning will be presented, including its use to reveal foodborne pathogen indicators and the potential to predict the presence of pathogens such as *Salmonella* and *Listeria monocytogenes* in agricultural water.

S68 Pesticides in Food – The Big Picture: Registration, Monitoring, Enforcement

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DAVID HRDY: *U.S. EPA, Washington, DC, USA*
JOHN JOHNSTON: *U.S. Department of Agriculture – FSIS, Fort Collins, CO, USA*
CHERYL CLEVELAND: *BASF, Research Triangle Park, NC, USA*

Pesticides are central to modern food systems, and a range of federal and state agencies work together to ensure the safety of U.S. citizens who consume domestic and imported foods. Federal agencies and industry play a vital role in pesticide registration and monitoring. For the upcoming IAFP, we plan to bring experts from a variety of USDA, EPA and FDA programs to discuss how each agency coordinates with foreign governments, the agrochemical industry, and domestic and foreign food producers to provide continuous oversight of pesticides in foods. This panel will discuss pesticide registration, monitoring, and enforcement - a big picture of how the U.S. government coordinates the complex task of approving pesticides for use in the U.S., monitoring pesticide concentrations on foods, and enforcing pesticide regulations.

Roundtable Abstracts

RT1 Dirt on Our Boots: What We've Learned after More Than a Season of Produce Safety Rule Inspections

BOB EHART: *National Association of State Departments of Agriculture, Arlington, VA, USA*
SURESH DECOSTA: *Lipman Family Farms, Immokale, FL, USA*
BYRON BEERBOWER: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
ANITA MACMULLAN: *North Carolina Department of Agriculture and Consumer Services, Raleigh, NC, USA*
DAIN SATTERWHITE: *Kentucky Department of Agriculture, Frankfort, KY, USA*
WESLEY KLINE: *Rutgers Cooperative Extension, Millville, NJ, USA*
DONNA LYNN BROWNE: *Naturipe Farms LLC, Salinas, CA, USA*

The Food Safety Modernization Act was signed into law in 2011 which granted the U.S. Food and Drug Administration authority to regulate on-farm produce safety under the "Produce Safety Rule" (PSR), which was published in 2015. The earliest mandated implementation of the PSR for large farms began in January 2018, but inspections were delayed until the spring of 2019 to give farms, and regulators, more time to prepare. After years of hearing about the PSR, there is finally some movement around implementation. Now that the first season of domestic inspections is complete, what have we learned? After all this time, how prepared are the farms in meeting these new requirements? What challenges or surprises have been encountered? Have the expectations from both farmers and regulators been met? The purpose of this roundtable is to bring in the "boots on the ground" who have played key roles in implementing the PSR on U.S. farms during the initial seasons of PSR inspections to reflect on the early impact of PSR implementation and how to move forward from here. The roundtable comprises panelists with extensive and practical experience directly related to on-farm PSR implementation, including panelists from industry, state and federal regulatory bodies, and university extension services.

RT2 It's Complicated, Multi-year and Multi-pathogen Outbreaks in the Era of Whole Genome Sequencing and Culture-independent Diagnostic Tests (CIDTs)

SCOTT HOOD: *Consultant, Shoreview, MN, USA*
LAURA GIERALTOWSKI: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
KARI IRVIN: *U.S. Food and Drug Administration, College Park, MD, USA*

Improvements in the technologies to detect, identify, and characterize foodborne pathogens such as whole genome sequencing (WGS) and culture independent diagnostic tests (CIDTs) have had wide ranging impacts on outbreak investigations, response, and retrospective analysis. These powerful tools are leading to the identification and investigation of more complicated outbreaks, such as those lasting multiple years and with multiple pathogens. These more complicated outbreaks present challenges for retrospective analyses, such as evaluation of trends over time or foodborne illness source attribution, and for industry to develop targeted preventive controls if the pathogen picture is foggy.

WGS is an increasingly cost-effective method of microbial subtyping that dramatically improves the ability to detect and investigate outbreaks. WGS allows isolates to be linked across space and time. For example, in 2015, a number of cases of listeriosis from that year and the year prior were linked to historical isolates in 2010, 2011, and 2012; all of these cases were eventually linked to Blue Bell ice cream products.

Likewise, CIDTs are increasingly used by clinicians to detect foodborne pathogens because they are often cheaper and faster than traditional culture-based methods leading to more immediate patient treatment, but the lack of isolate information hinders the ability of public health officials to identify clusters or outbreaks. Multiplex panels can identify multiple pathogens in a single test, leading to outbreaks with more complicated etiology.

This roundtable panel will discuss how outbreak investigations are changing under this new era of WGS and CIDTs, and the implications of both for regulatory agencies, industry, and consumers.

RT3 Pre-harvest Food Safety Challenges and Research in Developing Economies

EWEN TODD: *Ewen Todd Consulting, Okemos, MI, USA*
BASSAM A. ANNOUS: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
NATALIE DYENSON: *Dole, Charlotte, NC, USA*
SANTOS GARCIA: *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, NL, Mexico, Mexico*
ISSMAT KASSEM: *American University of Beirut, Beirut, Lebanon, Lebanon*

Countries with developing economies continue to play a key role in the production of raw materials for food production. Due to their unique circumstances, developing countries might require particular attention regarding pre-harvest food safety, especially as they enter the global supply chain. Implementing appropriate food safety measures at a pre-harvest level is dependent on extensive food safety infrastructure, research, and education tailored to the given population to ensure food safety. Several initiatives exist across relevant industries highlighting challenges, best practices, measurement tactics, successful tools, and analysis to effectively assess the impact of pre-harvest food safety measures.

This session will focus on strengthening the global scope of IAFP by raising awareness on the following areas of discussion:

The most common pre-harvest food safety challenges observed in developing economies; both in produce and in animals.

Specific cultural, economic, educational, and research needs to implementing food safety measures at the pre-harvest level in developing economies.

Lessons learned: experience with successful pre-harvest food safety measures in developing economies.

The aforementioned topics will be discussed by panelists with experience working in the Middle East and North Africa, Mexico, Africa, and other countries.

RT4 Creating Awareness within IAFP Regarding Food Safety in Africa

ABDOULIE JALLOW: *Food Safety & Quality Authority of the Gambia, Serre Kunda, KMC, Gambia, Gambia*
MOSES GATHURA GICHIA: *Food Safety Consultant, Nairobi, Kenya, Kenya*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia, Ethiopia*
ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture Abeokuta, Abeokuta, Nigeria, Nigeria*
LUCIA ANELICH: *Anelich Consulting, Pretoria, South Africa, South Africa*

During the 2019 IAFP Annual Meeting, the International Food Protection Issues (IFPI) PDG discussed how the PDG and its members could help strengthening food safety in Low- and Middle-Income Countries (LMICs). For a first focus, Africa was chosen since at the global level the region bears

a disproportionally high burden of foodborne disease. In response to a call for speakers through IAFP Connect (Open Forum and IFPI) as well as discussions during IAFP meetings, several food safety experts from Africa committed to participate in a roundtable discussion with the aim to create a better awareness in the IAFP food safety community of the needs and opportunities in terms of bolstering food safety in Africa.

Panel members will jointly illustrate the food safety situation on the Africa continent and/or specific situations within countries and/or trading blocks concerning various aspects such as: foodborne hazards and illnesses, food safety in trade, regulatory and in public health protection contexts, as well as food safety capabilities and innovations.

Individual panel members will focus on what they see as priority needs and opportunities regarding the necessary strengthening and advancement of food safety.

Aim is to initiate an interactive discussion with the audience on potential networking and collaboration between food safety professionals in Africa and the IAFP food safety community.

This RT session proposal relates to "Creating Awareness" in the IAFP community regarding food safety in Africa. It will help assure a better shared understanding within the food safety communities of IAFP and Africa food safety professionals as a basis for further engagement and collaboration. The interactive format will stimulate the interaction between the panel and the audience.

A companion session proposal (S6491: Passport to Food Safety in Low- and Middle-Income Countries: Rationale, Results and Reflections for Recent Research Initiatives) will discuss examples of ongoing collaborations between research in Africa and experts in other parts of the world. Key learnings/insights on food safety in Africa from both sessions will be compiled and used as input in further IAFP (PDG) discussions and engagements. The sessions will also investigate the possibility and stakeholder support for staging an IAFP regional meeting in Africa under the auspices of the African Continental Association for Food Protection.

RT5 A Balancing Act: Minimizing Food Waste While Striving to Maximize Food Safety

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KEVIN SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
ANDY HARIG: *FMI, Washington, DC, DC, USA*
ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*
BRIAN ROE: *The Ohio State University, Columbus, OH, USA*

According to the U.S. Department of Agriculture, nearly a third of the U.S. food supply for human consumption goes uneaten and is discarded at both the retail and consumer levels. There are many reasons why food is wasted. Studies have shown that confusion about the shelf life of foods and the various date labels that appear on packaged foods contributes to food waste. Manufacturers and retailers apply date labels to packaged foods for various reasons, most often for quality assurance and inventory control purposes. While it is rarely the case, many consumers believe that foods are unsafe or otherwise unfit for consumption after the date on a label and therefore discard the product unnecessarily. Concerns about food safety and liability and the limited availability of collection and distribution infrastructures can discourage food donation and the effective recovery and diversion of surplus foods from landfills to those in need. Inefficiencies in food production and distribution systems also contribute to food loss and waste domestically and internationally.

This roundtable will discuss how efforts to minimize food waste interface with efforts to make the U.S. food supply as safe as it can be. Panel members will share the perspective of industry, regulators, economists, and academia on reducing the burden of food waste. Attendees will hear about approaches to addressing food insecurity, economic efficiency, and environmental sustainability while continuing to improve food safety.

RT6 A Practical and Science-based Performance Standard as an Alternative to Zero Tolerance

STEVEN MUSSER: *CFSAN-FDA, College Park, MD, USA*
CATHERINE DONNELLY: *University of Vermont, Burlington, VT, USA*
DONALD W. SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
DEANN AKINS-LEWENTHAL: *Conagra Brands, Omaha, NE, USA*
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Listeria monocytogenes (*Lm*) is a unique pathogen that has challenged global regulatory agencies and food manufacturers alike. On one end, a hazard-based approach that has motivated a zero tolerance policy for the presence of *Lm* in foods and on the other policy that provides industry incentives for aggressive environmental monitoring and elimination of *Lm* on food contact surfaces. Ironically, these policies present a serious incompatibility in industry application, as a zero tolerance standard serves as a strong disincentive to both food contact surface and finished product testing. Even as *Listeria* science, prevalence and risk assessment knowledge for many food categories has grown significantly, *Lm*-related outbreaks continue to occur and the pursuit of public health goals relative to listeriosis incidence has remained challenging. This roundtable will present a practical and science-based regulatory framework that will allow food processors to operate in a proactive and transparent manner and contends with the true public health risks associated with the presence of *Lm* in foods. Specifically, panelists will review risk assessment and modeling studies and discuss alternative regulatory concepts such as application of quantitative *Lm* performance standards, and the benefits and limitations of these approaches.

RT7 What Don't We Know? Cultured Meat vs. Traditional Meat and Fish Food Safety Concerns

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This Roundtable seeks to build upon the baseline presented in the 2019 Symposium "Is Cell-Cultured Meat *Really* Meat?" about how technology is making possible meat production through non-traditional means.

This group of experts will discuss the differences in the production models between traditional agriculture and the production of cell-cultured meat and fish, including how the safety concerns are different and potentially unknown. As cell-cultured meat and fish move from possible to introduction to the consumer market, the question of safety becomes pressing. As technology is scaled up, are the issues the same, or are there additional food safety concerns? Do the current food safety laws adequately protect the public?

RT8 New Insights on Bridging Risk Assessment and Hazard Analysis – How Can We Really Do Both?

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WILLIAM WEISSINGER: *FDA ORA, Chicago, IL, USA*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands, Netherlands*
DONALD W. SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
ROBERT BRACKETT: *Institute for Food Safety and Health, Bedford Park, IL, USA*

This roundtable is a sequel to a 2018 roundtable that drew a large and engaged audience. New insights on how to reconcile differences between risk assessment and hazard analysis will be presented. Hazard analysis is widely used to determine hazards requiring preventive controls and is typically performed in a qualitative/semi-quantitative manner. Risk assessment is typically quantitative, often involves complex mathematical modeling and is often perceived as too difficult to apply. Technical experts and risk managers sometimes find the outcomes of a quantitative risk assessment difficult to understand. Conversely, qualitative hazard analyses may miss significant risks or identify inadequate preventive controls resulting in an ineffective food safety plan. Finding ways to bridge and reconcile these different approaches will foster a risk-reduction mindset, bring clarity on how to best translate risk assessment and hazard analysis results into actual reduction in risk of foodborne illness. This roundtable brings together a panel of six experts with broad and deep knowledge and experience in risk assessment and preventive controls, microbial and chemical hazards, as applied in large and small firms. The organizers and the panel will present brief remarks to spark discussion and the audience will be invited to pose questions. The panel members and audience will share insights and lessons learned on solutions to address underlying issues from lack of knowledge and technical expertise to real-world constraints to make a difference when moving towards application of risk-based preventive controls. The panel discussion will highlight available tools and resources to link quantitative risk assessment and hazard analysis approaches to risk reduction, and suggest methods to integrate more quantitative elements in analyses to strengthen risk-based preventive controls.

RT9 Interpreting Results from Enteric Virus Testing: Can Evidence of Viral Nucleic Acid Serve as an Indicator of Human Fecal Contamination or Defined Public Health Risk?

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Human norovirus is the most common cause of acute gastroenteritis worldwide, and amongst the most prevalent of the foodborne pathogens. Hepatitis A virus, while less common but also transmitted by contaminated foods, has recently surged to epidemic proportions in parts of the developed world. Concerns about these pathogens have led to calls for more routine testing across the food supply chain. However, since wild type stains of these viruses are not culturable, testing relies on the sequential steps of virus concentration and purification, nucleic acid extraction, and amplification, usually by RT-qPCR. The results are expressed as presence or absence of amplified viral nucleic acid, sometimes expressed quantitatively. Since no culture can be obtained, food safety management and regulatory decision-making must be based on evidence of viral nucleic acid alone. However, what is the public health impact associated with the detection of viral nucleic acid, be that in environmental, water, and/or food samples? Making these sorts of interpretations is complicated by factors such as (i) multiple testing protocols that are not well harmonized; (ii) the infectivity dilemma, i.e., the fact that only a (frequently small) proportion of viral particles are actually infectious; (iii) limits of detection and quantification as reflected by Ct values; (iv) the need for definitive confirmation of nucleic acid sequence, frequently when present in low copy number; (v) interpreting the presence of a small fragment of nucleic acid as compared to a full genome; and (vi) the absence of a virus culture. In this roundtable, a panel of global viral experts will discuss their experiences with the methods, data interpretation, and risk-based decision making relative to these issues, with a goal of identifying key issues that need to be addressed to aid in developing guidelines for the most prudent use of virus detection data so as to protect public health while at the same time preventing over-interpretation or pushing the test beyond its technical capabilities.

RT10 Synthesizing Food Defense Programs for FSMA and Third Party Audits

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RAQUEL MAYMIR: *General Mills, Minneapolis, MN, USA*
KARLEIGH BACON: *Kraft Heinz Company, Glenview, IL, USA*
JOEL MARTIN: *Cargill, Inc., Wayzata, MN, USA*
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The Food Safety Modernization Act (FSMA) Intentional Adulteration Rule of the requires certain manufacturing facilities to prepare and implement food defense plans based on vulnerability assessments that identify specific vulnerabilities to insider attackers in the manufacturing process itself. However, the standards audited by third parties including ISO and GFSI remain focused on foundational, facility-wide food defense programs. These standards require risk and threat assessments be performed rather than vulnerability assessments. As a result, the industry finds itself at a tension between different risk assessment theories and in the midst of an evolution of food defense theory.

This disparity precipitates the question "How will the global food industry reconcile these various food defense requirements in an efficient, robust way?" This session will convene a multi-disciplinary panel of food defense experts to discuss best practices and examples for how the industry can meet the many different standards for food defense programs to both comply with the applicable legislation and satisfy customer and third party standards.

RT11 This is How We Do It: Challenges and Strategies for Implementing Water Treatment in the Field

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Because water is one of the most likely routes of pathogen contamination during fruit and vegetable production, the FSMA Produce Safety Rule calls for rigorous water testing in order to verify the microbial quality of surface water that will contact the edible portion of the plant during growing, unless a water treatment method is used. This in addition to recent market-driven pressures, are pushing growers towards treatment of any surface water

that will come into contact with produce prior to harvest. This includes the treatment requirement for surface water coming into contact with produce recently approved in the Leafy Greens Marketing Agreement (LGMA) water metrics. The potential trickle-down consequences of these moves across the produce industry, cannot be overstated. If a grower does explore treating water, they face a myriad of options often with confusing (or conflicting) guidance on the requirements needed for successful implementation considering the array of experience and resources available to them and the variability in source water quality. With limited guidance, water treatment decisions are likely to be unsuccessful and expend both excess time and money without the ultimate outcome of reducing generic *E. coli* and potential pathogen loading within a water source. Under this scenario, the result is little to no reduction in microbiological food safety risk, inconsistent outcomes and potential damage to irrigated crops and long-term soil health. Grower guidance is needed on effective treatment and monitoring strategies to ensure adequate treatment that will reduce environmental impact and will ultimately protect public health. The overall goal of this round table is to discuss the strategies and challenges of applying water treatment standards across the whole produce industry.

RT12 NGS Identification as an Alternative for Classic Microbiological Subtyping Techniques: What Do We Need to Make This Happen?

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DEANN AKINS-LEWENTHAL: *Conagra Brands, Omaha, NE, USA*
THOMAS HAMMACK: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*

Development of sequence-based diagnostic platforms and devices that can detect and characterize foodborne pathogens is occurring at a fast pace, and global efforts to modernize food safety with genomics and bioinformatics have been impressive. It has become available for use in research and reference laboratories, and have been utilized as a real time tool to sequence etiological microorganisms during an outbreak investigation. Although, it is well-recognized that NGS can lead to a faster outbreak response, and produces subtyping and phylogenetic resolution that are quite reliable, there remains to be some challenges and opportunities for the food industry to fully embrace the concept and technology. Can next generation sequencing (NGS) technique become an alternative for identification and characterization of pathogen isolates? The current analytical capacity for pathogen testing used by many commercial laboratories still utilize the rapid molecular or immunoassay detection methods, culturing, identification and subtyping of bacterial isolates. Confirmation of presumptive pathogen results continue to utilize the classic reference methods – biochemical characterization and subtyping techniques, which can take several days or weeks. Use of the traditional cultural detection and subtyping techniques is also preferred in some countries as they are fairly inexpensive. The magnitude of loss to a company, however, can be enormous, if we factor in the costs associated when a production line shuts down due to an unfavorable result of finding a pathogen in a finished food and/or Zone 1 environmental samples. Timely delivery of goods and loss of a company's credibility to meet customer needs could also be at stake. Use of NGS for identification and subtyping promises to drastically reduce the overall cost and effort required to perform conventional test methods if a presumptive result is detected. How can we move pathogen testing into utilizing high-throughput and modern analytical instrumentation? Should characterization, identification and subtyping of pathogens become a regulatory mandate? Can food companies expect ingredient suppliers to take presumptive pathogen detection to full rapid confirmation testing and subtyping? Hear from a panel of experts present their insights on the latest development on sequence-based identification and subtyping and how NGS can be beneficial in responding to outbreak investigation in the global market.

RT13 Beyond the Lab, What Does Culture-independent Diagnostic Tests (CIDTs) Mean for Industry and Public Health Officials?

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CARRIE RIGDON: *Minnesota Department of Agriculture, Saint Paul, MN, USA*
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Going beyond the laboratory science aspects of Culture Independent Diagnostic Tests (CIDTs), the use of these advanced technologies to identify foodborne pathogens in clinical specimens has wide-ranging implications for public health officials, but also on how industry may respond to an outbreak investigation.

When an illness cluster has been identified by public health officials, the laboratory evidence is increasingly comprised of both traditional culture-based methods and CIDTs. While the testing methods have advanced there may be skepticism about the validity of the results due to the lack of "socialization" of such tests. For food regulatory officials, there are considerations for the strength of the epidemiology and laboratory evidence that helps decide which points of exposure to trace back to find the source of contamination in the supply chain. A food company that is identified as the "common source" in that outbreak may have questions about the human illness cases and how strong a link there is to a company's product and how does CIDT results factor into these discussions. Additionally, multiplex panels can identify multiple pathogens in a single test which may be confusing to that company and slow down their internal investigation.

This roundtable panel will discuss how advances in human illness diagnostic testing has both excited and challenged public health officials, and what this means for industry in response to a foodborne illness investigation.

RT14 Mutual Reliance – FDA's Vision for an Integrated Food Safety System

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The growing awareness of the need for food safety regulators to work together has led to the emergence of new models of cooperation. One such model is for FDA and trusted government partners to rely upon each other to increase inspection, surveillance, and response resources.

This model is referred to as mutual reliance which involves the ability of federal and state partners to rely on each other's food safety work as competent regulatory authorities. This work includes regulatory inspections, sample collections, illness outbreak investigations, recalls and consumer complaint data. Mutual reliance is also dependent upon the standardization of laboratory capabilities and competencies to provide confidence in the integrity, scientific validity, and consistency of laboratory analytical data in order to provide assurance and trust in the quality of data submitted to the end user.

To create trust among partners, FDA has collaborated with local and state partners and their associations to develop three sets of regulatory program standards to date for manufactured food, animal feed, and retail food regulatory programs. FDA is also using a similar systems-based approach for establishing reciprocity with other countries looking to export food to the U.S.

These regulatory program standards not only play a critical component for establishing trust among government agencies and advancing mutual reliance, but they play a major role in a national integrated food safety system envisioned by federal, state, and local government agencies.

In addition to the regulatory program standards, three mutual pilots were conducted by federal-state field staff as follows:

1. California Department of Health Services and ORA San Francisco's and Los Angeles' District Offices for egg safety data exchange
2. Wisconsin Department of Agriculture and ORA Minneapolis District Office for data sharing
3. New York Agriculture and Markets and ORA New York District Office for imports and recalls

All three pilots contained a lab component to test several unique elements of mutual reliance.

FDA is committed to building a nationally Integrated Food Safety System (IFSS) of high quality regulatory programs responsible for protecting public health from human and animal foodborne illness and injury. The inclusion of Mutual Reliance programs domestically will greatly advance this commitment and serve as a model for global consideration.

Technical Abstracts

T1-01 Genetic Characterization of Multidrug-resistant *S*. Typhimurium Harboring IncHI2-Class 1 Integron-IS*26*

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* Typhimurium is one of the most important serovars among nontyphoidal *Salmonella*. Multidrug resistant *S*. Typhimurium is prevalent throughout the world. The resistance genes on conjugative plasmids can be transferred under the transposition of mobile genetic elements (MGEs) including insertion sequences and integrons.

Purpose: The purpose of this study was to screen and analyze the structure characteristics of MGEs including IncHI2 plasmid, class 1 integron and IS*26* harbored by *S*. Typhimurium as well as to investigate the composition of resistance genes and the genetic relationship of isolates from different sources.

Methods: *S*. Typhimurium isolates were screened for the presence of plasmid incompatibility group IncHI2, *intI1* integrase and IS*26* by PCR amplification. The whole genome sequencing (WGS) and annotation was carried out among those isolates which IncHI2 plasmid with IS*26*-class 1 integron successfully transconjugated into E. *coli* C600.

Results: Among 255 *S*. Typhimurium isolates, sixty-two isolates were screened for the presence of harboring IncHI2 plasmid (62/255, 24.3%) of which fifteen isolates (15/62, 24.19%) harbored transmissible IncHI2 plasmids as well as IS*26*-*intI1* integrase. WGS analysis revealed that no significant difference ($P > 0.05$) was found among the numbers of antimicrobial resistance gene category in the fifteen isolates, and MLST analysis revealed ST34 was predominant. Four different complex IS-class 1 integron arrangement patterns were identified in fifteen harboring multidrug resistance region IncHI2 plasmids, of which three complex IS*26*-class 1 integron arrangements were characterized, including *aac(6')-Ib-cr, bla*$_{\text{OXA-1, catB3, arr-3, dfrA12}}$ and △*aadA* (5'-153bp truncated) resistance genes. The other one was IS*256*-class 1 integron arrangement (12/15, 80.0%) with *estX-psp-aadA2-cmlA1-aadA1* variable region gene cassette arrays.

Significance: IncHI2-IS*26*-class 1 integron was prevalent in *S*. Typhimurium. Fifteen *S*. Typhimurium isolates containing transmissible IncHI2 plasmid have a similar evolutionary origin. Horizontal transfer of multiple resistance genes may contribute to the spread of multidrug resistance *S*. Typhimurium.

T1-02 Influence of Acid Adaptation, Cold Adaptation on Barotolerance on Survival *of E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. during HPP Treatment of Apple Juice

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❖ ***Developing Scientist Entrant***

Introduction: High pressure processed (HPP) juice validation requires a 5-log reduction of the pertinent microorganism to comply with FDA Juice HACCP. Currently, there is no standardization among validation protocols for bacterial strain selection and preparation or parameters contributing to an effective process.

Purpose: Compare HPP inactivation and post-HPP recovery of matrix-adapted *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in apple juice using different cell preparation methods and treatment pressures.

Methods: Bacterial strains were grown using three different conditions: neutral, cold-adapted, and acid-adapted. Cold-adapted cells were prepared at 17°C. Acid-adapted cells were prepared in either intermediate pH TSBYE or in TSBYE with 1.00% glucose. Individual bacterial strains were inoculated into apple juice (pH 3.50 ± 0.20) at approximately 6.00 log CFU/mL and treated at pressures of 200-600 MPa (180 s, 4°C initial). Inoculated, untreated controls were included. Analyses were conducted 0 h, 24 h and 48 h (4°C storage) post-HPP on TSAYE.

Results: From 0 h analyses, <1.00-log reduction of neutral and acid-adapted *E. coli* O157:H7 TW14359 and approximately 2.00-log reduction of *E. coli* O157:H7 SEA13B88 were observed at 600 MPa. Reduction of acid-adapted *E. coli* O157:H7 TW14359 was observed (3.94 ± 0.22 log) only after 48 h cold storage. At 600 MPa, acid-adapted *S.* Cubana showed significant resistance ($P < 0.05$) (2.13 ± 0.13 log reduction) compared to neutral grown cells and *S.* Anatum. Cold-adapted *E. coli* O157:H7 and *Salmonella* strains showed significant reductions from all pressure levels. For all cell preparation methods, 5.00-log reductions of *L. monocytogenes* CDC and MAD328 were achieved at 0 h post-HPP, while 5.00-log reductions of *Salmonella* strains (500 MPa) and *E. coli* SEA13B88 (600 MPa) were achieved after 24 h cold storage. Cells prepared with glucose (acid-adapted) were significantly barotolerant for both *E. coli* O157:H7 strains.

Significance: These results suggest the efficacy of HPP inactivation during validation studies is influenced by bacterial strain preparation method and post-HPP recovery time.

T1-03 Construction of a Surface-scanning Detection System for the Direct and Automatic Detection of *Salmonella* Typhimurium on Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Rapid microbial methods including PCR, ELISA, and biosensor have been developed for the detection of foodborne pathogens. However, they still require complicated and/or tedious sample preparation procedures. Minimization of these redundant procedures is necessarily required for using as on-site applicable detection method.

Purpose: The purpose of this study was to construct the surface-scanning detection system combined with *S*. Typhimurium-immobilized sensor for the direct and automatic detection of *S*. Typhimurium on fresh produces.

Methods: Sensor and detector were fabricated with dicing and deposition, and microelectronic fabrication methods, respectively. The detector surrounded by magnetic boards was connected with sample stage, joystick and support jack for the automatic movement of the system. After dividing the zone of detector, a sensor was placed. A sensor was also placed on a surface of apple and the signal amplitude of sensor was measured by moving the detector for the determination of appropriate distance between sensor and detector. Finally, *S*. Typhimurium-specific phage-immobilized sensor and phage-devoid sensor were placed on the surface of apple contaminated with serially diluted *S*. Typhimurium for the measurement of resonant frequency shift of sensor.

Results: When sensor was placed on the zone of A and C, the system was able to detect the resonant frequency of sensor. As the distance between sensor and detector increased, the signal amplitude of sensor decreased with the ratio of approximately -21.07 Hz/mm. The maximum distance between detector and sensor was determined to be 1.3 mm. The resonant frequency shift of each phage-immobilized sensor increased significantly according to the increase of *S*. Typhimurium ($P < 0.05$). Detection limit and sensitivity were determined to be 2.17 ± 0.31 log CFU/mL and 531.00 ± 68.84 Hz/log CFU, respectively.

Significance: This study demonstrated the successful construction of surface-scanning detection system for direct and automatic detection of *S*. Typhimurium on fresh produces.

T1-04 Rapid Detection of Pathogenic Bacteria Using Engineered Bacteriophage

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❖ ***Developing Scientist Entrant***

Introduction: The limitations of pathogen detection in the food industry include long turnaround time for the conventional methods, complexity of sample preparation for microbial analysis, and high-cost of advanced instruments. Bacteriophage-based bacteria detection methods offer rapid detection of specific bacteria. Genetically modified bacteriophage induced expression of alkaline phosphatase allows sensitive fluorescent and colorimetric detection of pathogenic bacteria applicable for detection of bacteria in complex food matrices.

Purpose: The purpose of this study was to develop isolation free rapid bacteria detection methods for fresh produce and beverages based on engineered bacteriophage induced alkaline phosphatase fluorescent and colorimetric assays.

Methods: *E. coli* BL21 was inoculated on beverage and fresh produce samples. Without isolation of bacteria, the food samples were added to the bacterial enrichment media. Engineered bacteriophage T7 carrying *phoA* gene was added to the enrichment media for infection and alkaline phosphatase expression of bacteria. The samples were then filtered through a 0.2-micron polycarbonate filter to capture the infected bacteria. Fluorescent substrate (ELF-97) and colorimetric substrate (NBT/BCIP) of alkaline phosphatase were directly added onto the filter. Upon interactions with the released enzymes, the substrate converts from soluble to precipitated form enabling rapid detection using fluorescence or colorimetric measurements as well as visual analysis of colored products. All experiments were done in triplicate. For image analysis, the total number of 15 images were analyzed ($N = 15$). The color values were measured at 5 random positions on the filter and the mean and standard deviation values were calculated within the samples.

Results: The detection method using the fluorescent substrate successfully detected *E. coli* 10^2 CFU/mL with 4 hours of enrichment. This method has been verified in beverage matrices including coconut water and apple juice. The detection limit of the method using the colorimetric substrate was 10 CFU/mL of *E. coli* in coconut water and 10^2 CFU/g of *E. coli* on baby spinach leaves with 5 hours of enrichment.

Significance: The results demonstrate the effectiveness of the bacteriophage-based bacterial detection methods to rapidly detect specific target bacteria in complex food matrices with high sensitivity as this technique offers low detection limit of 10-10^2 CFU/mL of bacteria. The results also illustrate the potential for industrial translation.

T1-05 Evaluation of the Neogen Soleris® *Enterobacteriaceae* for Rapid Detection of *Enterobacteriaceae* in Dairy Products

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Introduction: Advances in microbiological analysis has enabled the rapid detection of dairy indicator organisms such as *Enterobacteriaceae*. The commercial availability of the automated Neogen Soleris® *Enterobacteriaceae* system offers laboratories a potential reduction in time-to-results from 3 days for culture-based methods to 18 h.

Purpose: This study evaluated the Neogen Soleris® system to detect *Enterobacteriaceae* in dairy products at ≥ 10 CFU/g as stated in EU microbiological criteria 2073 for pasteurized dairy products. The Soleris® S2-EBAC9 vial dilute to specification protocol is a semi-quantitative method which enables the detection of *Enterobacteriaceae* at defined thresholds.

Methods: The performance of Soleris® *Enterobacteriaceae* S2-EBAC9 vials was compared to the direct plating reference method ISO 21528-2:2017 following the validation procedure ISO 16140-2 (2016). A single plate of the reference agar was used with the presence of one or more colonies being equivalent to the detection of *Enterobacteriaceae* at >10 CFU/mL. This approach enabled the plate count to be used as a qualitative rather than a quantitative result.

Results: Data from the study revealed that the Soleris® S2-EBAC9 vials were 100% specific and 100% selective for *Enterobacteriaceae*, with a false positive ratio of 0% in the sensitivity study. The results also showed that Soleris® S2-EBAC9 vials were more sensitive than ISO 21528-2:2017 (in the relative limit of detection study) and no significant differences was seen between results obtained by reference method and Soleris® S2-EBAC9 vials in the interlaboratory trial.

Significance: Neogen Soleris® *Enterobacteriaceae* S2-EBAC9 vials offers a novel approach for *Enterobacteriaceae* detection in dairy products to meet legislative requirements, with equivalent performance to ISO 21528-2:2017. This is the first MicroVal certification of a semi-quantitative method as a qualitative method using the independent validation method protocol ISO 16140-2 (2016).

T2-01 Mold Remediation in Cannabis for the Food and Beverage Industry

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Introduction: The industry has strict limits for allowable levels of molds, yeasts, coliforms, and bacteria on cannabis and hemp plant matter, and there is need for technology to better eliminate such organisms.

Purpose: To reduce microbial counts on cannabis, so that contamination levels are under the allowable levels, if not eliminated completely.

Methods: The method of contamination control tested is chlorine dioxide (CD) gas. Cannabis crop was placed in a shipping container and treated with a dosage of chlorine dioxide gas at a level of 720 ppm hours. The gas chamber was then aerated of the gas, and product was able to be sent for lab testing.

Results: The usage of chlorine dioxide gas resulted in a 93.33% decrease on TYM, eliminated coliforms, and eliminated gram negative bacteria. The gas decontamination process also did not adjust the terpene levels significantly which would possibly be a sign of degradation in quality. Initial levels of THC were 17.25%, Terpenes 1.2%, TYM 420,000 CFU, Coliforms 200 CFU, and Gram-Negative Bacteria 400 CFU. After exposure levels of THC were 18.25%, Terpenes 1.17%, TYM 28,000 CFU, Coliforms 0 CFU, and Gram-Negative Bacteria 0 CFU. This testing was completed using a concentration of chlorine dioxide gas at 1 mg/L at dosage of 720 ppm hours.

Significance: With an increasing presence recreationally and numerous mainstream food and beverage companies dipping their toes into cannabis-infused products, the microbial counts on cannabis and hemp plant matter need to be closely monitored to ensure the safety of consumers. Levels of CFUs

and overall quality are extremely strict, with failing lab tests resulting in thousands of dollars lost if cannabis cannot be sufficiently remediated. Chlorine dioxide gas is proving to be an effective solution. The CD dosage can be monitored and adjusted to provide decontamination at a level to meet state restrictions.

T2-02 Application of Eugenol Nanoemulsion for Controlling *Listeria monocytogenes* Biofilms in Food Processing Environment

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (LM) biofilms are a significant food safety hazard in the food processing environment.

Purpose: The efficacy of eugenol nanoemulsion (EGNE) in reducing LM biofilm formation and killing mature biofilm on stainless steel surface at ambient (25ºC) and refrigeration abuse (10ºC) temperature was investigated.

Methods: EGNE consisting of Eugenol-1.25%, Gum-Arabic-0.5%, Lecithin-0.5%, and Ethanol 16.67% (co-surfactant) was prepared by high-speed homogenization and sonication. For investigating EGNE efficacy to inhibit biofilm formation, LM Scott-A (~10^6 CFU/mL) was inoculated on stainless steel coupons with or without the presence of sub-inhibitory concentrations of EGNE (600, 700 ppm) and allowed to form biofilms at 25°C for 2 days or 10°C for 16 days. The biofilms associated LM were enumerated using a bead-based quantification assay and plating on Oxford and TSA agar. For studying inactivation efficacy of EGNE, LM Scott-A biofilms were developed as described above followed by EGNE treatments (2,000, 2,750, 3,500 ppm) for 1, 5, 15, 30 or 60 minutes and LM enumeration. All experiments had duplicate samples, repeated twice and data were analyzed using one-way ANOVA.

Results: EGNE had a particle size of 96.39 nm, Poly Dispersity Index of 0.267 and a high negative surface charge. At 700 ppm, EGNE inhibited LM biofilm formation by at least 1.5 log CFU/mL in 48 h at 25°C (control ~ 6.0 log CFU/mL) and ~1 log CFU/mL on 16th days of incubation at 10°C (control ~ 5.5 log CFU/mL; $P < 0.05$). EGNE, at 2,000 ppm, completely inactivated (detection limit-1 log CFU/mL) LM in biofilm developed at 25°C or 10°C by 30 minutes of treatment time ($P < 0.05$). Higher concentrations of EGNE (2,750, 3,500 ppm) completely inactivated biofilm associated LM (25°C and 10°C) by 1 minute of treatment ($P < 0.05$).

Significance: EGNE could potentially be used as a natural sanitizer to control LM biofilms in food processing environments.

T2-03 Efficacy of Sodium Hypochlorite against Quaternary Ammonium Compound (QAC)-tolerant *Pseudomonas aeruginosa* and *Listeria monocytogenes* Co-culture Biofilms

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❖ ***Developing Scientist Entrant***

Introduction: Because biofilms facilitate prolonged survival of microorganisms in food production environments, repeated exposure to chemical sanitizers may enhance their tolerance to subsequent treatments.

Purpose: To determine if QAC- tolerant *Pseudomonas aeruginosa* (*Pa*) and *Listeria monocytogenes* (*Lm*) biofilms demonstrate cross-tolerance to sodium hypochlorite (SH).

Methods: Co-culture biofilms were generated in the CDC Biofilm Reactor on stainless steel coupons at 21°C and treated daily with a commercially available QAC sanitizer (1000 ppm, 10 minutes) under agitation. Following six successive QAC exposures, biofilms were then challenged with sodium hypochlorite (1000 ppm, 10 minutes). *P.aeruginosa* and *Lm* concentrations were determined using standard cultural methods by plating on TSA and MOX, respectively. Biofilm thickness was quantified using confocal microscopy and IMARIS 9.0 software.

Results: *P. aeruginosa* sensitivity to QAC diminished with repeated exposure, evidenced by 1.2 ± 0.2 and 0.3 ± 0.2 log CFU/coupon reductions after one and six successive QAC exposures, respectively ($P < 0.0001$). *L. monocytogenes* was reduced by 1.3 ± 0.1 and 0.5 ± 0.3 log CFU/coupon after one and six successive QAC exposures, respectively ($P > 0.05$). Sodium hypochlorite inactivated QAC-tolerant *Pa* in co-culture biofilms by 3.2 ± 0.3 log CFU/coupon but had no discernable effect on *Lm* (-0.1 ± 0.3 log CFU/coupon reduction). Regenerated biofilms increased in thickness from 126 ± 12 to 441 ± 32 mm after one and six QAC exposures respectively ($P < 0.05$), even though total viable cell counts were stable. This suggests an extracellular matrix-driven QAC tolerance phenotype.

Significance: Cross-tolerance between QAC and SH sanitizers was not observed in *Pa* and *Lm* co-culture biofilms. Implications of QAC tolerance in *Pa* and its protective effect towards *Lm* in co-culture biofilms remains under investigation.

T2-04 Efficacy of Nanobubbles in Removing Biofilms Formed by *Escherichia coli* O157:H7, *Vibrio parahaemolyticus*, and *Listeria innocua*

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Introduction: Biofilm formation in food processing facilities is recognized as one of the major food safety concerns. Compared to planktonic cells, biofilms are more resistant to the environmental stresses and disinfectants; therefore, decontamination of biofilms in food processing facilities is an important food safety challenge.

Purpose: This study was initiated to develop a novel antibiofilm approach by combining nanobubble (NB) and electrolyzed water (EW) technologies to remove different microbial biofilms including *Escherichia coli* O157:H7, *Vibrio parahaemolyticus*, and *Listeria innocua* on plastic and stainless-steel (SS) surfaces.

Methods: Biofilms were grown on sterile plastic and SS disc coupons. EW containing 10 ppm free chlorine concentration, NB (Dissolved oxygen = 40 mg/L), and a combination of NB and EW (NBEW) (10 ppm free chlorine concentration) were used to compare the efficacy of these solutions to remove bacterial biofilms. Bacteria were recovered on tryptic soy agar supplemented with rifampicin (50 µg/mL), PALCAM agar, and TCBS for *E. coli*, *L. innocua*, and *V. parahaemolyticus*, respectively, and compared using ANOVA.

Results: NB alone and in combination with EW, significantly reduced bacterial biofilms on plastic and SS coupons ($P < 0.05$). *V. parahaemolyticus* biofilm was the most sensitive and *L. innocua* biofilm was the most resistant biofilm. NB alone, was able to completely (more than 7-log reduction) remove *V. parahaemolyticus* biofilm on both plastic and SS coupons after 5 min. However, NB reduced 1 to 3 log CFU/cm^2 of *E. coli* and *L. innocua* biofilms, and complete reduction (more than 6-log reduction) only observed after 5 min exposing to the combined NB and EW treatments (NBEW).

Significance: This study demonstrates that NB could be a reliable technology for removing microbial biofilms from surfaces and enhancing the efficacy of conventional sanitizers.

T2-05 Formulation Matters – Efficacy of Hand Sanitizers against Human Norovirus is Highly Variable

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Introduction: Human norovirus (HNV) remains the leading cause of foodborne illness and poor hand hygiene is known to contribute to the spread of disease. While hand washing is considered the gold standard in hand hygiene, there remains a need for more efficacious hand sanitizers to aid in controlling the spread of HNV.

Purpose: To conduct an extensive comparison of hand sanitizers and a 60% ethanol benchmark for their efficacy against HNV using the ASTM E1838-17 fingerpad method.

Methods: The hands of ten volunteers were inoculated with 10 µL of HNV GII.4 Sydney positive stool (a 20% suspension in PBS), and the efficacy of 60% ethanol and seven representative, commercially-available hand sanitizers was determined using the E1838-17 method, which includes a rubbing step. Remaining HNV after treatment was enumerated using RT-qPCR with an RNase pre-treatment. Log reduction (LR) of HNV was determined by comparing to the input virus control and expressed as LR of HNV genome equivalent copies (GEC).

Results: After a 30 s exposure, the 60% ethanol benchmark produced a 1.5±0.5 LR of HNV GEC using the E1838-17 method. LR of HNV GEC ranged from a low of 0.4±0.2 to a high of 3.2±0.3 for the seven hand sanitizer products. Of those, only one commercially-available hand sanitizer resulted in a significantly greater reduction in HNV GEC compared to the 60% ethanol benchmark ($P < 0.05$).

Significance: These data show the highly variable efficacy of hand sanitizers against HNV, and that product formulation matters. The best hand sanitizer tested resulted in a 3.2±0.3 LR of HNV GEC. Additional studies benchmarking hand sanitizers against hand washing and hand hygiene regimens using methods based on ASTM E1838-17 are in progress.

T2-06 Tracing Back Food Spoiling Bacteria during Enzymatic Cleaning with 16S rDNA Metagenetic

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Introduction: *Leuconostoc* spp. was described as spoiling bacteria in several food products. These bacteria have the particularity to grow very quickly and to dominate the bacterial flora throughout the shelf life even if the initial concentration is very low. *Leuconostoc* spp. is present in the environment of the food industry and could be harbored in biofilms. The biofilms are very resistant to conventional sanitizing methods and new strategies are proposed to eradicate them such as enzymatic cleaning.

Purpose: This study aims to analyze the microbial flora from the environment of a food process during conventional and enzymatic cleaning to identify sources of spoiling bacteria and their impacts on the food products.

Methods: Conventional and enzymatic cleaning was applied in a food company producing Italian dishes during 3 months. A total of 244 samples were analyzed, including surface samples, cleaning-in-place systems and food products throughout the shelf life by classical microbiology and 16S rDNA metagenetics. Statistical analysis was carried out with the R project software with different packages.

Results: During conventional cleaning, *Leuconostoc* spp. became the most predominant bacteria at the end of the shelf life (55.0±24.1%). After the implementation of the enzymatic cleaning, the proportion of this bacteria is reduced in the food products at the end of the shelf life (0.62 ±1.23 %) with a significant difference between conventional and enzymatic cleaning ($P < 0.05$). *Leuconostoc* spp. was also detected on some equipment during conventional cleaning and was reduced after implementation of enzymatic cleaning ($P < 0.05$).

Significance: The 16S rDNA metagenetic analysis is a useful tool to identify the source of contamination by spoiling bacteria from the food equipment. Enzymatic cleaning reduces the proportion of spoiling bacteria from installations and improves the microbial quality of the food products.

T3-01 Temporal Distribution and Characterization of *Listeria monocytogenes* and *Listeria* species in a Produce Packinghouse

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❖ ***Developing Scientist Entrant***

Introduction: The residence of *Listeria monocytogenes* in the postharvest environment represents a risk for produce contamination.

Purpose: The objective of this study was to investigate potential sites where *L. monocytogenes* and *Listeria* species (excluding *L. monocytogenes*) were repeatedly detected and isolated.

Methods: Environmental swab samples ($n = 402$) from a produce packinghouse were screened for *Listeria*, and up to eight isolates were characterized by *sigB* sequencing to determine species and allelic type (AT). Samples were collected on three separate visits over a 3-week period during the packing season. The same sixty-seven sites were sampled at two time-points: pre-operation and 4 h into operation ($n = 134$ each visit). Sites were determined based on prior results from environmental samplings or attributes conducive to *Listeria* harborage. Univariate associations of *Listeria*-positive samples between visit and time-point were determined using chi-square tests. *P*-values < 0.05 were considered significant.

Results: No significant differences were observed between *Listeria*-positive samples and visits and time-points ($P > 0.05$). Among the 144 *Listeria*-positive samples, 98 (24.4%) and were positive for *L. monocytogenes* only and 91 (22.6%) were positive for *Listeria* spp. (excluding *L. monocytogenes*) only, with 45 (11.2%) samples positive for both *L. monocytogenes* and another species of *Listeria*. *L. monocytogenes* was the most prevalent species identified in samples, followed by *L. seeligeri*, *L. innocua*, *L. ivanovii*, *L. marthii*, and *L. welshimeri*. Sixty-six of the 144 (45.8%) *Listeria*-positive samples contained two or more ATs, indicating a diversity of *Listeria* within the packinghouse. Five sites yielded *Listeria*-positive samples during both time-points and across all visits (two drains and three cooler floor/wall junctions). Upon isolate characterization from *Listeria*-positive samples, various ATs were identified within samples from the site.

Significance: Cases of *Listeria* repeat isolation were identified in certain sites, indicating those sites may have factors that promote *Listeria* establishment. Existence of such sites may increase the potential for resident *Listeria* in the postharvest environment.

T3-02 Genetic Diversity of *Listeria monocytogenes* Isolated from Three Commercial Tree Fruit Packinghouses and Evidence of Persistent and Transient Contamination

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Introduction: A longitudinal survey on the incidence and prevalence of *Listeria monocytogenes* (*Lm*) in 3 commercial apple packinghouses (i.e., P1, P2 and P3) with similar workflows was conducted. Sampling in Year 1 recovered 139 positive environmental sponge samples.

Purpose: To identify the genetic diversity of the *Lm* isolates in the apple packinghouses, to determine the profiles of genes associated with virulence and persistence, and to assess the tolerance of isolates to quaternary ammonium compounds (QAC).

Methods: Core genome multilocus sequence typing (cgMLST) was performed to identify the genetic relatedness of isolates from the packinghouses. *In silico* analyses were performed to determine the PCR serogroup, MLST sequence type and clonal complex. BLAST was used to identify the plasmids and genes associated with virulence and persistence. Growth analysis was performed to assess the tolerance of select isolates to QAC.

Results: The facility P2 had the least genetic diversity with a Shannon's index of 0.38; P2 contained a Clonal Complex (CC) 554, serogroup IVb-v1 strain that persisted through the year and spread across the entire facility. The facilities P1 and P3 had much higher diversity of *Lm* clones (a Shannon's index of 2.49 and 2.10, respectively), as a result of transient contamination. Facilities P1 and P3 had the highest percentage (43.1%) of lineage III isolates followed by lineage I (31.3%) and lineage II (25.5%) isolates. Analysis of select isolates did not reveal difference in their tolerance to QAC.

Significance: Our results provided new insights on the genotype diversity and distribution of *Lm* in tree fruit packing environments.

T3-03 Survival and Growth of Listeria monocytogenes on Whole Cucumbers under Dynamic and Static Temperatures during Industrial Post-harvest Handling Conditions in Australia

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❖ ***Developing Scientist Entrant***

Introduction: Food safety risks associated with *Listeria monocytogenes* contaminating cucumbers is of rising concern to the Australian fresh produce industry due to the lack of killing step to reduce potential contamination.

Purpose: The purpose of this study was to determine survival and growth of *L. monocytogenes* on whole cucumbers affected by different postharvest distribution conditions.

Methods: Five-strain cocktail of *L. monocytogenes* were spot inoculated on whole Lebanese cucumbers before exposing to different industrial postharvest handling conditions. In order to simulate these industrial practices in the laboratory, environmental conditions (temperature and RH%) of the produce (core and surface) was monitored from packhouse to retail, and used to define dynamic supply chain (SC1: 9-23°C; and SC2: 9-14°C), and static conditions (lowest: 5°C and highest: 40°C recorded; and optimum: 12°C). Cells were recovered by using ALOA medium. Generalised least squares and post hoc Tukey's honest significance test was used to compare the different treatments on *L. monocytogenes* survival.

Results: It was observed that temperature has a significant effect which promoted L. monocytogenes growth on some conditions (SC1 and 12°C), whilst inhibition was observed in SC2, 5°C, and 40°C. There was 0.90 log CFU/g increase for SC1. In contrast, there was 0.52 log CFU/g decrease for SC2 after 46 hours. When comparing between dynamic and static conditions (5°C, 12°C, and 40°C), only optimum (12°C) and lowest (5°C) were not significantly different ($P \geq 0.05$) than SC1 and SC2 after 46 hr, respectively. This demonstrates that L. monocytogenes presence pose a higher risk across different supply chains (9-23°C) and survival at optimum (12°C) temperatures, and monitoring transportation conditions is crucial to control population growth on cucumbers.

Significance: The data suggests that L. monocytogenes survive and grow when conditions are favourable, highlighting the need to reduce contamination after postharvest to identify and monitor conditions that promote growth and survival of the pathogen.

T3-04 Risk Factors Associated with Prevalence of Foodborne Pathogens in Manured Soils from USDA-NOP-Certified Organic Farms in Four Regions of USA

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Introduction: Application of untreated manure on cropland can introduce foodborne bacterial pathogens to soil and produce. Management practices and environmental factors can influence such contamination.

Purpose: To assess predictors for prevalence of foodborne pathogens in amended soils from USDA-NOP (National Organic Program)-certified farms in four USA regions.

Methods: A longitudinal, multi-regional study was conducted on nineteen USDA-NOP-certified farms in 4 USA regions (9 CA, 4 ME, 5 MN and, 1 MD). Untreated manure (cattle, horse, poultry), soil, and agricultural water collected during the 2017-2018 growing seasons were enrichment cultured for Shiga toxin-producing *Escherichia coli* (non-O157 STEC), *E. coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* to determine prevalence before and up to 180 days post-manure amendment (dpa). Mixed effect logistic regression was used to analyze risk factors for non-O157 STEC, *Listeria* and *Salmonella.*

Results: Observed soil prevalence was 0.04% (1/2460) for *E. coli* O157, 7.3% (179/2460) for non-O157 STEC, 1.1% (26/2460) for *Salmonella* and 5.0% (122/2460) for *L. monocytogenes* during this 2-year study with samples from 0-180 dpa. When accounting for clustering in time and space with random effects, adjusted prevalence was 2.7% for non-O157 STEC, 0.1% for *Salmonella,* 3.4% for *L. monocytogenes*. For all 3 pathogen groups analyzed, prevalence peaked significantly just after manure application and decreased significantly ($P < 0.05$) by 30 dpa and onwards. A second peak for non-O157 STEC was observed after 120 days. Generic *E. coli* in the soil and presence of pathogen in manure samples were significant predictors of pathogens in the soil. Soil humidity, wind, and soil temperature were significantly increased with pathogen prevalence, whereas snow was associated with lower prevalence. Animal manure type showed moderate evidence of association with pathogen prevalence.

Significance: This study provides science-based information to identify potential risk factors influencing foodborne pathogen persistence in pre-harvest produce production environments for soils amended with raw manure on organic production systems.

T3-05 Assessing Microbial Quality of Agricultural Water Used for Irrigation of Produce on Small Alabama Farms and Alabama Agricultural Experiment Stations

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❖ ***Developing Scientist Entrant***

Introduction: Produce can become contaminated with foodborne pathogens from water applied in the field. Therefore, the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) includes regulations on microbial quality of applied irrigation water

Purpose: Objectives of this research were to determine: 1) average *E. coli* presence in agricultural water used to irrigate produce during two growing seasons from eight locations across Alabama; 2) whether the water sources evaluated met the PSR criteria of 126 CFU/100 mL generic *E. coli* and 3) differences between three approved generic *E. coli* enumeration methods.

Methods: Sampling locations consisted of three locations (identified as A, B and C) at Alabama Agricultural Experiment Stations (AAES) and five locations (identified as D, E, F, G and H) at small Alabama farms. Irrigation water came from either surface water (Locations A, B, D, E, G, H) or ground water (Location A, B, C, F, G). Each location was sampled at least 3 times in a growing season. Generic *E. coli* were enumerated using EPA 1103.1, EPA 1604 and Hach method 10029. A complete randomized block design was used for the statistical analysis.

Results: *E.coli* in surface water was 2 - 460 CFU/100 mL at AAES locations and, from 2 - 118 CFU/100 mL on small farms. There was no detectable *E. coli* in the ground water at any locations. All of the water sources met the PSR criteria and there was no statistical difference ($P > 0.05$) between the three enumeration methods.

Significance: Identifying generic *E. coli* levels can aid identifying further actions to reduce produce contamination and foodborne outbreaks in the state of Alabama. Overall, the results from this research indicated that all the locations evaluated met the requirements with ground water having no detectable *E. coli*. from the PSR and the 3 enumeration methods produced equivalent results.

T4-01 Validation of a Simple Technique to Predict Cooling Rates of Cooked Foods in Retail Establishments

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❖ ***Developing Scientist Entrant***

Introduction: The FDA food code states that restaurants must cool hot foods within 6 hours. However, it is often not possible for inspectors to be present for a full 6 hours to make this assessment.

Purpose: This project validates a simple two-point modeling approach to predict cooling compliance using real cooling profiles collected in more than two dozen different foods under real-world restaurant conditions.

Methods: Cooling time-temperature profiles of 29 different foods were collected in 25 different restaurants. The estimated cooling rate was calculated by: [Log(T1-Tdf)-Log(T2-Tdf)]/t, where T1 and T2 are two temperatures measured during cooling, Tdf is the driving force temperature, and t is the time between the two temperature measurements. Cooling rate compliance was calculated for foods that had smooth (log-linear) cooling profiles. Monte Carlo simulations were used to determine whether the 2-point method would successfully predict food code compliance for foods with erratic (non log-linear) cooling profiles.

Results: Cooling rates were highly ($R^2 > 0.96$) log-linear for most (15/21) foods and generally linear ($R^2 > 0.87$) for all foods with smooth profiles. The two-point approach successfully predicted that 6 of 9 foods would not meet the food code cooling rate. In three cases the model predicted that foods cooling at a rate slightly slower than allowed would comply. The model correctly predicted that 10 of 12 foods would meet the food code cooling requirement. A majority (8 of 11) of slow cooling foods had a product depth greater than 3 inches. Monte Carlo simulation estimated that the two-point method would (on average) successfully identify 2 of 6 non-compliant cooling foods as well as 2 of 2 compliant cooling foods.

Significance: A simple two-point method that could be implemented during routine inspection is generally able to predict whether cooling foods will either pass or fail to meet food code cooling requirements.

T4-02 Heterogeneous *Bacillus* Spore Germination and Superdormancy under High Pressure

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Introduction: High pressure (HP) of 150 MPa can trigger the germination of spores, making them lose their extreme resistance, thereby increasing their susceptibility to milder inactivation strategies. However, germination response within a population is heterogeneous and of special concern are high-pressure superdormant (HPSD) spores, i.e., spores that remain dormant after exposure to the germination trigger.

Purpose: The purpose of this study was to provide suitable tools to study germination heterogeneity and investigate HP superdormancy.

Methods: *Bacillus subtilis* spores were HP treated at 150 and 37°C, stained with SYTO16 and PI, and analyzed via flow cytometry to visualize different subpopulations. Each subpopulation was single-cell sorted with fluorescence-activated cell sorting and characterized for its cultivability and heat resistance (80°C/10 min). The influence of heat activation (75°C/30 min) on HP germination was further investigated. The role of germination proteins was studied using isogenic mutants lacking specific germination proteins. Results are expressed as the mean value of $n = 3 \pm$ standard deviation.

Results: Four subpopulations were detected including heat-resistant and mostly cultivable HPSD spores, heat-sensitive and cultivable germinated spores, heat-sensitive and partially-cultivable germinated spores, and membrane-compromised cells with barely detectable cultivability. Heat activation did not increase germination; it slowed down germination with 23 and 13 % more HPSD spores after a 2 and 6 min HP treatment, respectively. Elevated SpoVA levels had no influence on germination. Germinant receptors strongly influenced HP germination; wildtype spores showed 1 ± 0.35 % HPSD spores after 16 min and the mutant lacking germinant receptors still showed 93.1 ± 0.6 % after 40 min HP treatment.

Significance: This research provides a better understanding of heterogeneous spore behavior under HP, and contributes to further investigations of HP germination mechanisms and the development of mild HP-based spore control strategies.

T4-03 Characterizing the Gut Microbiome of Commercial Broilers Raised with and without Antibiotics

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❖ ***Developing Scientist Entrant***

Introduction: Alternative poultry production systems are becoming increasingly popular. Consequently, the interest in the microbial profiling of non-conventional broilers is growing. Little research has been conducted to evaluate potential microbial community shifts occurring under such systems.

Purpose: The aim of the study was to characterize the gut microbiome of commercial broilers raised under no antibiotics ever and conventional industry practices.

Methods: Two conventional and two no antibiotics ever (NAE) farms were included in this study. Cecal (n = 224) and ileal (n = 224) contents were collected from birds during the grow out phase, with every feed change and following transportation to the processing plant, to evaluate temporal changes in the microbial communities. Sample DNA was extracted using the QIAmp DNA stool mini kits and the V3-V4 hypervariable region of the 16S rRNA gene was sequenced on the Illumina HiSeq 2500 platform. The DADA2 software package was used for analysis of microbiome data.

Results: The cecal microbiome was dominated by genera *Escherichia, Faecalibacterium* and *Bacteroides,* whereas *Lactobacillus*, *Streptococcus* and *Psychrobacter* were the most abundant genera in the ileum. The composition of cecal microbial communities of broilers raised under NAE practices was significantly different ($P \leq 0.05$) from conventionally raised broilers. Similar results were observed for ileal microbial communities. For both systems, microbial community composition changed as the birds aged ($P \leq 0.05$). Diversity of the cecal microbiome increased during early growth stages ($P \leq 0.05$); whereas, ileal microbiome diversity remained relatively stable ($P > 0.05$). Transport to the processing facility influenced the microbial composition of the ileum ($P \leq 0.05$) with *Psychrobacter* becoming the dominating genus.

Significance: Poultry production practices such as feed supplementation with antibiotics and changes in feed formulation affect the gut microbiome. These results fill knowledge gaps to further understand the influence of food animal production environments on microbial communities.

T4-04 Analysis of *Listeria monocytogenes* Strains Isolated from Food and Clinical Sources Uncovers Naturally Occurring Mutations Responsible for Tolerance and Sensitivity to Nisin

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Introduction: *Listeria monocytogenes* possesses numerous response mechanisms for resistance to different stresses, including nisin, a widely used bio-preservative produced by *Lactococcus lactis;* however, most nisin response mechanisms in this bacterium remain unknown thus necessitating novel approaches for their identification.

Purpose: Analysis of nisin growth phenotypes and genotypes in a diverse collection of *L. monocytogenes* to identify genes and mutations contributing to nisin response.

Methods: Growth phenotypes (based on area under the growth curve (AUC) determined by OD_{600} absorbance measurements for 24 h at 37°C) in BHI and BHI-nisin (12.5 ppM) were determined for 356 *L. monocytogenes* field strains. Absorbance data were modeled using spline fitting method and nisin induced change in area under the growth curve (ΔPAUC) determined for each strain. The maximum likelihood estimate method was used to cluster ΔPAUC values into nisin tolerant, intermediate and sensitive phenotypic groups. Targeted sequencing and comparison of 30 known *L. monocytogenes* nisin response genes were performed between nisin tolerant and sensitive strains using CLC Genomics Workbench.

Results: Sixty-six percent (236/356) of the strains were nisin tolerant (-2.4% ≤ ΔPAUC ≤ 38.2%), whereas 26% (91/356) and 8% (29/356) were intermediate (38.7% ≤ ΔPAUC ≤ 70.7%) and sensitive (74.7% ≤ ΔPAUC ≤ 100%) to nisin, respectively. Targeted gene sequence comparison between nisin sensitive and tolerant strains uncovered $_{229}$Guanine>Adenine and $_{718}$Guanine>Thymine missense mutations in *rsbU* and *PBPB3* genes, respectively. Missense mutations including $_{851}$Cytosine>Thymine, $_{506}$Cytosine>Thymine, $_{142}$Thymine>Adenine and $_{392}$Guanine>Adenine in *dltB*, *dltD*, *virR* and *virB* genes, respectively, were uncovered in nisin-sensitive strains. Nonsense mutations were also uncovered in *rsbU* and *virB* genes of nisin-sensitive strains leading to truncated respective proteins.

Significance: These data show that a combination of growth parameter-based phenome and gene sequence analysis can be used to identify nisin response genes in *L. monocytogenes* that can be further targeted to improve food safety.

T4-05 Fabrication of Biomimetic Spinach Leaves and the Role of Surface Microstructure on Decontamination Efficacy during the Washing Process

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❖ ***Developing Scientist Entrant***

Introduction: The role of plant surface microstructure on the persistence of pathogenic microbes and antimicrobial deposition during washing was characterized.

Purpose: In this study, a biomimetic leaf replica was developed to understand the role of surface microstructure on the physical removal and chemical disinfection of attached microbes. Various chemical deposition methods were characterized to improve the inactivation of microbes on leaf surfaces.

Methods: Biomimetic spinach leaf replicas were developed by a two-step casting process with polydimethylsiloxane (PDMS). Flat PDMS surfaces were used as control. The role of leaf surface microstructure on *Escherichia coli* O157:H7 decontamination efficacy was determined by understanding i) the influences of physical forces (shear stress, ultrasound) on microbial removal, ii) the influences of surfactants (Tween-20) on locally clustered microbes, and iii) the reaction with antimicrobials (free chlorine, particle-based sanitizer) deposited using dip, electro-spraying and ultrasound-assisted misting in the system. Viable microbes were enumerated by plate counting, and the spatial localization was characterized using fluorescence imaging. All experiments were done in triplicate (n = 27; 3/treatment).

Results: The results illustrate significant improvement (>1 log) in the mechanical removal of inoculated microbes from leaf replicas using ultrasound compared to mechanical shear generated by water. The application of surfactants further enhanced the removal of inoculated microbes by 0.5 log CFU/cm^2. Disinfection with 5-ppm free chlorine and particle-based sanitizers resulted in 1.66- and 5-log reduction of inoculated microbes, respectively. In the presence of particle-based sanitizers, the viable microbes in the valleys of leaf replica surfaces were still observed but significantly reduced. The use of electro-spraying and ultrasound-assisted misting improved the uniformity of microbial inactivation.

Significance: The study characterizes the role of microstructure in limiting the efficacy of the washing and sanitation process. It also illustrates the improvement in both removal and antimicrobial inactivation of inoculated microbes using advanced washing and sanitation solutions.

T4-06 Risk Factors for *Salmonella* Contamination in Poultry Products Following Changes in U.S. Oversight Programs

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is a leading cause of foodborne illness in the U.S. with salmonellosis often being attributed to poultry products. U.S. poultry processing is highly automated increasing opportunities for using predictive modeling to inform food safety initiatives. Previous studies have associated *Salmonella* contamination with meat processing facility characteristics including product class (i.e., broiler, steer, etc.) and establishment size. An evaluation of risk factors for *Salmonella* contamination in U.S. poultry has not been performed since implementation of the New Poultry Inspection System in 2014.

Purpose: The goal of this study was to determine if risk factors for *Salmonella* contamination changed following the 2014 modifications in poultry oversight.

Methods: The presence/absence of *Salmonella* in poultry products was modeled using microbiological testing data collected by U.S. Department of Agriculture's Food Safety Inspection Service between May 2015 and December 2019 from 210 poultry processing establishments. A generalized linear marginal model was fit for weekly presence/absence with establishment size, geographic location, season, and history of *Salmonella* contamination included as potential covariates. Odds ratios (OR) and 95% confidence intervals (CI) for potential risk factors were calculated from the marginal model.

Results: Of the 40,616 analyzable samples, 4.34% were positive for *Salmonella*. Several establishment characteristics were significantly associated with contamination. Odds of contamination was higher among small (OR = 1.503; 95% CI: [1.116, 2.024]) and very small (OR = 4.235; 95% CI: [3.208, 5.592]) establishments than large establishments. Odds of contamination was also higher among establishments which process turkey and chicken (OR = 2.853; 95% CI: [2.140, 3.802]).

Significance: This work confirms the risk factors for *Salmonella* contamination following implementation of changes in federal oversight of poultry while applying analytical methods appropriate for longitudinal data. These results support continuation of targeted food safety policies and initiatives promoting pathogen reduction by small processors and establishments producing multiple product classes.

T5-01 Phenotypic Testing and Comparative Genomics of Antibiotic and Heavy Metal Resistance of *Salmonella enterica* and *Escherichia coli* isolates from U.S. Swine Feed Mills

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❖ ***Developing Scientist Entrant***

Introduction: Antibiotics and heavy metals are commonly used in the animal feed industry. Some of these compounds can persist in the environment and evidence of co-occurrence in exposed organisms exists. Whole genome sequencing (WGS) technology can be used for better genetic characterization, including identification of resistant determinants in environmental bacteria isolates.

Purpose: The goals of this study were to: *i)* use phenotypic screening of antibiotic and heavy metal susceptibilities to characterize *S. enterica* and *E. coli* isolates from US swine feed mills; *ii)* identify resistance genes by WGS, and *iii)* examine the utility of WGS in predicting antimicrobial resistance (AMR) phenotypes.

Methods: This study included 27 *S. enterica* and 16 *E. coli* strains isolated from swine feed mills between 2018 and 2019. The isolates susceptibility patterns against a panel of 9 antibiotics were determined by microbroth dilution assay. Samples were also subjected to heavy metal testing by agar dilution method. Identification of resistance genes was performed using Resistance Gene Identifier (RGI) and BLASTn search engine.

Results: Susceptibility testing identified 12 *Salmonella* and 7 *E. coli* isolates resistant to at least one antibiotic. Isolates that possess copper resistance gene operon, were found to be viable at the highest concentration tested. Several genes conferring resistance to antibiotics were found: 33% of *Salmonella* and 13% of *E. coli* isolates displayed resistance to four or more antibiotics, including cephalosporin, colistin, streptomycin, and tetracycline. Plasmids containing metal resistance cassette were present in all isolates. Eighty-one percent of *Salmonella* and 19% of *E. coli* isolates showed resistance to three or more metals, one of them being copper.

Significance: Our study shows a good correlation between predicted and measured resistances when comparing genotypic and phenotypic data, indicating the potential role of feed mills as AMR entry route into the food chain.

T5-02 Rapid Luminescent Detection of *E. coli* in Drinking Water Using Click-conjugated Bacteriophage-based Magnetic Nanoprobes

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❖ ***Developing Scientist Entrant***

Introduction: Fecal contamination in drinking water causes hundreds of thousands of deaths annually. Regulations mandate zero detectable CFU of the fecal indicator organism *E. coli* in 100 mL of drinking water. Diagnostic assays rapidly giving observable signal for low levels of *E. coli* in drinking water are therefore crucial in limiting the impact of waterborne illnesses.

Purpose: Since standard culture-based methods require prolonged incubation times that allow pathogens to spread, we developed a T4 bacteriophage-based biosensor that gives signal for <10 CFU *E. coli* in 100 mL water within 7 hours.

Methods: We start with T4 bacteriophages expressing a luminescent reporter enzyme fused to a cellulose binding module. By engineering the phage capsid proteins to incorporate alkyne groups, we create a handle to conjugate the phages to azide-decorated magnetic nanoparticles. The resulting bioaffinity nanoprobes allow for detection of bacteria in liquid samples using two separate concentration steps (magnetic separation of bacteria from solution and cellulose immobilization of the reporter enzyme).

Results: Engineered phage capsid proteins bear significantly more alkyne groups than the WT control as shown by fluorescent cycloaddition (649 ± 52 rfu vs. 329 ± 34 rfu, $P < 0.05$). Alkynylated phages conjugate significantly more efficiently to azide-decorated nanoparticles than WT phages (5.10 ± 0.17 log PFU/mL recovered vs. 4.00 ± 0.13 log PFU/mL, $P < 0.001$). The resulting phage-conjugated nanoprobes give significantly higher luminescent signal for samples containing 7 ± 2 CFU *E. coli* than for samples containing 0 CFU *E. coli* (29 ± 3 rlu vs. 7 ± 3 rlu), indicating detection is sensitive enough to meet safety standards. Significance was determined by one-way ANOVA in all cases.

Significance: The developed assay is designed to be field-ready for water testing. It can also be generalized, with appropriate sample preparation techniques, to bacterial detection in complex matrices such as produce, dairy products, and meat.

T5-03 Evaluation of Zero-valent Iron Filtration in the Removal and Persistence of *Escherichia coli* in Non-traditional Irrigation Water Sources: A Conserve Study

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❖ ***Developing Scientist Entrant***

Introduction: Groundwater depletion is a critical agricultural irrigation issue and can be mitigated by supplementation with water of higher microbiological risk (e.g., surface, reclaimed) to support crop irrigation needs in the US. Zero-valent iron (ZVI) filtration may be an affordable and effective treatment technology for reducing pathogen contamination during irrigation of crops.

Purpose: To determine the effects of ZVI filtration on the removal and persistence of *E. coli* in irrigation water, and transfer to crops and soils.

Methods: *E. coli* inoculated water, ~4 log MPN/mL, was filtered through a ZVI filtration unit consisting of 4 columns: sand, 2-ZVI:sand (50:50), and sand. Diluted pond water was used for filtration and irrigation events. Plots, 3-square meters each, were irrigated with 2 L of filtered or unfiltered water every 10 days. Filtered and unfiltered water samples (n = 168), soil (n = 144), and cucurbit and cruciferous leaves (n = 40) were collected from each plot throughout the study. *E. coli* enumeration was performed via MPN using tryptic soy broth (TSB) and MacConkey agar. *E. coli* die-off was calculated, and one-way ANOVA performed to determine significance.

Results: ZVI filtration significantly ($P < 0.05$) reduced *E. coli* levels in filtered (2.97 ± 0.33 log MPN/mL) vs. unfiltered (4.46 ± 0.34 log MPN/mL) water. Die-off intervals of remaining bacteria in water were decreased by 2.22 days in filtered (2.66 days) vs. unfiltered (4.89 days) water. ZVI treatment significantly reduced *E. coli* transfer to leave and soil by 1.6-1.7 log MPN/g. *E. coli* levels in soil remained significantly lower in plots irrigated with filtered water (1.14 ± 0.56 log MPN/g) compared to unfiltered water (1.97 ± 1.15 log MPN/g) throughout the study.

Significance: ZVI filtration may be a suitable technology for decontamination of surface water. The reduction of *E. coli* in irrigation water, as well as on crops and in soils, by ZVI filtration is indicative of its potential to remove pathogens in produce pre-harvest environments.

T5-04 Prevalence of Ciprofloxacin-resistant Genes in *Campylobacter* Isolated from Poultry Breeder Farms and Processing Plants

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❖ ***Developing Scientist Entrant***

Introduction: While the use of fluoroquinolones (FQ) in poultry production has been banned, evidence suggests that ciprofloxacin (CIP) resistance has remained relatively constant. FQ-resistance in *Campylobacter* is a major concern as FQs are widely used for treating severe *Campylobacter* infections.

Purpose: The aim of this study was to identify CIP-resistance in *Campylobacter* isolates obtained from poultry breeder farms and processing plants and determine the gene responsible for CIP-resistance in *Campylobacter.* Concordance between the phenotypic and genotypic resistance was also evaluated.

Methods: Cloacal and boot swabs (n =1,296) from poultry breeder farms, and carcass rinses and ceca samples (n = 1,350) from poultry processing plants were collected in the Southeast U.S. during 2017 and 2018. *Campylobacter* isolation was performed according to the USDA-FSIS methods. Confirmed *Campylobacter* isolates were analyzed for antimicrobial resistance according to the National Antimicrobial Resistance Monitoring System (NARMS) protocol. Whole Genome Sequencing (WGS) was conducted on the CIP-resistant isolates using the Illumina MiSeq® platform and antimicrobial resistance genes were identified by running assembled WGS data through ResFinder.

Results: A total of 902 samples (69.5%) from breeder farms and 839 samples (62%) from the processing plant were positive for *Campylobacter*. Phenotypic resistance to CIP was observed in 5.65% (51/902) and 0.48% (4/839) of isolates from breeder farms and processing plants, respectively. WGS analysis identified a mutation in the *gyrA* gene at position 86 in 88.2% (45/51) and 75% (3/4) of CIP-resistant *C. jejuni* isolates from breeder farms and processing plants, respectively. Concordance between all the CIP-resistant phenotypes and genotypes was 87.7%.

Significance: This study indicates a higher prevalence of CIP-resistance inducing mutations in *C. jejuni* isolated from breeder farms as compared to the processing plants. Knowledge about genes and mutations causing resistance can be further used to develop and implement strategies to lower the prevalence of antimicrobial resistance of *Campylobacter* on poultry breeder farms and processing plants.

T5-05 *Listeria monocytogenes* Comes in Different Shades: Clinical and Food Associated Strains Vary in Virulence, Stress Resistance, and Carbon Source Metabolism

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a public health and food safety challenge due in part to its natural stress resistance and virulence phenotypes.

Purpose: Evaluation of phenotypic and genotypic diversity underlying variable *L. monocytogenes* distribution in foods and clinical cases.

Methods: Sixty-two *L. monocytogenes* isolates of food and clinical origin were characterized based on growth in brain heart infusion (BHI) with and without 8% NaCl (osmotic) and benzalkonium chloride (BC) [MIC in BHI] stress tolerance, virulence (zebrafish embryo microinjection and hemolysis), phenotype microarrays (carbon-source utilization, osmotic and pH stress), and whole-genome analysis. ANOVA was used to determine the statistical significance of differences between the strains in virulence and growth kinetics under stress.

Results: Despite a 76.0% core genome conservation, the strains differed significantly ($P < 0.05$) in osmotic and BC stress tolerance, and zebrafish pathogenicity. Clinical lineage I serotype 4b, CC1, CC2, CC4, and CC6 ($n = 28$) strains displayed significantly higher zebrafish pathogenicity [100% mortality ($n = 30$ embryos per strain) at 24 h post-infection (hpi)], whilst food associated lineage II, CC8 ($n = 5$) and CC9 ($n = 11$) strains were less virulent [≤30% mortality at 24 hpi]. Lineage I, CC2 and CC4 strains were significantly ($P < 0.05$) more tolerant whereas lineage II, CC9 strains were more sensitive to osmotic stress [2.1 vs 4.13-fold increase in lag phase]. Phenotypic microarrays revealed significant variation ($P < 0.05$) in C-source utilization and confirmed osmotic and pH stress resistance variation. A lineage II, serotype 1/2a outbreak strain utilized more C-sources (51 vs 34-39) than other strains. Strain-dependent alkaline stress inhibition patterns upon inclusion of β-phenylethylamine (2/8 strains inhibited) were observed indicating a potential for its exploitation in *L. monocytogenes* control.

Significance: We provide evidence of both virulence and stress resistance stratification with *L. monocytogenes* genetic backgrounds. Phenotypic microarray data generated provides a potential basis for improved *L. monocytogenes* detection media design and novel listeriosis control strategies.

T5-06 Radio Frequency (RF) Pasteurization and Drying of Fresh Inshell Hazelnuts Inoculated with *Salmonella*

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Introduction: Radio frequency (RF) heating is a novel thermal processing with advantages of volumetric heating and high penetration depth. Inshell hazelnut has air-gap between shell and kernel, which hurdles conventional heating as an efficient pasteurization and drying method. *Salmonella* outbreak has been frequently associated with nuts. No studies have been reported for RF pasteurization of *Salmonella* in inshell hazelnuts. Thermal resistance parameters (*D*- and *z*-values) of *Salmonella* in hazelnuts need to be determined for developing an efficient RF pasteurization protocol.

Purpose: The purpose of this study was to develop an efficient RF pasteurization protocol for inshell hazelnuts.

Methods: Either 1.5 g hazelnut shell powder ($a_w = 0.91$) or 2 g ground kernel ($a_w = 0.92$) (original water activities) inoculated with a 5-strain *Salmonella enterica* cocktail were packed in an aluminum pouch and sandwiched in a heating block for measuring *D*- and *z*-values under different temperatures. The sample pouches were heated for specific time intervals determined by the preliminary experiments before being transferred to the ice-water bath. After cooling, survival cells were enumerated. Microbial validation of RF pasteurization of *Salmonella* in inshell hazelnuts will be conducted based on the thermal death kinetics obtained from this study. Quality attributes (cracking ratio and lipid oxidation) of hazelnuts will be evaluated after RF pasteurization and drying.

Results: The *D*-values of *Salmonella* in hazelnut shells ($a_w = 0.91$) at 55, 60 and 65°C were 33.96 ± 0.41, 5.11 ± 0.22 and 1.08 ± 0.06 min, respectively. The *D*-values of *Salmonella* in hazelnut kernels ($a_w = 0.92$) at 60, 65 and 70°C, were 15.44 ± 1.66, 2.87 ± 0.08 and 0.19 ± 0.01 min, respectively. The *z*-values of *Salmonella* in hazelnut shells and kernels were 6.80 and 5.23°C, respectively.

Significance: The thermal resistances of *Salmonella* in hazelnut shells and kernels were determined. This study provides a protocol for developing efficient RF pasteurization and drying of inshell hazelnuts for the nuts industry.

T6-01 Heat Resistance in *Escherichia coli* from Cattle and Beef Packing Plants in Canada

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Introduction: Decontamination practices are routinely performed in beef packing plants and have largely improved beef safety in North America in recent years; however, these antimicrobial interventions may select for resistant bacteria, for example, *Escherichia coli,* which comprises pathogenic strains often concerning public health.

Purpose: To investigate the heat resistance in *E. coli* recovered from their natural reservoir, cattle, and various processing stages in meat plants in Canada.

Methods: *Escherichia coli* isolates recovered from cattle ($n = 750$) and meat packing plants ($n = 700$) were included in the study. Cattle *E. coli* isolates included seven serogroups (O103, O103, O111, O121, O145, O157, O26 and O45) and meat plant isolates were generic *E. coli* collected before and after/during antimicrobial interventions including hide-on wash, carcass chilling and equipment sanitation. The heat resistance of each isolate in Lennox broth was measured at 60°C, and *D*-value ($D_{60'}$ min) was calculated. Each isolate was also screened for the locus of heat resistance (LHR) conferring heat resistance to *E. coli* via real-time PCR.

Results: Prevalence of *E. coli* with $D_{60°C}$-values > 2 min was not significantly different ($P > 0.05$) among cattle and meat plant isolates. The *E. coli* recovered from equipment before sanitation (median, 1.03 min) were more resistant to thermal treatment than those recovered after sanitation (median, 0.9 min). No significant differences in heat resistance were observed between *E. coli* recovered before and after hide-on wash or before and during carcass chilling. Among 1,450 isolates, 28 (1.97%) had LHR and the heat resistance of LHR positive *E. coli* (median, 3.25 min) was significantly greater than LHR negative isolates (median, 0.96 min). The prevalence of LHR in cattle and meat plant isolates was not significantly different.

Significance: The decontamination practices currently applied in meat packing plants may not contribute to the selection of heat resistant *E. coli*.

T6-02 Promoting Food Safety Training in a Multicultural Workforce: Concept, Methodologies, and Approach

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Introduction: The food industry operates on hiring multinational migrant workers. However, a weakness in training programs is the absence of a curriculum that caters to multicultural, multiracial, and multilingual variations. The present reality is that the food industry must be ready to accommodate the rapidly growing needs of multicultural workers to adequately develop a viable food safety culture and run a seamless operation.

Purpose: The purpose of this presentation is to expose the benefits of multi-cultural training strategies to enhance food processing operations, break down operational barriers, and improve food safety culture as a whole. This study further discusses the need for training methodologies to include cultural diversity concepts based on an in-plant trial.

Methods: Fifty-eight employees in a food processing facility were provided with food safety pieces of training in 9 major languages (Arabic, Burmese, English, French, Nepal, Somali, Spanish, Swahili, and Vietnamese). Each employee took classes in their language of choice to include: GMP, SSOP, allergen awareness, pest prevention, traceability, and food safety basics.

Results: Seventy-six percent of participants noted that receiving the training in their language of choice enhanced their food safety education. Eighty-five percent reported that the targeted training had a positive impact on their knowledge of food handling operations. Sixty-nine percent of the participants have

reported food safety near miss or incident because of this training. Ninety-seven percent of the participants want to continue to receive subsequent training in their language of choice.

Significance: Overall, multicultural employees possess many of the skills and abilities necessary to succeed in complex food operations. However, companies must invest in strategies for multicultural training that include language, social system, and cultural values. Multicultural training can increase awareness, impart knowledge, and educate employees on necessary food safety and operation programs for the business to remain profitable.

T6-03 Strategic Allocation of Sampling Resources at the United States Department of Agriculture's Food Safety and Inspection Service

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Introduction: The U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) uses robust sample scheduling and collection protocols to verify the safety of regulated products. In fiscal year 2018, FSIS inspectors collected over 120,000 samples for microbiological and chemical residue analysis at FSIS labs and also collected and analyzed almost 4,000 samples for pathology. FSIS reported over 500,000 different analyte results from these samples.

Purpose: Historically, each FSIS sampling project was designed independently, with different purposes and goals. As part of an agency-wide effort to maximize resources, and because sampling projects are resource intensive, FSIS undertook a comprehensive evaluation to identify gaps and provide recommendations to strengthen the sampling programs.

Methods: A semi-quantitative approach was developed to normalize rankings for each of the sampling projects and facilitate direct comparison between them.

Results: The evaluation proceeded with the underlying premise that sampling only fulfills its purpose when the data generated is used by the agency. Process-related recommendations highlighted the need to continue integration of decisions on sample program development into the agency's formal governance process, thereby allowing greater transparency of decision-making criteria for allocation of sampling resources. Statistics-related recommendations highlighted the need to develop innovative solutions to interpret data sets where traditional methods have not been helpful. Each of the findings, and progress on recommendations, will be presented for discussion.

Significance: This evaluation has provided FSIS the data needed to maximize the efficiency, effectiveness and value of sampling projects, while ensuring that each continues to function as intended when initially developed.

T6-04 Validation of Innovative Tools to Assess and to Improve Microbiological Safety in the Food Chain (VITAL)

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Introduction: Microbiological food safety is of paramount importance for the food producing companies, which must guarantee absence of hazards in the foods distributed on the market. However traditional microbiology does not comply any more with the needs of the food producers in terms of time-to-results. Non conventional and fast methods have become more and more relevant in microbiological testing, however they must be validated.

Purpose: The purpose of this study was to develop a framework for accelerated method validation. More specifically with the use of artificial intelligence (AI) it was possible to analyze data from existing official validation of alternative methods to generate tools which can use past evidence to revise and refine the validation criteria so that alternative methods can benefit from a more efficient validation.

Methods: AI was exploited to analyze all available data on rapid methods validation to generate a tool (a recommender system) which enabled the definition of the criteria to be used in order to validate a new rapid method, which was represented by a lab-on-chip device for the detection of Salmonella. Two food ingredients were identified and used for the study. The validation procedure proposed by the AI was fully validated in laboratory settings following the ISO guidelines.

Results: The results of the validation showed a good agreement between the rapid method and the traditional culturing supporting the possibility to exploit non conventional validation schemes based on AI.

Significance: The results of this study underline how validation of rapid methods can be performed and verified by taking advantage of innovative approaches such as AI. It is expected that due to the lower demand requested for the validation of new rapid methods, those possibilities will be more often employed at industrial level resulting in better assessment of foodborne pathogens in the food chain and safer foods for the consumers.

T6-05 Bayesian Statistical Modeling for Describing Uncertainty of Bacterial Spore Inactivation Behavior

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❖ ***Developing Scientist Entrant***

Introduction: In a quantitative microbial risk assessment, survival cell density should be estimated as a probability distribution instead of deterministic conventional point estimation. Since conventional predictive models treat parameters as fixed values, uncertain variation in bacterial inactivation behavior is not evaluated. In contrast, because Bayesian statistics treats parameters as random variables, uncertain variation in bacterial inactivation behavior could be described by using random variables. The Bayesian statistics will be able to incorporate some uncertainty into a model, which leads to estimate survival cell density as a probability distribution.

Purpose: The objective was to develop a predictive model that enables one to explain the uncertainty of bacterial spore survival.

Methods: Bacillus simplex spores in 100 µL of pH-adjusted tryptic soy broth (pH: 5.4, 5.8, 6.2, 6.6, and 7.0) were heated at 80°C, 82.5°C, 85°C, 87.5°C, and 90°C in a thermal cycler. The survival spore counts were determined on a tryptic soy agar after 24 h incubation at 37°C. The 25 conditions were divided into 15 conditions for model development and 10 condition for model validation. The survival kinetics of the spores were fitted by Weibull model and the parameters of the Weibull model were described as a function of temperature and pH by using Bayesian regression. Based on the posterior distribution obtained from the Bayesian regression, the numbers of survival spore were calculated.

Results: The estimated parameter distributions were successfully converged since the R-hut values were less 1.01. The prediction of spore survival kinetics was successfully described as 95% prediction band by using the estimated parameters' distribution. The 90% of the independent validation data agreed within 95% prediction band.

Significance: The Bayesian model enabled one to explain the uncertainty of spore survival by using random variable in parameters, which will be useful for estimating whole inactivation behavior instead of point estimation.

T6-06 Risk Ranking of Food Categories Associated with *Salmonella* spp. Contamination in Central Mexico Using Multi-criteria Decision Analysis

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❖ ***Developing Scientist Entrant***

Introduction: To prevent and control foodborne diseases, there is a fundamental need to identify the food categories that are more likely to cause illness.

Purpose: The goal of this study was to rank 25 food categories (including meats, processed-meats, produce, dairy products and seafood) associated with *Salmonella* spp. contamination in the central region of Mexico (CRM).

Methods: A multi-criteria decision analysis framework was developed to obtain a *Salmonella* risk score for each food category by using four criteria: (i) the probability of *Salmonella* exposure at home (*Se*); (ii) *Salmonella* growth potential during storage at home (*Sg*); (iii) *per capita* consumption (*Pcc*), and (iv) the probability of a *Salmonella* outbreak caused by the food category (*So*). *Se* was estimated considering the prevalence of the pathogen and the food handling practices related to cross-contamination or the survival of *Salmonella* in each food category. *Sg* was estimated from secondary growth models considering reported refrigerator temperatures at homes in CRM. *Pcc* and *So* were obtained from previous studies in CRM and Latin America. The risk scores for each food category were calculated by the equation: Risk = $Se*W_1+Sg*W_2+Pcc*W_3+So*W_4$, where each criterion was assigned a normalized score (1 to 5) and the relative weight (W) was defined by experts' opinion. Variability and uncertainty around the weight scores were characterized by a Beta-Pert distribution and included in a Monte-Carlo simulation (10,000 iterations).

Results: *Se* had the largest effect on the risk score followed by *So*, *Sg* and *Pcc*. The results identified chicken (4.46±0.57), pork (4.27±0.56), and beef (4.21±0.51) as the highest risk foods. Seed (3.66±0.47), tropical (3.43±0.44), dry (3.37±0.45) and citric (2.72±0.37) fruits, as well as leafy greens (2.87±0.39) were in the top ten of the ranking.

Significance: This information can help government authorities to prioritize resources to control *Salmonella,* focusing on the highest risk-food categories.

T7-01 Mature Biofilms of *Listeria monocytogenes* Isolated from Vermont Dairy Production Environments are Resistant to QACs in Nutrient Rich Media

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (Lm) is known to form biofilms in food processing environments, particularly on stainless steel. Quaternary ammonium compounds (QACs) are sanitizers commonly used on food processing equipment but have been shown to have limited effect on mature biofilms.

Purpose: The objective of this study is to determine planktonic QAC tolerance, attachment capacity, and efficacy of QACs on mature biofilms for eleven Lm strains isolated from Vermont dairies.

Methods: Sanitizer MIC: isolates were incubated statically in 1x and 1/20x Brain Heart Infusion broth (BHI) in polystyrene microtitre plates for 24 hours (22°C) with 3.125, 6.25, 12.5, and 25 ppm QAC (DiverseyTM J-512TM/MC Sanitizer) to determine minimum inhibitory concentrations. A standard crystal violet assay was performed, with isolates grown in 1x and 1/20x BHI at 22°C for 1, 3, and 5 days. Sanitizer efficacy on mature biofilms: isolates were grown on one cm stainless steel coupons with 1x or 1/20x BHI. Coupons were incubated statically (22°C) for 10 days, rinsed 3 times with phosphate buffered saline, and placed into 0, 50, 100, or 200 ppm QAC for 60 seconds. QAC was neutralized with Dey-Engley broth, and adherent cells were removed for enumeration. Significant differences for MIC, attachment, and biofilm survival were assessed using Analysis of Variance in R (v. 3.6.1).

Results: At 22°C, isolate MIC ranged from 3.125 to 25 ppm. Attachment on polystyrene varied; however, all isolates reached ~6 to 8 log CFU/coupon on stainless steel. In 1x and 1/20x BHI, QAC application at 200 ppm reduced viable Lm numbers by ≤ 0.59 log CFU and ≤1.63 log CFU, respectively. Reductions from different QAC concentrations differed significantly ($P_{adj} < 0.05$) in 1/20x BHI, but were not significantly different in 1x BHI.

Significance: Mature biofilms from both persistent and transient Lm environmental isolates from Vermont dairies are resistant to working concentrations of QAC sanitizer.

T7-02 Effects of Commercially Available Antimicrobials on the Inhibition and Inactivation of Listeria monocytogenes Biofilms

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (*Lm*) contamination of food often occurs in food-processing environments where cells attach to various surfaces. Hydrogen peroxide (HP), ε-polylysine (EPL), and lauric arginate ethyl ester (LAE) are commercially available antimicrobials with demonstrated antimicrobial activity against *Lm* in foods. However, the impact of these antimicrobials on biofilm formation and inactivation is not well known.

Purpose: The purpose of this project was to determine the effects of EPL, HP, and LAE on the inhibition and inactivation of *Lm* biofilms on polystyrene (PS) and stainless steel (SS).

Methods: Subinhibitory (SIC) and minimum bactericidal (MBC) concentrations of each antimicrobial were determined through microdilution assays in brain heart infusion (BHI) broth with enumeration on BHI agar. For inhibition assays, *Lm* Scott A or 2014L-6025 were resuspended at 6 log CFU/mL in BHI broth containing SICs of each antimicrobial. Aliquots were then added to 96-well polystyrene (PS) plates or tubes containing SS rounds, which were incubated at 37°C for 48 h. Biofilm-associated cells were enumerated at 24 and 48 h. Mature biofilms for inactivation experiments were formed on PS and SS by incubating *Lm* strains resuspended in BHI at 6 log CFU/mL at 37°C for 7 days. The MBC, 10xMBC, and 100xMBC of each antimicrobial were then added and biofilm-associated cells were enumerated at 1 and 24 h.

Results: No differences were observed between treatments or control for biofilm inhibition. Significant reductions in biofilm-associated *Lm* on SS were observed for LAE treatments at 10xMBC ($P \leq 0.01$) and 100xMBC ($p \leq 0.01$). There were significant treatment*time interactions for HP and LAE against mature biofilms on PS ($P \leq 0.01$) and application of HP or LAE at 100xMBC resulted in greater reductions over 24 h ($P \leq 0.05$) compared to control.

Significance: These data demonstrate that although SICs of these antimicrobials may not inhibit biofilm formation, HP and LAE are promising strategies to combat mature *Lm* biofilms.

T7-03 Development of a Dry Surface Biofilms Rapid Model for Disinfectant Testing

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Introduction: EPA-registered disinfectants are tested against planktonic bacteria or wet surface biofilms (WSB) although under actual use conditions, bacteria mainly exist as dry biofilms (DB) which are harder to inactivate than WSB.

Purpose: This study aimed to develop dry surface biofilm models of *Staphylococcus aureus* and *Pseudomonas aeruginosa* for subsequent use in EPA disinfectant testing, which requires six log CFU per coupon.

Methods: *S. aureus* ATCC-6538 and *P. aeruginosa* ATCC-15442 WSB were grown on glass coupons following EPA MLB SOP MB-19. Rods holding coupons were harvested from a CDC bioreactor and dried at 25°C or 30°C for *S. aureus* and at 16°C or 21°C for *P. aeruginosa* for 24 to 120 h. Three coupons with DB were harvested every 24 h for five days, processed to release DB from glass coupons, and grown on TSA or R2a agar for 48 ± 4h at 36°C following EPA MLB SOP MB-20. Scanning electron microscopy was used to visualize biofilm matrixes on coupons (N = 100) pre- and post-DB development.

Results: Overall, we achieved an average ≥6 log CFU per coupon biofilm post-drying regardless of temperature and dry time for both organisms. For *S. aureus,* significantly higher mean log densities per coupon were achieved after 24 h compared to 96 h and 120 h of drying ($P < 0.05$). For *P. aeruginosa* grown at 21°C, there were no significant differences in DB CFU density irrespective of time, ($P > 0.05$). Overall, 86% of coupons (86/100) with DB had a visible biofilm matrix.

Significance: The developed method is a viable *in vitro* model for disinfectant testing against DB, which represent a systematic challenge to the food industry.

T7-04 Meta-regression Models Describing the Effects of Essential Oils and Added Lactic Acid Bacteria on *Staphylococcus aureus* Inactivation in Cheese

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Introduction: Biopreservation methods based on the use of natural extracts and starter cultures have been proposed as hurdles to increase the microbiological safety of many food products, including cheese.

Purpose: This study's objective was to extract all published findings on *Staphylococcus aureus* (SA) inactivation in cheese containing essential oils (EOs) and added lactic acid bacteria (LAB) and unify them by constructing two separate meta-regression models.

Methods: Suitable primary studies were identified through exhaustive literature search. Twenty studies were considered appropriate for inclusion (N = 299), and the following information was extracted: antimicrobial class (EO or LAB) and name, mean log reduction, storage temperature, exposure time, antimicrobial application (i.e., cheese mixture, cheese surface, milk, or film), and antimicrobial and pathogen's inoculum concentrations. Studies were assigned weights according to the sample size (n) used along the experiment to evaluate microbial inactivation.

Results: The EOs model revealed the significant impact of application type ($P < .0001$), storage temperature ($P < .0001$) and inoculum concentration ($P = 0.019$) on SA microbial reduction. Additionally, exposure time and antimicrobial concentration affected SA inactivation, although those effects were dependent on the type of application ($P < .0001$). Cheese mixture and milk were found to be the matrices promoting the highest microbial reduction, whereas incorporation in films presented the lowest inhibitory effect. Among the types of EOs meta-analyzed, lemon balm and sage produced the greatest mean bactericidal effects. The LAB model did not show differences ($P = 0.091$) in the inhibitory effect achieved by different applications (milk or cheese mixture), but revealed the interaction between this term and exposure time ($P = 0.040$). Heterogeneity analysis showed that the moderators of the EO and LAB models explain >95% and 11.80% of the between-antimicrobial variability, respectively.

Significance: The meta-regression models produced provide valuable insight on the main causes of variability in microbial reduction, which is vital when implementing and optimizing biopreservation hurdle technologies for pathogen control in foods.

T8-01 GenomeTrakr Best Practices for Uploading Sequence Data to NCBI: Assuring Good Sequence Quality and Proper Data Curation

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Introduction: The GenomeTrakr surveillance network of laboratories collects genomic data for foodborne pathogens isolated from non-clinical sources (e.g., food, environmental, water). These data are submitted to NCBI's pathogen surveillance platform called NCBI Pathogen Detection (NCBI-PD) to aid in traceback and regulatory actions. Until 2019, the FDA brokered most of these submissions for nearly 50 GenomeTrakr laboratories, receiving genomic data and metadata, performing quality control, and submitting to NCBI.

Purpose: As founders of GenomeTrakr, with extensive experience in managing an open genomic surveillance platform for foodborne pathogens, we are pleased to share our Best Practice guidelines here.

Methods: In 2019 GenomeTrakr released an SOP for PulseNet laboratories to independently submit their GenomeTrakr isolates to NCBI-PD through the software platform, BioNumerics. With the release of "Optimizing open data to support One Health: Best practices to ensure interoperability of genomic data from microbial pathogens" (2020), GenomeTrakr laboratories, or any laboratory interested in participating in open genomic pathogen surveillance, will find detailed guidance for directly contributing sequence and metadata to NCBI-PD.

Results: These Best Practices include four new protocols, hosted on a version-controlled web platform, protocols.io. They include detailed step-by-step protocols to assess sequence quality, to populate the metadata template using standardized vocabulary, to submit genomic data and metadata to NCBI, and to maintain and curate public data submitted by your laboratory.

Significance: The best practices document will improve workflow efficiency by eliminating the FDA as a data broker as well as assure that acceptable quality standards are preserved as the process becomes decentralized. Importantly, these tools and SOPs are written for *any* laboratory wishing to submit microbial pathogen data to NCBI, which, we hope, will democratize the process, increasing participation for all open genomic pathogen surveillance efforts.

T8-02 Two-year Monitoring of Environmental Microbial Communities in Three Apple Packing Facilities and Their Association with the Presence of *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (Lm) has been implicated in outbreaks and recalls of apple products and can survive in food processing environments that are cold and wet, such as apple packing facilities.

Purpose: Through a two-year longitudinal study, we aimed to determine the occurrence of Lm in three apple packing facilities and identify associated microbial communities.

Methods: Three apple packing facility (F1, F2 and F3) environments were sampled bimonthly between November 2017 and April 2018 (Y1), and between November 2018 and April 2019 (Y2). Swabs were collected from floors under the washing, drying and waxing area (n = 225). The presence of Lm was determined following the FDA-BAM protocol and microbial community profiles were characterized by 16S rRNA V4 and ITS2 sequencing. Alpha and beta diversity indices were calculated, and PERMANOVA was used to compare the microbiomes among facilities. One sample from each facility was sequenced using Nanopore to obtain greater taxonomic resolution.

Results: The occurrence of Lm increased from Y1 to Y2; from 28.2% to 41.7% in F1 and from 41.0% to 86.1% in F3. All samples collected from F2 were positive for Lm in both years. The mean alpha diversity of bacterial communities measured using Shannon index decreased from 4.92 ± 0.66 in Y1 to 4.24 ± 0.56 Y2. Samples collected in Y1 in F2 had a distinct bacterial community profile compared to samples collected from F1 and F3 (P = 0.001). In Y2, the microbial communities in F3 and F2 became more similar, which coincided with a significant increase in the occurrence of Lm in F3. Nanopore showed consistent results with amplicon sequencing and allowed for detection of Lm present in low concentrations.

Significance: This study indicated associations between high occurrence of Lm and environmental microbial communities in apple packing facilities, warranting further investigation of the role of microbial communities in the persistent contamination with Lm.

T8-03 Inferred *Salmonella enterica* Serotype from Whole Genome Sequencing Data Using SeqSero2

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Introduction: *Salmonella* serotype is traditionally determined by agglutination reactions using antisera specific to O-antigens and H-antigens. This method is laborious, expensive if all antisera are maintained, and can be subjective due to analysts' perception of agglutination. SeqSero2, a bioinformatics tool which uses whole genome sequencing (WGS) data to determine *Salmonella* serotype, recently became available. In FY2017, FSIS began performing WGS on 100% of *Salmonella* isolated from both regulatory and non-regulatory (cecal) programs. During this time, both molecular serotyping and WGS were performed on *Salmonella* isolates, and subsequently a comparison of molecular serotyping vs WGS-inferred serotype was completed.

Purpose: The objective of this work is to compare the results for determination of *Salmonella* serotype from molecular serotyping vs WGS serotype determination using SeqSero2 for *Salmonella* isolates from FSIS samples.

Methods: A total of 9,842 *Salmonella* isolates were compared based on serotype determined by molecular serotyping or traditional agglutination testing by the USDA National Veterinary Services Laboratories (NVSL) and SeqSero2. SeqSero2 was performed on assemblies using the k-mer based method. Isolates were considered to have matching results if the exact same serotype was called for molecular serotyping vs WGS, or the exact factor combination was obtained by both NVSL and WGS.

Results: For 98.21% (9,666/9,842) of the isolates sequenced, the serotype determined by WGS matched the serotype determined by molecular serotyping or by NVSL. Serotype inference from WGS data was successful at determining 109 distinct serotypes. For 1.79% (176/9,842) of the isolates the serotype determined by WGS did not match the molecular serotyping result or NVSL determined result. The most common reason for non-matching results (102/176) was due to antigenic factors not detected by WGS.

Significance: WGS serotype prediction is a step towards a single streamed characterization method which can lead to both cost and time savings.

T8-04 Getting a Handle on *Listeria* in New Zealand – Developing a Shared Whole Genome Sequence Database for Food Safety Applications

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Introduction: In New Zealand (NZ), listeriosis cases are sporadic and outbreaks are rarely identified but they remain the third highest foodborne human health burden for the country. There has been increased use of whole genome sequencing (WGS) to identify risks and control *Listeria* across multiple food manufacturing sectors in NZ.

Purpose: The NZ Food Safety Science and Research Centre (NZFSSRC) has created a research program to establish a shared and secure database containing *Listeria* WGS data for use in food safety applications.

Methods: To date, there have been three main areas of development. First, major contributors of the database including regulators and industry groups and companies were consulted to seek permission to use their WGS data and establish an agreed level of metadata to be included. Secondly, a database was established using the Research Electronic Data Capture (REDCap®) software. A user-friendly dashboard to view the data was created using RStudio Shiny applications which includes interactive phylogenetic trees (using Phylocanvas) to visualize comparisons between isolates. Thirdly, collation of existing WGS data and sequencing of historical *Listeria monocytogenes* was undertaken.

Results: The current database contains WGS data for over 800 *L. monocytogenes* isolates from clinical and non-clinical sources and continues to be populated. Users can select and compare isolates of interest using the seven-loci or core-genome (1,741 loci) multilocus sequencing typing (cgMLST) using phylogenetic trees. Some of the most common sequence types (STs) identified using seven-loci MLST represent STs that are distributed worldwide and reported to be involved in human infections and outbreaks. cgMLST demonstrates significant genetic diversity between isolates within the same ST, offering greater discrimination than the seven-loci MLST schema.

Significance: The database will serve as an ongoing resource for New Zealand and will facilitate the development of robust *Listeria* risk reduction strategies.

T8-05 Community of Fermenting Microorganisms during Spontaneous and Kefir Fermentation of Soy Milk

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Introduction: Natural or kefir fermentation of foods is characterized by rich diversity of microorganism, whose identities may not be fully revealed by culture-based approaches. Natural fermentation of water extracts of soybeans (soymilk) is yet to be extensively studied.

Purpose: This study sought to investigate the succession of lactic acid bacteria (LAB) and yeasts during the fermentation of different soymilk products using a metagenomic approach

Methods: Soybeans was sprouted at optimized conditions of soaking (12 h) and germination (52 h) and used to produce soymilk. A portion of soymilk obtained from sprouted soybeans was subjected to spontaneous fermentation (for 48 h) and another portion was fermented by wet kefir grains (for 24 h), with samples drawn at 6 h interval in each case. Fermented soymilk from unsprouted beans served as control samples. Bacterial and yeast communities were characterized using tag-encoded Nextseq and MiSeq-based high throughput amplicon sequencing (HTS).

Results: Dominant LAB found include *Weissela cibaria, Lactococcus lactis, Leuconostoc lactis, Leuconostoc mesenteroides,* while yeast species are *Saccharomyces cerevisiae, Pichia fermentans* and *Torulospora delbrueckii*. HTS revealed the presence of *Bacillaceae* and other bacteria as well as *Zygosaccharomyces* and *Dekerra*, with other unidentified fungi involved in spontaneous and kefir fermentation of soymilk. Alpha diversity and weighted unifrac PCoA biplots also showed the diversity and interrelationship of microorganisms as fermentation progressed.

Significance: HTS approach provided detailed information of the community of fermenting organisms in naturally and kefir-fermented soymilk. It also revealed that LAB and yeast isolated could find further applications as starter cultures.

T8-06 Keeping up with the *Bacillus cereus* Group: Leveraging Genomic Data to Counter Bacterial Taxonomic Ambiguity from Farm to Clinic

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Introduction: The *Bacillus cereus* group comprises numerous closely related species, including foodborne pathogen *B. cereus*, biocontrol agent *B. thuringiensis*, and bioterrorism weapon *B. anthracis*. Differentiating isolates which can cause illness or death from those which have important industrial applications is essential for proper risk evaluation; however, there are numerous ambiguities, misnomers, and inaccuracies in the current *B. cereus* group taxonomy which can hinder communication between microbiologists, clinicians, and industry professionals.

Purpose: The purpose of this study was to develop a standardized, genomics-based taxonomic nomenclature for the *B. cereus* group.

Methods: All publicly available *B. cereus* group genomes (n = 2,231) were queried for virulence factors using BTyper version 2.3.2, and FastANI version 1.0 was used to calculate pairwise average nucleotide identity (ANI) values between all genomes. Species clusters formed at various species thresholds (e.g., 95 and 92.5 ANI) were identified using average linkage hierarchical clustering implemented in R version 3.6.0.

Results: Current *B. cereus* group species definitions lead to overlapping species clusters, in which 66.2% of genomes belong to multiple species at a conventional 95 ANI threshold. Medoid genomes identified at a species threshold of ≈92.5 ANI are shown to yield resolvable species clusters with minimal overlap (six of 2,231 genomes assigned to multiple species; 0.269%). Using these findings, we provide a standardized nomenclatural framework for the *B. cereus* group comprising eight species, two subspecies, and three biovar terms to account for heterogeneity of clinically and industrially relevant phenotypes. We additionally discuss how historical species names are affected, with an emphasis on lineages relevant to agriculture, the food industry, and public health.

Significance: The work presented here provides a standardized taxonomy for the *B. cereus* group, which will remain interpretable and actionable to those in industry and public health, without sacrificing genomic definitions of bacterial species.

T9-01 Role of Edaphic Soil Factors and Climatic Conditions in Pathogen Survival on the Farm

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Introduction: Complex soil-environmental interactions associated with application of Biological Soil Amendments of Animal Origin (BSAAO) enhance soil nutrients and may support pathogen survival and transfer.

Purpose: Evaluate effects of edaphic soil factors on *E. coli* survival in BSAAO-amended soil.

Methods: Over two years, twenty plots (3 m^2) were amended with four amendments: poultry litter (PL), heat-treated-pelletized poultry litter (HTPP), composted PL (CPL), or unamended inorganic fertilizer (UN) with (M) or without plastic mulch (NM). Plots were spray-inoculated with *E. coli* TVS355 (1L, 6 log CFU/mL per plot) in June of 2018 (Y1) and 2019 (Y2). Every 10 days post-inoculation (dpi), composite soil samples were collected (n = 468) along with mature cucumbers (n = 320), and evaluated for soil parameters (temperature, soil moisture, conductivity, soluble carbon, C:N) and *E. coli* survival. Data were analyzed using one-way ANOVA (P < 0.05).

Results: Results indicated yearly effects on soil parameters and *E. coli* survival. In Y1 and Y2, soil temperature (27.2±7.0°C, 21.6±6.2°C) and solids content (85±4.0%, 89.1±6.2%) did not vary significantly by amendment type over 120 dpi. Soil moisture in Y2, reduced significantly (P < 0.05) by 120 dpi (2.5-3.5%) in CPL, UN and HTPP amended NM plots compared to 0dpi (13-15%), while in Y1 the change was insignificant (15±4.0%). In Y1, soil conductivity reduced significantly (P < 0.05) by 40 dpi and soluble carbon by 30 dpi compared to 0 dpi across all amendment types. While in Y2, no significant change was observed in soil conductivity and soluble carbon levels. In Y2, *E. coli* populations reduced to below detection limit by 60 dpi across amendments while in Y1 populations were variable but detectable. *E.coli* was detected on cucumbers in all plots in Y1 but not in Y2. Changes in bacterial survival and transfer are likely due to higher precipitation in Y1 (6.33-7.29 in.) than Y2 (0.77-2.11 in.), supporting the yearly effect in data.

Significance: Results suggests that climatic conditions, especially precipitation, are critical in pathogen survival and transfer.

T9-02 Influence of Soil Microbiota on *Escherichia coli* O157

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❖ ***Developing Scientist Entrant***

Introduction: Soil microorganisms can impact pathogen survival and identifying specific microorganisms in the soil that reduce survival time may be used as biocontrols.

Purpose: The objective of this research was to determine the influence of soil microbiota on *E. coli* O157 survival in an artificial model system and in soil rinsate.

Methods: *E. coli* O157 log CFU g^{-1} counts were determined in artificial soil (50 g) supplemented with soil rinse (filtered and unfiltered) (5 mL) for 42 days at 30ºC. Additionally, *E. coli* O157 log CFU mL^{-1} over 7 days at 30ºC was performed in soil rinses (50 mL) treated with cycloheximide (200 mg mL^{-1}), vancomycin (40 mg mL^{-1}), heat (80ºC, 15 min), and no treatment (control). Liquid (1 mL) or soil samples (1.0 g) were diluted and plated to determine counts at each sampling interval. A Welch's two-sample *t*-test was performed comparing mean *E. coli* O157 log CFU g^{-1} in soils. A one-way ANOVA of mean log CFU mL^{-1} was performed at sampling intervals for soil rinse treatments. Tukey's honest significant difference test was performed when statistical significance was observed. All experiments were performed in triplicate at a statistical significance of $P = 0.05$.

Results: There was a significant difference ($P = 0.027$) in *E. coli* O157 log CFU g^{-1} after 35 days between soils supplemented with filtered (4.45 ± 0.29) and non-filtered (1.83 ± 0.33) soil rinse. There were significant differences ($P < 0.05$) in *E. coli* O157 log CFU mL^{-1} after 3 days of incubation between soil rinse treatments (heat (7.04 ± 0.03), cycloheximide (6.94 ± 0.05), vancomycin (4.26 ± 0.98), and no treatment (5.00 ± 0.93)).

Significance: *E. coli* O157 survival influenced by natural soil microbiota may help identify certain microorganisms that can be used as biocontrols to reduce pathogen survival in manure amended soil.

T9-03 Survival of Salmonella enterica Subsp. Javiana and Listeria monocytogenes is Dependent on Type of Soil-free Hydroponic Growing Medium

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Introduction: The production of microgreens in controlled–environment agricultural (CEA) settings is increasing. These systems utilize soil alternatives such as fibrous or synthetic mats, peat, perlite, or coco coir. It is not well understood how the risk of foodborne pathogen transmission may be affected by the type of soil–free growing medium (SFGM).

Purpose: This measures survival of *Listeria monocytogenes* and *Salmonella enterica* subsp. Javiana over a 10–day microgreen growing period on four SFGM types.

Methods: Samples of coco coir, a *Sphagnum* peat/vermiculite mix, Biostrate® mats, and hemp mats were inoculated with a bacterial cocktail of 3 x 10^6 CFU/mL per sample and a control of bacteria in PBS. Samples were incubated at room temperature for 10 days with sample collection on day 0, 1, 3, 6, and 10.

Results: Statistically significant differences in pathogen survival were observed across multiple time points for hemp mats and Biostrate® mats compared to coco coir, peat, and bacteria in PBS ($P < 0.05$). *Salmonella* showed greater overall survival compared to *Listeria* ($P < 0.0002$). For hemp and Biostrate®, there was an initial increase in growth (~1 log) for both *Listeria* and *Salmonella* after 1 day while both pathogens declined on coco coir, peat, and in PBS. By day 10, *Salmonella* persisted at the initial inoculum concentration for hemp and Biostrate® while declining by 1–2 log CFU/mL on coco coir, peat, and in PBS. *Listeria* also persisted at the original inoculum level of 10^6 CFU/mL in hemp and Biostrate®. Conversely, *Listeria* decreased to 1 log CFU/mL for peat and below the detection limit for coco coir and control.

Significance: Overall, there are survival differences between bacterial pathogens in soil–free microgreen systems using different growing medium. This impacts the microgreen industry as media selection may be used to reduce foodborne illness risk.

T9-04 Drought Stress Affects Kale Leaf Phytochemical Profiles and *Salmonella enterica* Leaf Association

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Introduction: Abiotic stresses such as drought affect plant physiology. The impact plant abiotic stresses have on *Salmonella enterica* leaf surface association is not understood.

Purpose: Evaluate the effect of drought on the kale leaf metabolome and association with *Salmonella*.

Methods: Kale cultivar 'Improved dwarf' plants were grown for 2 or 8 weeks post-germination in a greenhouse (23°C, 16 h L:8 h D) before being subjected to drought for 6 or 2 days, respectively, depending on age, or regularly watered (control). About 10^6 *Salmonella* Newport cells were inoculated onto the adaxial surface of the third true leaf of plants and kept at room temperature. Inoculated leaves were clipped 24 hours post-inoculation (hpi), for *Salmonella* quantification. Leaf samples from 20- or 59-day-old control and drought-subjected plants were flash-frozen and ground into powders and mixed with 70% methanol with 0.5% formic acid. Solutions were used for biochemical analyses and subjected to electrospray ionization-mass spectrometry (ESI-MS) profiling. Multivariate data was analyzed using Multidimensional Scaling (MDS) ordination and Analysis of Similarity (ANOSIM) using Primer 6.

Results: In the control group, 20-day-old plants supported higher epiphytic *Salmonella* growth than 59-day-old plants 24 hpi ($P < 0.05$). Control 20-day-old plants yielded higher *Salmonella* counts than drought-subjected plants ($P < 0.05$). This drought effect was not detected in 59-day-old plants. Regardless of treatment, younger plants had lower antioxidant capacity, and flavonoid and total phenolic levels ($P < 0.05$), and higher glucosinolate levels ($P < 0.05$), compared to older plants. Drought-subjected 20-day-old kale plants had higher accumulations of glucosinolates, flavonoids and antioxidant capacity than control ($P < 0.05$). The leaf metabolome profiles of 59-day-old control and drought-subjected plants clustered together on MDS plots. However, 20-day-old control and drought-subjected leaf metabolome profiles clustered separately and diverged from 59-day-old samples ($P < 0.05$).

Significance: Levels of flavonoids, total phenolics and antioxidant capacity in kale leaves may affect ability of *Salmonella* to associate with leaf surfaces.

T9-05 Evaluation of a Commercially Available Irrigation Water Chlorination System for Leafy Green Production in the Everglades Agricultural Area (EAA)

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Introduction: The Leafy Greens Marketing Agreements (LGMA) require growers treat surface water that is applied in a way that contacts the crop ≤21 days prior to harvest to no detectable generic *E. coli* and ≤99 total coliforms per 100 mL of water.

Purpose: The purpose was to evaluate the efficacy of a commercially available calcium hypochlorite tablet-based water chlorination system for EAA canal water applied through overhead irrigation.

Methods: During each of five trials, surface water was injected with dissolved calcium hypochlorite from a tablet chlorinator while being pumped into ca. 470 feet of tubing connected to a pivot sprinkler. When the target chlorine concentration (2-5 ppm) was reached at the sprinkler, water samples (250 mL) were collected at 0, 20, 40, 60 minutes from the canal and from the end of the 470 feet of tubing immediately prior to the sprinkler. Free chlorine concentration was measured just prior to the sprinkler, and 0.12% w/v sodium thiosulphate immediately added to deactivate chlorine. Samples were analyzed for turbidity, total dissolved solids (TDS), total soluble solids (TSS), chemical oxygen demand (COD), temperature, pH, and chlorine breakpoint; total coliforms and generic *E. coli* were enumerated.

Results: Turbidity, TDS, TSS, COD, temperature, free chlorine concentration, and pH of the treated water ranged between 10.7-21.0 FAU, 384-424 ppm, 0.01-0.24 ppm, 27-88 ppm, 21-21.5°C, 2.6-3.3 ppm, and 7.7-8.1, respectively. Average breakpoint to 4 ppm free chlorine was 16.8 ppm. In untreated water, coliform populations ranged from 9,075 to 90,135 MPN/100 mL and *E. coli* populations ranged from 3.1 to 66.9 MPN/100 mL. Post treatment, generic *E. coli* was not detected and coliform populations ranged from <1 to 35.4 MPN/100 mL, meeting LGMA requirements.

Significance: Careful application and monitoring of a calcium hypochlorite treatment system (2-5 ppm at emitter) was an effective method to meet revised LGMA metrics of non-detectable generic *E. coli* and 99 or fewer total coliforms per 100 mL when applied to EAA canal waters.

T9-06 The Effectiveness of Vegetative Buffer Zones to Reduce the Risk of *Salmonella* and STEC Transmission from Animal Operations to Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Due to the recent outbreaks of *Salmonella* and STEC in fresh produce in the United States, it is apparent that the transfer of foodborne pathogens between animal feeding operations (AFO) and fresh produce continues to be a considerable risk.

Purpose: The purpose of this study is to determine if the establishment of a vegetative buffer zone (VBZ) at AFOs could prevent the transfer of *Salmonella* and STEC to nearby fresh produce fields.

Methods: A 5-layer VBZ (31X49 m) consisting of hardwood trees, two rows of evergreen trees and shrubs, a non-manicured grass strip, and a row of pollinator plants was planted between produce fields and dairy or poultry AFOs. Samples were collected from manure, air, soil, barrier, and fresh produce (romaine lettuce and tomato) sources for 15 months. Four replicates of soil and fresh produce samples were taken from plots located 32, 200, and 400 feet away from cattle/poultry AFOs. Air and vegetative strip samples were sampled at 15-day intervals.

Results: A total of 175 presumptive positive *Salmonella* and STEC isolates were retrieved from the soil, produce, air, and manure samples from both dairy cattle and poultry farms. The bulk of these isolates were recovered in 2019. Interestingly, 17.1% of presumptive *Salmonella* and STEC isolates are from produce samples (n = 30). All of the isolates were collected from lettuce (n = 30) samples. Surprisingly, *Salmonella* isolates (n = 9) were only found in manure samples from dairy cattle.

Significance: Preliminary data analysis suggests that the source of presumptive STEC and *Salmonella* contamination is not coming from the feed operations, but rather the contamination was already present in the soil. This suggests the need for more effective bioremediation practices to decontaminate agricultural soils. The data also suggest that there is a difference in the recovery rates of *Salmonella* and STEC depending on the produce type.

T10-01 Can Bacteriophages Contribute Massively to the Food Safety Future? Bacteriophages as a Biosensor Tool for the Detection of Foodborne Pathogens with Emphasis on Immobilization of Bacteriophage for the Detection of Non O157:H7 Shiga Toxin-producing *E. coli*

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Introduction: Foodborne pathogens are a major cause of disease and death among the global population. Illnesses related to contaminated food may vary from person to person from temporary to long-term complications. Nevertheless, the rapid rise of multidrug-resistant bacteria worldwide with a declination in antibiotics developments and production make bacteriophages an attractive tool to overcome bacterial resistance. Bacteriophages have become widely recognized for several potential applications in food industry. They represent an ideal tool for a rapid and sufficient diagnostic assays with great potentials in controlling the spread of harmful pathogens. Their abundance in nature of and high specificity against a specific host bacterium allow them to eradicate, prevent foodborne illness and recalls and provide safe food to consumers.

Purpose: The purpose of this topic is to shed a light on the existing phage based application such as immobilization of phages and using them as a biosensor for foodborne pathogen detection in food.

Methods: In our research, a phage capture-amplification assay based on the phage immobilization on bioactive paper were used. Experiments started with as isolation of very specific phage against one or more of Non-O157:H7 *E. coli* in food. Isolated phages are screened and made to undergo a variety of phenotypic and genotypic characterization to make sure that they meet the desirable requirement For example host range experiment, efficiency of plating, phage adsorption, growth curve of phage, whole genome sequencing, immobilization phage into colorlok paper (dipstick approach) using electrostatic properties of phages and the surface. The data were collected from three independents trials where the averages and standard deviations were determined. Detection limits were calculated and compared using an Independent-Samples t-Test using IBM SPSS.

Results: The result have statistically significant differences (P<0.05) in the detection of *E. coliO45:H2.* The cycle threshold (Ct) values were averaged for each concentration and compared to the average Ct values for the incubated control paper without phage. Using phages as biosensor enabled the detection

of as few as 10CFU/mL of the Big Six Shiga toxin-producing *E. coli* strains in both TSB media and ground beef using both a plaque assay and real-time PCR to detect phage progeny.

Significance: These data suggest that the immobilization of bacteriophages onto paper offers a promising approach for the detection of foodborne pathogens. The bacteriophage dipstick assay had the ability to detect as few as 10 cells of *E. coli*O45:H2 in only 8 hours in both TSB and ground beef.

T10-02 Conjugated Linoleic Acid Over-producing *Lactobacillus casei* Reduced Colonization of *Campylobacter jejuni* in Chicken

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter jejuni* (CJ) is one of the major foodborne-zoonotic, specifically poultry-product-borne pathogens. Our laboratory-generated probiotic strain *Lactobacillus casei* (LC) with *mcra* (myosin-cross-reactive-antigen) over-expressed, known as LC^{+mcra}, can produce higher amounts of bioactive compounds specifically conjugated-linoleic-acid. LC^{+mcra} can reduce growth, survival and pathogenic traits of CJ.

Purpose: In this study, control of CJ colonization in gut with LC^{+mcra} was evaluated in chicken-model.

Methods: A total of 60 chickens were raised in duplicate-trials up-to 28-day to determine effect of LC^{+mcra} on reduction of CJ colonization using marker-strain CJRMKm (grown on Kanamycin containing media). For chicken gut microbiome, 16S-metagenomic analysis of cecum-contents of 28-day old chickens were analyzed. ANOVA was used to determine differences in treatments/control groups.

Results: We observed higher number of LC^{+mcra} colonization in chicken gut compared to wild-type LC. LC^{+mcra} competitively reduced colonization of both natural and challenged *Campylobacter*. We recorded that LC^{+mcra} reduced CJRMKm colonization by >1.0 log ($P < 0.05$) in various parts of 28-day-old chicken gut including cecum, ileum and jejunum compared to control group. Natural colonization of *Campylobacter* was also reduced significantly ($P < 0.05$) in different intestinal portions. We also observed reduction of contamination of *Campylobacter* in drinking water. At phylum level, we observed 84.86%, 81.16% and 83.39% of Firmicutes in control, LC-fed, and LC^{+mcra}-fed group, respectively. There was an increasing pattern at level of Bacteroidetes and Tenericutes, when both LC-fed and LC^{+mcra}-fed group were compared to control. Differences at various genus levels among chicken groups were detected. At species level, diversity was determined for observed number of species and alpha indices (Shannon-index, Simpson-index, and Margalef's-richness). Though no significant ($P < 0.05$) differences in diversity among groups were found for observed indices.

Significance: This study reveals bioactive probiotic strain LC^{+mcra} could be used as sustainable alternative to reduce colonization of *Campylobacter* in poultry through microbiome modulation and produce safer poultry products along with other beneficial attributes.

T10-03 Antimicrobial Efficacy of Probiotic *Lactobacillus rhamnosus* GG in *Salmonella*-infected Chickens

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Introduction: Poultry products are the most common sources for human *Salmonella* infection. Use of antibiotics in production animals has been attributed to more antibiotic resistant *Salmonella*.

Purpose: Antibiotic independent therapeutics are needed to combat *Salmonella* in young poultry to minimize infection. Thus, we tested the efficacy and characterized the antimicrobial properties of known probiotics (*Lactobacillus rhamnosus* GG (LGG), *Lactobacillus* acidophilus (LA) and *Bifidobacterium animalis* subsp. Lactis (Bb12)) against *Salmonella* in young chickens.

Methods: Probiotics were incubated in mixed media co-cultures to analyze antagonistic effects against *S.* Typhimurium (ST) and *S.* Enteritidis (SE). LC-MS/MS analysis was used to quantify organic acids present in probiotics cultures. Additionally, probiotic derived peptides were tested for effectiveness against *Salmonella* (~5*10^5 CFU/mL ST and SE) at 37˚C for 12 hours. The most successful *in vitro* candidates, LGG and Bb12, were chosen to analyze efficacy against *Salmonella* in poultry. Chickens in experimental groups were treated with free or encapsulated probiotics daily (n=10/ group) from 1- 16 days of age and infected with 1x10^4 CFU/bird of ST at 7 days of age. On day 17, tissues were collected for the presence and quantification of *Salmonella*.

Results: LGG treated birds had significantly less (~2 log, P<0.05) ST in cecum than untreated control. In LGG treated birds, fewer number of spleens (30% less) were positive for *Salmonella* compared to untreated group. LC-MS/MS analysis showed lactic acid as the predominant organic acid present in probiotic culture. Additionally, LGG derived peptides P1 and P2 showed 100% inhibition of ST and SE at 18mM and 15mM respectively. These results imply that organic acids and small peptides play a role in *Salmonella* inhibition.

Significance: Our studies suggest that LGG and LGG derived peptides are promising food safety candidates for pre-harvest control and can potentially be implemented to prevent *Salmonella* transmission.

T10-04 Phenotypic and Genotypic Characterization of Extended-spectrum Beta-lactamase (ESBL)-producing *Escherichia coli* from Sheep and the Abattoir Environment in North Carolina: A Serial Cross-sectional Study

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❖ ***Developing Scientist Entrant***

Introduction: Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (ESBL-EC) are increasingly detected on retail meats from cattle, chicken, turkey and pork in the US.

Purpose: The purpose of the study was to characterize ESBL-EC isolated from sheep and their associated abattoir environment by antimicrobial susceptibility test (AST) and whole genome sequencing (WGS).

Methods: We conducted a serial cross-sectional study in an abattoir in North Carolina from March to November 2019. Samples included 186 carcass swabs, 157 cecal contents, 155 feces, 79 lairage swabs, 56 soil samples, 55 resting area feces, 51 feed and 51 water samples. Presumptive ESBL-EC isolates (n = 378) were isolated on Chrom agar with 4 µg/mL cefotaxime and confirmed as ESBL using the CLSI combined-disk diffusion test. Antimicrobial susceptibility testing employed the NARMS broth-microdilution method. Twenty-five ESBL-EC isolates were sequenced using the Illumina miSeq WGS platform.

Results: A total of 215/378 (56.9%) isolates were confirmed as ESBLs. One hundred forty-eight isolates were tested for AST presenting 25 phenotypically different resistance profiles. The most common profiles were AMP-AXO-AZI-CHL-FIS-STR-TET-XNL (n = 31, 20.9%), AMP-AXO-CHL-FIS-STR-TET- XNL (n = 28, 18.1%) and AMP-AXO-AZI-CHL-FIS-STR-SXT-TET-XNL (n = 26, 17.6%). More than 85% (127/148) of the isolates were multidrug resistant and four isolates were resistant to ciprofloxacin (2.7%). Sequencing 25 ESBL-EC isolates identified eight different serotypes: O10:H25, B2:H32, O9:H30, O7:H7,:H34, O8:H9,:H26 and

O17 or O77:H45. ESBL-genes detected included bla_{CTX-M} (21 isolates), bla_{TEM} (11 isolates) and bla_{CARB-2} (2 isolates). Other genes included bla_{CMY-2}, *qnrB19*, *qnrA1*, *qnrS1*, *floR*, *tet(A)*, *tet(B)*, *sul1*, *sul2*, *mdf(A)*, *mph(A)*, *aadA2*, *aph(6)-Id* and *aph(3")-Ib*.

Significance: This study detected ESBL-EC from sheep and their abattoir environment. This may pose a public health risk and serves as baseline information for decision makers.

T10-05 Quantification of Antimicrobial Resistance in Locally-grown Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce is increasingly recognized as a source of antimicrobial-resistant bacteria and antimicrobial resistance (AMR). However, limited studies have been conducted in this area.

Purpose: To determine the extent of AMR in locally-grown fresh produce.

Methods: A total of 48 vegetable samples were collected from Detroit urban gardens. Fifty grams of the sample was mixed with 450 mL of brain heart infusion to collect the vegetable rinse. Total DNA was extracted using DNeasy power water DNA Kit. bla_{TEM}, *IntI*-1, *sul*2, and *tet*M were quantified by qPCR using standard curves based on cloned target genes in *E. coli* DH5α. The 16S rRNA gene was included to normalize the abundance of antimicrobial resistance genes. *E. coli* and *Enterococcus* were isolated from vegetable rinse. Disk diffusion test was conducted to identify the AMR phenotype of the isolates. The ARG profile was obtained using whole genome sequencing (WGS) on selected *E. coli* and *Enterococcus* isolates.

Results: The relative abundance of bla_{TEM}, *IntI*-1, *sul*2, and *tet*M was 2.09×10^{-3}, 9.17×10^{-2}, 4.92×10^{-3}, and 3.52×10^{-3}, respectively. *IntI-1*, a gene marker for class-1 integron, showed higher abundance than the other three genes ($P < 0.05$). *E. coli* was isolated from 23 of 48 (48%) samples whereas *Enterococcus* was isolated from 38 of 48 (79%) samples. *E. coli* was resistance to ampicillin (60.8%) and *Enterococcus* isolates showed resistance to streptomycin (78.9%) and ampicillin (31.6%). Multidrug efflux pump genes, beta-lactamase genes, and prophage were common in *E. coli* as identified by WGS. *Enterococcus* isolates carried efflux pump genes, macrolides-lincosamides-streptogramines (MLS) resistance genes, tetracycline resistance genes, and transposons.

Significance: The data suggest that locally-grown fresh produce may serve as a source and vehicle of antimicrobial-resistant microorganisms. Mobile genetic elements, such as *IntI*-1, prophage, and transposons, may play an important role in AMR dissemination in the environment.

T10-06 Antimicrobial Efficacy of Pecan Shell Extracts Incorporated in Pullulan Film against Bacterial Pathogens and Molds

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❖ ***Developing Scientist Entrant***

Introduction: Pecan shell, considered as waste, constitute about 50% of the pecan mass. However, studies have found that it contains various bioactive compounds that could have potential inhibitory activity against microorganisms.

Purpose: This study evaluated the antimicrobial efficacy of pecan shell extract in pullulan film as an antimicrobial coating against various bacterial pathogens and mold.

Methods: Freeze-dried aqueous (A-PSE) or ethanol pecan shell extract (E-PSE) was obtained from Caddo variety pecans. The antimicrobial activity of PSE in pullulan film was determined using agar disc diffusion method. A coat forming solution (10 mL) comprising extract (5 or 10%), pullulan (10%) and glycerol (4-8%) was spread on sterile Petri dish, dried (35-37°C) for 12 h, and 6 mm diameter discs were cut. The antimicrobial and antifungal property of PSE in pullulan film disc was tested against two Gram-positive *Staphylococcus aureus, Listeria monocytogenes;* two Gram-negative *Salmonella* Enteritidis, *E. coli* O157 pathogens; and four fungal strains *Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus niger and Fusarium solani.* The diameter of growth inhibition (mm) around discs was measured (n = 3).

Results: PSE significantly ($P < 0.05$) inhibited the growth of Gram-positive organisms *Staphylococcus aureus* (15.2 ± 0.7 - 19.6 ± 2.9 mm) and *Listeria monocytogenes* (15.7 ± 1.3 - 18.5 ± 1.3 mm) followed by Gram-negative *Salmonella* Enteritidis (9.6 ± 0.8 - 11.1 ± 1.4 mm) and *E. coli (*6.0 ± 0.0 - 8.7 ± 1.4 mm). The inhibition was highest ($P < 0.05$) for *Staphylococcus* (19.6 ± 0.4 mm) treated by E-PSE (10%) and lowest/no inhibition for *E. coli* treated by 5% A-PSE or E-PSE. Ethanol extracts showed higher inhibition at 5% PSE, however at 10% PSE no difference was observed between extraction methods. Increasing the concentration of aqueous or ethanol extracts from 5 to 10% increased ($P < 0.05$) the inhibition zone. No antifungal property was shown by pullulan film with A-PSE or E-PSE at tested concentrations.

Significance: Pecan shell extracts in pullulan film shows promise as a potential antibacterial coating whose application on various food products or packaging material could be further explored.

T11-01 Evaluating the Effectiveness of Vegetative Buffer Zones at Reducing Transmission of *Salmonella* and STEC: Challenge Study

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❖ ***Developing Scientist Entrant***

Introduction: Due to the recent outbreaks of *Salmonella* and STEC in fresh produce in the United States, it is apparent that the transfer of foodborne pathogens between animal feeding operations (AFO) and fresh produce continues to be a considerable risk.

Purpose: The final step of our two-year study was to challenge the vegetative buffer zones (VBZ) with *Salmonella* and STEC at a concentration of log 6. This was done to determine the effectiveness of the VBZ at stopping pathogen transmission, the carrying capacity of the VBZ, and the survival rate of *Salmonella* and STEC in the piedmont region of North Carolina.

Methods: A 5-layer VBZ (31X49 m) consisting of hardwood trees, two rows of evergreen trees and shrubs, a non-manicured grass strip, and a row of pollinator plants was planted between produce fields and dairy or poultry AFOs. Samples were collected from the VBZ, air, soil, and produce sources for a period of 21 days. Four replicates of soil and fresh produce samples were taken from plots located 32, 200, and 400 ft. away from the VBZ. Samples were taken on before and after inoculation as well as seven and 14 days after inoculation.

Results: A total of 403 (1st trail, n=268; 2nd trail, n=135) environmental samples were collected from soil, produce, air, and VBZ samples from both dairy cattle and poultry AFOs. Twenty presumptive *Salmonella* and STEC isolates were retrieved from the VBZ, produce, and soil samples. Interestingly, the majority of isolates were recovered from samples taken on Day 7 of both trails. These isolates were found at distances of 32, 200, and 400 ft. away from the VBZ.

Significance: Preliminary REP-PCR data suggest that three isolates retrieved from both trials matched the banding pattern of the lab strain of *Salmonella* and STEC used to inoculate the VBZ.

T11-02 Detection of Norovirus, Hepatitis A and Rotavirus in Vegetables and Their Correlation with the Presence of Somatic Coliphages as Viral Contamination Indicators

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❖ ***Developing Scientist Entrant***

Introduction: Because of the difficulties associated with direct detection of viruses in food, such as norovirus, hepatitis A and rotavirus, bacteriophages are becoming attractive indicators of viral contamination.

Purpose: To investigate the potential of somatic coliphages as indicators of viral contamination of leafy green vegetables sold on local markets.

Methods: A total of 80 rinsates, 40 of lettuce and 40 of parsley (each one consisting of rinses with 50 mM glycine, 100 mM Tris, 1% beef extract [pH 9.5] buffer (TGBE) of four vegetable samples) were subjected to polyethylene glycol (PEG) precipitation and RNA extraction by Trizol/chloroform. Reverse transcription was performed for detection and quantification of Norovirus GI, Norovirus GII, Norovirus GIV, VHA and rotavirus by qPCR using G blocks as positive controls. The enumeration of somatic coliphages was according to EPA (1602 method). Results were analyzed by Pearson correlation.

Results: The presence of rotavirus estimated in lettuce was 22.5% (9/40) and 20% in parsley (8/40). Norovirus or hepatitis A were not detected in any samples. Rotavirus was present from 11 ± 16.98 to 45 ± 0.14 genomic copies in lettuce and 22 ± 1.97 to 44 ± 0.79 genomic copies in parsley. Somatic coliphages were present from 1-10^3 PFU/100 mL in lettuce and 1-10^3 PFU/100 mL in parsley. A statistically significant, moderate and inversely proportional linear association (r_p = -0.134, $P < 0.05$) was found between the number of genomic copies of rotavirus and somatic coliphages in lettuce samples. A high and inversely proportional linear association (r_p = 0.187, $P < 0.05$) was found between the number of genomic copies of rotavirus and somatic coliphages in parsley samples.

Significance: The presence of somatic coliphages shows a high correlation with the presence of rotavirus in parsley and a moderate correlation with lettuce, which suggest its potential as an indicator of viral contamination in fresh produce.

T11-03 Determination of the Levels and Population Composition of Microorganisms on Baby Spinach from Harvest through the End of Shelf Life

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❖ ***Developing Scientist Entrant***

Introduction: Spoilage of fresh produce is an important contributor to food waste. Identifying changes in the microbiota of baby spinach provides insight into the microbial basis of spoilage.

Purpose: To determine baseline levels and population composition of microorganisms on spinach collected across the supply chain and through shelf life.

Methods: Three composite samples of spinach were collected from a single lot after each of the following steps in the supply chain (in-process samples): harvest, transport to the storage shed, vacuum-cooling, storage at the processing facility, triple-washing and drying. Shelf life analysis was conducted by testing packaged spinach, stored at 4°C, from day 0 to day 20 on alternate days. Twenty-five grams of each sample was tested for aerobic plate count (APC), total Gram-negative count (GN), psychrotolerant count (PC), total yeast count (YC) and total mold count (MC). Isolates were collected from a subset of shelf life samples and characterized using 16s rDNA sequencing.

Results: APC, GN and PC levels were highest after storage at the processing facility. MC and YC levels were highest after vacuum-cooling and transport to the storage shed, respectively. APC, GN and PC levels were lowest after triple-washing, while YC and MC levels were lowest after drying. Overall, microbial levels on in-process samples were 5.44 ± 0.60 (APC), 5.10 ± 0.46 (GN), 5.44 ± 0.44 (PC), 4.53 ± 0.77 (YC) and 3.94 ± 0.90 (MC) log CFU/g. For shelf life samples, microbial levels reached maximum concentrations of 9.21 (APC), 8.92 (GN) and 9.64 (PC), while yeast and mold levels remained at 5.83 (YC) and 3.50 (MC) log CFU/g. Sequenced isolates were predominately *Pseudomonas* spp. followed by *Pantoea* spp.

Significance: This study provides a baseline understanding of microbial levels and populations on spinach from harvest through end of shelf life, which allows for identification of targeted intervention strategies to minimize microbial levels and extend shelf life.

T11-04 Extracellular Antibiotic-resistance Genes in the Cantaloupe Farm Environment

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❖ ***Developing Scientist Entrant***

Introduction: Extracellular antibiotic resistance (exARG) genes can cause resistance through the natural transformation of non-resistant cells, and can spread throughout the food chain.

Purpose: To determine and identify the presence of antibiotic-resistance genes and mobile elements involved in antibiotic resistance in the cantaloupe farm environment.

Methods: A total of 201 samples from farm-workers hands (n = 66), irrigation water (n = 35) and cantaloupe (n = 100) were collected on three farms in northern Mexico in 2017. Each sample was assayed for the presence of 13 genes: ten antibiotic-resistance genes (B-lactamase: *bla*CARB-4 and *bla*SHV; tetracyclines: *tetA* and *tetB*; macrolides: *ermB* and *ermF*; sulfonamides: sul1; quinolones: *qnrA*; polymyxins: *mcr1*; and glycopeptides: *vanB*), and 3 mobile elements (integrins: *intI* and *intII*; and plasmid: *oriV*) by PCR. The relationship between gene presence and sample type was evaluated using logistic regression.

Results: The exARGs most frequently found were *tetA* (23.9%, 48/201 total samples) mostly from cantaloupe (25%, 25/100), followed by *sul1* (13.4%, 27/201 total samples) mostly from farmworker's hands (21.2%, 14/66). Class 1 integron (int1) was the most widely distributed mobile element (23.9%, 47/201 total samples), frequently found in cantaloupe (30/100, 30%). The odds of finding any gene in hand samples was higher (OR 2.678 95% CI: 0.668, 10.74) than those in cantaloupe (OR 1.431 95% CI: 0.609, 3.36) compared to water. However, there was no statistically significant association ($P < 0.05$) between sample types and the presence of any gene analyzed.

Significance: These results indicate that exARGs are present and can be detected along the cantaloupe farm environment. Because these genes could transmit resistance among the bacteriome, it is important to reduce this possibility by pursuing better agricultural practices.

T11-05 Subtyping of Presumptive *Bacillus cereus* to Distinguish and Trace the Strain Used in SCUTELLO Biopesticide from Field to Fork

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Introduction: *Bacillus thuringiensis* (Bt) is a widespread spore-forming bacteria commonly found in soil. Due to its ability to produce parasporal crystalline inclusions that show insecticidal properties, it has become the main microorganism used for pest control in organic farming since the 1950s. Bt-based products containing crystal proteins and spores are applied to foliage, soil, water environments or even food storage facilities. While Bt-based products provide highly efficient crop protection, the persistence of spores on vegetables represents a major issue for Food Business Operator with raw materials showing *B. cereus* presumptive spores contamination surpassing 3 log CFU/g.

Purpose: The aim of this work was to distinguish and trace the *Bacillus thuringiensis* strain used in SCUTELLO for crop protection from field to fork.

Methods: Based on previous work on a well-characterized collection representative of the biodiversity of *B. cereus* Group, PFGE subtyping was used to acquire molecular fingerprints and trace strain from field to fork. Several case studies were performed with salad, parsley and broccoli with and without SCUTELLO pesticide. Sampling was performed upon vegetable harvest and after several processes to quantify bacterial spores and presumptive B. cereus but as well acquire molecular fingerprint of isolate.

Results: *bacterial* counts indicate a spore contamination on SCUTELLO treated vegetables that is twice more than for non treated vegetable. Since contamination remains as spore, few impact of processes is noted. Clustering of PFGE fingerprints enables to trace back the strain used in SCUTELLO but as well to identify a psychrotrophic *B. cereus* population naturally occurring in non-treated vegetable.

Significance: The clustering of PFGE fingerprints enables the distinction of strains used as bioinsecticides from foodborne contaminants, highlighting the persistence of spores in soil, on vegetable at harvest and all along the process and shelf-life of ready-to-eat. This case study is part of BtID project, supported by the French ministry CASDAR program.

T11-06 Development, Validation and Comparison of 24 Machine-learning Models That Predict the Presence of Foodborne Pathogens in New York Streams Used to Source Water for Produce Production

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Introduction: Since water quality varies based on environmental conditions, risk management strategies that account for this variation may improve growers' ability to identify and address food safety risks associated with preharvest water use in real-time. Due to the availability of spatial data, GIS-based analyses can be used to develop such approaches for individual water sources.

Purpose: To develop and compare the ability of different machine-learning models to identify when (i) *Salmonella* and (ii) *stx* and *eaeA* were likely to be detected in agricultural water.

Methods: This study used datasets collected in 2018 (68 streams, 196 samples) and 2017 (6 streams, 181 samples) to train and test the models, respectively. Twenty-four machine-learners (e.g., conditional forests) were used to develop separate models for each combination of outcome (*Salmonella* isolation, *stx-eaeA* codetection), and data type (microbial, physiochemical, weather, and spatial data). Baseline models where *E. coli* levels were the sole covariate were also developed.

Results: Overall, ensemble and regularized learners outperformed all other learners, including the baseline models. For *Salmonella* isolation, the top learner (ridge regression; Kappa=0.28) included all four data types. However, when a subset of data types was used, models that included microbial and physiochemical water quality performed better than other models built using the same learner. For *stx-eaeA* codetection, ensemble learners that included *E. coli* levels and spatial data (Kappa=0.42), or *E. coli* levels and physiochemical water quality data (Kappa=0.42) outperformed all other learners.

Significance: These findings suggest machine-learning can be used to develop tools to help growers identify when and where pathogens are likely to be detected in agricultural water. These findings also suggest that while microbial data is key to developing accurate models for predicting when foodborne pathogens are likely to be present in agricultural water, pairing microbial data with other data types produces superior models.

T12-01 Pre-growth Conditions and Genetic Variation Affect Nisin Treatment against *Listeria monocytogenes* on Cold Smoked Salmon

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (LM) is commonly found in environments associated with cold smoked salmon (CSS). Nisin is frequently used to control LM in CSS, however, environmental stresses encountered in CSS processing facilities might affect LM's nisin susceptibility.

Purpose: To investigate the effect of seafood-relevant pre-growth conditions and LM genetic variation on nisin treatment in CSS.

Methods: Six LM strains representing serotypes most commonly associated with CSS (1/2a, 1/2b, and 4b) were pre-grown in a variety of conditions prior to subculture into BHI with or without nisin. Pre-growth conditions with the lowest mean and the most strain-to-strain variability of nisin susceptibility were selected for experiments on CSS; these included: (i) 4.65% w.p. NaCl (NaCl); (ii) pH=6.1 (pH); and (iii) 0.5 µg/mL benzalkonium chloride (Quat). CSS slices with or without nisin were inoculated with LM pre-grown in one of the conditions above, vacuum-packed, and incubated at 7°C. LM population was enumerated on days 1, 15 and 30 with four biological replicates.

Results: Compared to the control (BHI), significant reduction ($P < 0.05$) in nisin susceptibility was induced by pre-growth in pH and Quat on both day 15 and 30, and in NaCl on day 30, indicating a time-dependent cross-protection effect. An effect of genetic variation on nisin susceptibility was observed; 1/2b strains were more susceptible to nisin compared to 1/2a and 4b strains. Across the 30-day storage time, an interaction between the effect of pre-growth condition and genetic variation was observed, as pre-growth in pH lowered nisin susceptibility specifically for strains from serotype 1/2a and 4b, but not for 1/2b strains. In contrast, pre-growth in Quat reduced nisin susceptibility indistinguishably for all strains.

Significance: This study indicates that pre-exposure to low acidic environment or quaternary ammonium compounds is likely to provide cross-protection against subsequent nisin treatment for LM in CSS.

T12-02 Prevalence, Antibiotic Resistance and Genetic Diversity of *Salmonella* Recovered from Imported and Domestic Seafood

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Introduction: Seafood importation and domestic aquaculture farming have increased. Recently, it has also been reported that multidrug resistant *Salmonella* may be associated with seafood. However, information is limited about the prevalence, antibiotic resistance and genetic diversity of *Salmonella* recovered from imported and domestic seafood.

Purpose: The present study investigated the prevalence, antibiotic resistance and genetic diversity of *Salmonella* isolated from three imported and domestic seafood species obtained from four retail stores located on the Eastern Shore of Maryland, U.S.

Methods: A total of 468 frozen catfish, shrimp, and tilapia imported (60, 85, 84) and domestic (96, 71, 72) samples were analyzed for *Salmonella* using standard methods. One isolate from each positive sample (n = 127) was tested for the presence multidrug resistance, serovar and genetic diversity using the Sensititre® micro-broth dilution method, serotyping, and pulsed-field gel electrophoresis, respectively. Measurement outcomes were evaluated by one-way ANOVA or t-test when quantitative and by Fisher's exact test when qualitative.

Results: For both domestic and imported seafood, 26% of shrimp, 28% of catfish, and 27% of tilapia were positive for *Salmonella*. In these three types of seafood, the average MPN/g of *Salmonella* ranged from 1-1.6, 1.4-2.1, and 2.6-3, respectively. There was a significant difference between *Salmonella* prevalence in imported (33.3%) versus domestic (19.4%) tilapia but not with shrimp and catfish. All isolates were *Salmonella* Typhimurium var-5 and genetically diverse. These isolates were uniformly susceptible to six (amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, imipenem, nitrofurantoin, and trimethoprim/sulfamethoxazole) of the 17 tested antimicrobials. Forty-eight percent of the *Salmonella* isolates were resistant to at least one antimicrobial and 45% were multidrug resistant.

Significance: These results indicate potential food safety hazards associated with domestic and imported seafood. Moreover, the analysis of the antibiotic resistance phenotypes of *Salmonella* recovered from domestic and imported seafood has provided useful information for the seafood industry and regulatory agency.

T12-03 Antimicrobial Effects of Nisin and Grape Seed Extract on *Listeria monocytogenes* on Cooked Shrimp ((*Litopenaeus vannamei*) by Metabolomics

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Introduction: Relatively high prevalence of *Listeria monocytogenes* has been reported in shrimp, especially ready-to-eat shrimp.

Purpose: This work aimed to investigate the antimicrobial effects of nisin and grape seed extract (GSE) against *L. monocytogenes* on cooked shrimp and to elucidate the underlying antimicrobial mechanism.

Methods: Nisin (2000 IU/mL) and GSE (1%, w/v) were applied to treat *L. monocytogenes* (SSA184, SSA97 and LM10) on cooked shrimp (*Litopenaeus vannamei*). The inactivation kinetics were characterized by linear, Weibull, and Huang's models while the residual effects of nisin and GSE during storage were demonstrated by Gompertz, exponential, Beta function and Baranyi models. NMR and atomic force microscopy were applied to monitor the metabolic and morphological changes, respectively. The shrimp color was assessed by L*, a*, b* and △E. All experiments were performed at least three times independently. Data were statistically analyzed by analysis of variance (ANOVA) using SPSS with $P < 0.05$ as significant.

Results: The combination treatment yielded enhanced and more lasting antimicrobial effects on *L. monocytogenes* on shrimp than single treatment, as it led to 1.66-1.88 log reductions in 15 min. The Weibull and Baranyi models best fitted the survival and growth kinetics of *L. monocytogenes*, respectively. Among all the strains, SSA184 was more susceptible under combined nisin and GSE, which required 6.90 min for 1 log CFU/g reduction and recovered at a rate of 0.11 log CFU/g/day. Interestingly, these treatments did not cause significant difference on color ($P < 0.05$). Mechanistically, the inactivation of *L. monocytogenes* SSA184 was a cooperation of cell membrane breakdown by nisin and disturbance in the intracellular metabolism (unbalanced glycolysis and amino acid metabolism) by both nisin and GSE.

Significance: The combined nisin and GSE could be a promising strategy for seafood industry to control *L. monocytogenes* contamination and maintain the seafood safety during storage.

T12-04 Frontiers in Pressure-based Pasteurization: Cost Optimization by Synergism with Natural Bactericidal and Bacteriocin Compounds

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Introduction: Pressure-treated products typically have slightly higher price per unit relative to those products treated by traditional methods. Stakeholders of the technology currently rely on treatments at intensity level of around 87K PSI (600 MPa) lasting typically for 3 minutes. Application of pressure at lower intensity levels could increase life of the pressure vessels and reduce the operation costs and enhance retention of nutrients and organoleptic properties.

Purpose: The current presentation discusses recent microbial challenge studies conducted at the Public Health Microbiology laboratory of Tennessee State University utilizing synergism of natural bactericidal and bacteriocin compounds with mild heat and mild hydrostatic pressure to assure microbial safety of the pressure-treated products at lower processing cost.

Methods: Studies are repetitions of two biologically independent trials each as blocking factor in a randomized complete block design. Each block is further consisted of three replications with each replication also repeated twice as microbiological replicates. Studies involved use of elevated hydrostatic pressure (200 to 600 MPa), mild heat (up to 55°C) with additional of bactericidal and bacteriocin compounds such as carvacrol, thymol, nisin, lysozyme, and citricidal for inactivation of epidemiologically significant foodborne pathogens and three microbial spores. Studies are statistically analyzed by ANOVA followed by Tukey- and Dunnett's-adjusted means separations.

Results: In presence of carvacrol at 450 MPa, >3-log reductions ($P < 0.05$) of O157 and non-O157 Shiga toxin-producing *Escherichia coli* were observed. Similarly, nearly 4-log reductions ($P < 0.05$) of *Listeria monocytogenes* were observed in presence of nisin at 400 MPa. Decontamination of non-typhoidal *Salmonella* and pressure and heat-resistant spores were also augmented ($P < 0.05$) in presence of the antimicrobials.

Significance: Results of current challenge studies indicate that through synergism with bactericidal and bacteriocin compounds, validated utilization of mild hydrostatic pressure could yield comparable microbial safety of the commodities at lower operation cost.

T12-05 Antimicrobial Activity of Hydrogen Peroxide, with and without Neutralization, against *Listeria monocytogenes* on the Surface of High-moisture Cheese

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (*Lm*) cross-contamination is a major concern in the dairy industry. Surface application of hydrogen peroxide (HP) has been shown to inactivate *Lm* on Queso Fresco (QF) and inhibit growth during storage. However, treatment conditions were not optimal for commercial application and did not address residual peroxide.

Purpose: The objectives of this study were to determine the antimicrobial effects of HP treatments optimized for commercial application; the transfer and degradation of peroxide; and the impact of peroxide neutralization.

Methods: Three independent batches of QF were produced. Samples (30 ± 2 g) were surface inoculated with *Lm* at ~4 log CFU/g and submerged in 10% HP for 5 seconds with (HP+cat) or without (HP) catalase neutralization at 30 min. Controls were no dip, deionized water dip (DI), and HP+DI dip at 30 min. Duplicate samples were vacuum-sealed and stored at 4°C. *Lm* counts and residual peroxide were determined at 30 min, 4h, 24h and 7d.

Results: HP, HP+cat, and HP+DI similarly reduced *Lm* counts by ~2 log CFU/g at 30 min. Catalase fully neutralized HP at 30 min. Counts in the HP treatment were lower at 4 h (1.04 log CFU/g) compared to HP+cat and HP+DI (1.89 and 1.74 CFU/g, respectively; $P < 0.001$) as peroxide levels in HP and HP+DI remained at 3,200 and 2,560 mg/L, respectively. Counts in the HP+DI decreased to 0.92 and 0.4 log CFU/g at 24 h and 7 d, respectively, while DI and no dip control counts (~4 log CFU/g) and HP+cat remained the same. Counts remained lowest in the HP treatment at 24 h (0.51 log CFU/g) ($P < 0.01$) and 7 d (0.1 CFU/g) as residual peroxide levels decreased to 400 and 25 mg/L, respectively.

Significance: These data further characterize the strong antimicrobial effects of HP against *Lm* on QF and highlight the role of residual peroxide as well as the impact of neutralization on efficacy.

T13-01 U.S. Consumers' Flour Handling and Recall Knowledge

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Introduction: Flour, a low moisture food product, was considered as high risk for microbial food safety by consumers. Recently, flour has been identified as a source of pathogenic bacteria, including *Salmonella* and *E. coli*.

Purpose: To understand flour users' knowledge and behavior of flour handling and flour recalls or outbreaks, and to evaluate the effectiveness of food safety messages.

Methods: Upon the Institutional Review Board (IRB) approval from Purdue University, flour-using consumers were recruited from an online panel managed by Qualtrics Inc. in May 2019. Survey questions were pilot tested for face and content validity.

Results: Flour-using consumers (n = 1,045), from the United States, reported that when using flour, they typically made cakes, cookies, and bread. Most consumers stored flour in sealed containers. Less than 1% kept the record of lot numbers, and less than 11% kept brand and use-by-date information. Many consumers (85%) were not aware of flour recalls or outbreaks and very few (17%) believed they would ever be affected by flour recalls or outbreaks. If the recall affected the flour they bought, half consumers (47%) would buy the same product from a different brand for a few months before they go back to the recalled brand. Among consumers who use flour to bake, 66% claimed they ate raw cookie dough or batter. Food safety messages were perceived less effective by those dough "eaters" than "non-eaters," who never ate raw dough. Food safety messages containing why and how to properly handle raw flour products were more effective in convincing consumers to change their practice and communicating the information.

Significance: These findings will provide insights in effective consumer flour safe handling education and assist in more accurate risk assessment model development.

T13-02 The Role of Hands in the Cross-contamination of Kitchen Surfaces When Preparing a Meal in a Consumer-style Kitchen

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❖ ***Developing Scientist Entrant***

Introduction: A significant amount of foodborne illness is acquired in the home and cross-contamination is a contributing factor to this. However, specific behaviors that lead to cross-contamination during meal preparation have not been adequately characterized using a combination of observational and microbiological methods.

Purpose: This project's purpose was to determine how human hands, with a focus on handwashing and touch-based behaviors, contributed to the likelihood and degree of cross-contamination to kitchen surfaces during preparation of turkey burgers and a salad.

Methods: Data were obtained from an existing study where consumers were observed preparing a meal in test kitchens. Turkey burgers were inoculated with MS2, a harmless bacteriophage, and cross-contamination was monitored by environmental swabbing of kitchen surfaces. Behavioral coding was performed to record handwashing and touch-based behaviors. Cross-contamination risk was defined as the likelihood and degree (contaminant concentration) of MS2 transferred to surfaces. Statistical analysis, regressions and ANOVAs, were performed in R or SPSS.

Results: Participants who attempted to wash their hands displayed significantly reduced risk of cross-contamination ($P < 0.0001$). Similarly, completing more handwashing steps resulted in a greater risk reduction ($P < 0.0001$) and an average scrub time of 5 seconds reduced the risk of cross-contamination ($P < 0.050$). Regression models were created using the three handwashing behaviors previously described to predict cross-contamination. The models revealed that greater attempted handwashing, more completed steps, and longer average scrub times resulted in a decreased risk of cross-contamination ($P < 0.050$). For touch-based behaviors, the best predictor of cross-contamination varied across the sampled surfaces.

Significance: These results improve our understanding of how consumer behaviors influence cross-contamination during meal preparation. This information can be used to improve consumer messaging and reduce home-acquired foodborne disease resulting from improper food handling.

T13-03 Determinants of Food Thermometer Use and Poultry Washing among Canadian Consumers

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Introduction: Previous surveys have found that few Canadians use a food thermometer to check the cooking doneness of meat and poultry, and many report rinsing or washing poultry prior to cooking.

Purpose: The study purpose was to identify the sociodemographic and psychosocial determinants of these targeted food safety behaviors among Canadian consumers. The study was guided by the Theoretical Domains Framework.

Methods: An online survey was administered to a panel of Canadian consumers on November 18, 2019. The questionnaire was informed by previous surveys and was pre-tested through 10 cognitive interviews. The outcomes of interest were participants' self-reported frequency of thermometer use and poultry washing, each measured on a 5-point Likert scale, and food thermometer ownership (yes/no). We measured and evaluated 7-8 psychosocial constructs and six sociodemographic characteristics as possible determinants of these three behaviors in a series of ordinal and logistic regression models.

Results: A total of 524 responses were obtained. Nearly two-thirds of respondents (64%; *n* = 333) reported owning a food thermometer, and ownership was more common among males (odds ratio [OR]=1.48, 95% CI=1.02, 2.15) and those with higher incomes. Approximately 45% of these respondents (*n* = 147) reported often or always using their thermometer to check cooking doneness. Thermometer use frequency was best determined by four psychosocial constructs: behavioral intentions, beliefs about consequences, self-efficacy, and habits. Nearly two-thirds of respondents (64%; *n* = 333) reported often or always washing their poultry before cooking it. This behavior was more frequently reported by males (OR=1.64, 95% CI=1.08, 2.50). It was also predicted by six psychosocial constructs: behavioral intentions, beliefs about consequences, self-efficacy, social influences, social responsibility, and habits. Habits had the largest influence on both behaviors.

Significance: The results can be used to inform the development of targeted education, communication, and outreach strategies with consumers to improve their use of these food safety behaviors.

T13-04 Consumer Preparation and Thermometer Use for Cooking Not-Ready-to-Eat Frozen, Breaded Poultry Products and Vegetables: Findings from an Observational Study

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Introduction: Frozen not-ready-to-eat (NRTE) chicken products have recently been associated with several foodborne outbreaks; although these products may appear fully cooked, they require cooking to 165°F for safety as measured by a food thermometer.

Purpose: This study measured rate of thermometer use by consumers when preparing packaged breaded stuffed chicken breasts from frozen and NRTE corn following exposure to food safety messaging.

Methods: Participants were randomized to a treatment group (*n* = 206) exposed to a passive video loop (playing in study waiting room) with a news segment on safely preparing NRTE foods that appear RTE, or the control group (*n* = 197) exposed to a video loop without the food safety segment. Participants prepared frozen NRTE chicken breasts and black bean salad with frozen corn in a test kitchen while being videotaped. Coders viewed the videos to determine adherence to recommended food safety practices and statistical testing was conducted for differences between the groups.

Results: Thermometer use for poultry was high among both the control (77%) and treatment (88%) groups; the difference between the groups was not statistically significant. Nearly all participants read the manufacturer's cooking instructions before preparation, which instructed consumers to use a thermometer to check for doneness. Among thermometer users, 86% checked the temperature of both breasts. The most common method to prepare the corn was stovetop (58%), followed by microwave (38%). Three percent of participants did not cook the corn. One participant in the control group and seven in the treatment group used a thermometer to check the corn for doneness.

Significance: Manufacturer's cooking instructions are an important source of information on how to safely prepare NRTE frozen poultry products but are less effective for providing information on how to safely prepare frozen vegetables.

T13-05 Content Analysis of Online Flour-based Recipes: Cookies, Cookie Dough, and Egg Noodles

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❖ ***Developing Scientist Entrant***

Introduction: Recipes and food-handling techniques are shared via online videos and blogs. Pathogen-contaminated flour incited 14 recalls in 2019, but few consumer-directed food safety campaigns emphasize safe flour handling.

Purpose: Evaluate the potential food safety risk of flour-handling content in popular online videos and blogs using cookies, cookie dough, and egg noodles as examples.

Methods: YouTube and Alexa Internet were searched for videos and blogs, respectively, using keywords specific to each recipe category. Only recipes that were conveyed in English, used flour, and had a minimum of 200 views (videos only) were selected. Videos (*n* = 146) and blogs (*n* = 85) were coded for general characteristics, user flour-handling practices, and food safety messages, including adherence to FDA recommended practices.

Results: Most videos (84%) were posted by consumers. Eating raw dough was demonstrated or mentioned in cookie (8%) and cookie dough (52%) videos. Flour was spread unintentionally while being transferred to mixing vessels in 25% of videos and during mixing in 26% of videos. No users were observed washing their hands after handling flour. Only one video showed cleaning flour-contacted surfaces, and none showed sanitized surfaces. Seventy-seven percent of videos depicted at least one cross-contamination event. Kitchen tools were the most commonly cross-contaminated item. Cookie dough videos recommended heat-treatment methods including baking (10%), and microwaving (3%) dry flour. Half (51%) of blogs were posted by consumers. Only cookie dough blogs (88%) mentioned eating raw cookie dough. Bloggers recommended drying egg noodles on a broom handle, storing egg noodles in newspaper, and removing cookies from the oven before baking finished or when "raw" in the middle. Flour heat-treatment methods were described in 24% of cookie dough blogs.

Significance: Misinformation related to and mishandling of flour-based products in shared recipes can increase consumers' foodborne illness risk. Strategies targeted at improving consumers' flour-handling practices need to be developed.

T13-06 Value of Interactivity in Online Training: Assessment of Interactivity Level in an Online Training Program

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❖ ***Developing Scientist Entrant***

Introduction: To reduce the risk of foodborne outbreaks, it is crucial for food safety training programs to impact behaviors. For many small manufacturers, online training can be an intriguing option since attending face-to-face trainings can be difficult due to time and money constraints. To make online training more engaging, instructional designers may include interactive components. Interactivity is the extent to which users perceive communication, stimuli, and control within an environment.

Purpose: The purpose of this study was to evaluate the impact of interactivity within an online training on students' knowledge, self-efficacy, behavioral intentions, and self-reported behaviors, and determine the value of creating highly interactive training programs.

Methods: Students were randomly assigned to one of the three treatment groups: low, moderate, or high interactivity. Analytics from the Learning Management Software (LMS) were used to measure students' actual level of interactivity, and Moore's Theory of Transactional Distance was used to measure the students' perceived level of interactivity. ANOVA was used to compare data from each treatment.

Results: There was no significant difference between the final average grade in each treatment group (low 80%, moderate 84%, high 85%, $P = 0.7989$). There was no significant difference between the lesson completion rates or amount of adaptive feedback used between the treatment groups. However, the low interactivity group had the highest rate of completion (96%, $P = 0.6407$) and used the most adaptive feedback (54.67%, $P = 0.4687$). Students spent the least amount of time on the high interactivity treatment (17.2 minutes, $P = 0.2747$).

Significance: It may not be necessary for companies to invest so much time and money into developing highly interactive training. By designing a low interactivity training, companies can keep development costs low, while still providing training that will have an impact on behaviors and help keep the food system safe.

T14-01 The Impact of Different Osmotic Stresses on the Survival, Growth and Detection of *Aeromonas hydrophila*

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Introduction: *Aeromonas hydrophila (Ah)* is an opportunistic zoonotic pathogen of aquatic animals and human. However, how do osmotic pressures present in the aquaculture and food processing environment impact the survival, growth, and detection of *Ah* remains largely unknown.

Purpose: To address this knowledge gap, this study evaluated the impact of salinity, sugar, ammonia nitrogen and sodium nitrite on the growth, the production of extracellular capsule and pili, and the antibody-antigen affinity of *Ah*.

Methods: Osmotic pressures were simulated by incubating *Ah* cultures in Luria-Bertani broth (LB) supplemented with different concentrations of sodium chloride, sucrose, ammonium chloride, urea, sodium nitrite and sodium nitrate. Cell growth was monitored via a plate reader at 600nm. The production of capsule was characterized with a microscope. The expression of OmpF was evaluated by quantitative Western Blot while the interaction between surface antigens and OmpF antibodies was investigated by an enzyme-linked immunosorbent assay.

Results: Among all tested conditions, 4% of NaCl inhibited the growth of *Ah*. More than 2% of NaCl significantly decreased the detection limit of *Ah*, although similar expression levels of OmpF were observed under different osmotic pressures. The sugar concentration (0-4%) did not affect the growth of *Ah*. Capsules were induced when more than 0.5% sucrose was added in LB. The formed capsules blocked the binding of OmpF antibody. Ammonia nitrogen (0-160mg/L) did not affect the growth and detection of *Ah*. When incubating *Ah* in LB broth with less than 0.5% NaCl and no sucrose, higher binding affinity of OmpF antibodies was observed.

Significance: This study not only identified factors that inhibit the growth of *Ah*, but also solicited conditions that can improve the recovery efficacy of *Ah* from food and environmental samples via immunoassays.

T14-02 Heavy Metal Tolerance of *Salmonella* Typhimurium Strains with *Salmonella* Genomic Island 3

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Introduction: *Salmonella* Typhimurium remains one of the top strains responsible for outbreak-associated foodborne illness cases. Heavy metal tolerance in *Salmonella* Typhimurium is concerning, as it could improve strain fitness in animal operations where heavy metals are added as feed supplements. *Salmonella* Genomic Island 3 (SGI3) is a mobile genetic element with heavy metal resistance genes that can be used to predict a strain's phenotype.

Purpose: To evaluate the phenotypic heavy metal tolerance of *Salmonella* Typhimurium strains which are positive or negative for SGI3.

Methods: Three groups of *Salmonella* Typhimurium strains (6 human isolates with SG13, 6 human isolates without SGI3, and 6 bovine isolates without SGI3) were tested for minimum inhibitory concentration (MIC) of zinc chloride and copper sulfate using a broth microdilution method (2-fold dilutions, 0 to 32 mM). Two inoculum concentrations were tested (10^5 CFU/mL and 10^7 CFU/mL). After incubation at 37°C under aerobic (24 h) or anaerobic (48 h) conditions, absorbance was measured at 600 nm, with growth represented by OD values > 0.2. MIC was defined as the lowest concentration that inhibited growth. Experiments were performed in triplicate.

Results: In aerobic conditions with the lower initial inoculum, MICs for copper and zinc were 16 and 4 mM for all strains, respectively. Anaerobically, the zinc MIC was 2 mM for all strains, while the copper MIC was 8 mM for SGI3-positive strains and 0.5 mM for SGI3-negative strains. Increasing the inoculum raised the zinc MIC by one dilution for all strains and the copper MIC by one dilution for SGI3-negative strains.

Significance: *Salmonella* strains with SGI3 have higher tolerance to copper under anaerobic conditions such as those found in an animal gut. Initial inoculum concentration can impact MIC values for SGI3-negative strains. Surveillance of SGI3 presence in *Salmonella* strains could help identify strains with higher fitness potential.

T14-03 Application of the Human Intestinal Enteroid System for Culturing Infectious Norovirus Recovered from Surface Swabs

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❖ ***Developing Scientist Entrant***

Introduction: Human noroviruses (HuNoVs) are the leading cause of foodborne illness and surfaces are a significant transmission route in food handling environments. Surface swabbing is critical for monitoring HuNoVs, but detection has been limited to molecular methods that cannot differentiate infectious viruses. HuNoV can be grown in human intestinal enteroids (HIEs), but the ability to grow HuNoV recovered from swabs and in the presence of the surrogate virus MS2 has not been examined.

Purpose: We used the HIE HuNoV culture system to evaluate infectivity of HuNoV recovered from surface swabs, both alone and mixed with MS2.

Methods: Veneered fiberboard, representing common high touch surfaces, was inoculated with HuNoV ($7.5x10^4$ – $6x10^6$ RNA copies/3 cm^2), MS2 (5 - 230 Plaque Forming Units/3 cm^2), or a 50/50 mixture. The surface was swabbed with macro-foam swabs pre-moistened in phosphate buffered saline (PBS) plus 0.02% Tween80. Swab eluate was tested for HuNoV and MS2 using molecular and culture methods.

Results: HuNoV recovered from swabs in PBS plus 0.02% Tween80 replicated in HIEs. Thirty-three swabbing experiments were conducted and average HuNoV recovery efficiency by molecular methods was 13%. HuNoV recovered from nine experiments grew in HIEs, with an average 250-fold increase in RNA copies between 1 and 72 hours post infection. A minimum of $1.5x10^5$ HuNoV RNA copies/3 cm^2 was needed to observe growth in HIEs. Recovered HuNoV grew in HIE culture in the presence of low amounts of MS2 surrogate inoculum (5 PFU/3 cm^2).

Significance: The HIE culture system can be used to monitor infectious HuNoV recovered from swabbing. However, high amounts of HuNoV are required to measure HIE growth and the presence of MS2 surrogate virus may inhibit the HIE system. This work can inform the application of the HIE culture system to measurement of HuNoV in a range of food handling and processing scenarios.

T14-04 *Lactobacillus casei* expressing Internalins AB Genes of *Listeria monocytogenes* Protects Caco-2 Cells from Listeriosis-associated Damages under Simulated Intestinal Conditions

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* has been implicated in a number of outbreaks including the recent largest outbreak in South Africa. It is an intracellular pathogen that survives ingestion, proliferates in the gut and subsequently cause listeriosis. Therefore, inhibition of its adherence to receptors on the intestine, under simulated intestinal conditions is crucial in controlling its infection.

Purpose: Here, the ability of *Lactobacillus casei* expressing the *L. monocytogenes* invasion proteins Internalin A and B (*inlAB*) to prevent infection under simulated intestinal conditions was investigated.

Methods: Recombinant *L. casei* adhesion to, invasion and translocation through enterocyte-like Caco-2 cells was examined under simulated intestinal conditions. Three different mechanisms of inhibition; competitive adhesion, inhibition and displacement of adhesion; of *L. monocytogenes* by the *L. casei* strains were analyzed. Effects of Caco-2 exposure time to *L. casei* strains, on the inhibition of *L. monocytogenes* adhesion was also analyzed. Furthermore, its mediated cytotoxicity on Caco-2 cells and impact on tight junction integrity were analyzed.

Results: The recombinant *L. casei* (Lbc^{InlAB}) strain showed significantly higher ($P < 0.0001$) adherence to, invasion and translocation through Caco-2 cells than the wild-type *L. casei* strain (Lbc^{WT}). All *L. casei* strains were able to compete and inhibit adhesion of *L. monocytogenes*, however, there was no significant difference in the displacement. Over time, pre- exposure to Lbc^{InlAB} showed enhanced reduction of *L. monocytogenes* adhesion, invasion and translocation through the cell monolayer than Lbc^{WT}. Furthermore, pre-exposure of Caco-2 cells to Lbc^{InlAB} significantly reduced *L. monocytogenes*-induced cell cytotoxicity and epithelial barrier dysfunction.

Significance: Recombinant *L. casei* expressing internalin AB shows potential for use as a prophylactic intervention strategy for targeted control of *L. monocytogenes* intestinal infection phase.

T14-05 Two Multiplex Real-time PCR Assays for the Detection of > 30 Beverage-relevant Beer Spoilage Bacteria

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Introduction: A spoiled beer may be recognized in different ways. In less severe cases, unwanted turbidity may be observed, either due to the high number of microorganisms or as a result of pH changes. In other cases, microorganisms cause an undesired change of flavor.

Purpose: *Lactobacillus*, *Pediococcus*, *Megasphaera* and *Pectinatus* (LPcMPn) are the most troublesome microorganisms for breweries. Some species tolerate hop acids, and especially the presence of horA and horC genes has been shown to correlate with the ability of isolates to grow. The purpose was to develop two assays to detect multiple parameters in parallel.

Methods: BIOTECON Diagnostics has developed two BeerScreening LyoKits. Both provide all necessary reagents and a control template for reliable interpretations of results. To prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included in both assays.

Results: We successfully established two multiplex assays: With the foodproof BeerScreening LyoKit the bacterial DNA is detected in channels FAM (>30 beer spoilage bacteria (LPcMPn)), HEX (identification of *L. brevis*) and ROX (horA and horC). A negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of DNA of beer spoilage bacteria and hop-tolerance related genes in the sample. With the foodproof BeerScreening 2 LyoKit the bacterial DNA is detected in channels FAM (*Lactobacillus* and *Pediococcus* spoilage bacteria (LPc)), HEX (*Megasphaera* and *Pectinatus* spoilage bacteria (MPn)) and ROX (horA and horC). Both kits are tested using the LightCycler 480 System, the Dualo32 instrument and other platforms.

Significance: These are the first two lyophilized qPCR kits on the market detecting >30 beer relevant microorganisms in a single reaction plus horA / horC. Since conventional microbiological methods for the detection and identification of beer spoilage bacteria are very time-consuming, qPCR as a highly sensitive and specific detection method has been introduced into the beverage industry.

T15-01 Environmental Sources of Lymph Node Infections with Non-typhoidal *Salmonella* in Calves

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❖ ***Developing Scientist Entrant***

Introduction: The inclusion of peripheral lymph node (LN) tissue in ground beef contributes to contamination and foodborne transmission of non-typhoidal *Salmonella* (NTS). However, the source and timing of LN infections in cattle are unclear. Previously, our lab recovered multi-drug resistant NTS serovars in the LN tissue of 20-week old veal calves, despite a low prevalence in on-farm samples, suggesting other exposures were responsible for infections.

Purpose: Therefore, the objective of this prospective cohort study was to assess the prevalence and strain types of NTS at additional points in veal production. We hypothesized that NTS strains present in LNs would be indistinguishable from strains present in the trailer or holding pen environments.

Methods: Nine cohorts of roughly 82 calves each were enrolled between November 2018 and July 2019. Environmental swabs were taken in the source barn (*n* = 6), livestock trailer used to haul calves to the harvest facility (*n* = 8), and harvest facility holding pens (*n* = 8). Trailer and pen samples were collected before and after calf entry. We collected mesenteric LNs from 35 calves per cohort and pooled prefemoral LNs from 25 calves per cohort. Sample culture, enrichment, and analysis were conducted by Wisconsin Veterinary Diagnostic Laboratory.

Results: In general, environments were highly contaminated with NTS isolated from 70.8% (51/72) of trailer and 91.7% (66/72) of holding pen samples. NTS was confirmed in 30.8% (91/295) of mesenteric LNs and in the prefemoral LNs of three cohorts. NTS prevalence in LNs was variable between cohorts, ranging from 0% to 80%. For two cohorts, matching serotypes (Agona, Typhimurium) were recovered from trailer and pen environments and calf LNs.

Significance: Whole genome sequencing of the matching serotypes confirmed lymph node strains were indistinguishable from trailer or holding pen strains within the same cohort, suggesting that mitigation of these exposures could be used to reduce the transmission of NTS through ground beef.

T15-02 Prevalence of *Salmonella enterica* in Backyard Chickens in Vermont and Survey of Owners' *Salmonella* Knowledge and Biosecurity Practices

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Introduction: Backyard flocks of chickens are increasingly common in Vermont and across the U.S. Concurrent with that, the number of outbreaks of salmonellosis associated with live poultry has also increased, with seventy outbreaks of salmonellosis from backyard chickens occurring since 2000. During 2016-2019 an average of 19 Vermonters became ill from *Salmonella enterica* associated with backyard chickens each year. However, the prevalence of *S. enterica* in backyard chickens has not been adequately studied.

Purpose: The purpose of this study was to determine *S. enterica's* prevalence in backyard flocks of chickens in urban and rural settings across Vermont, as well as owners' practices and knowledge.

Methods: We tested 233 chickens from 28 farms across Vermont. We performed an owner survey on husbandry, biosecurity practices and knowledge of *S. enterica* and took cloacal swabs from each chicken. These were enriched in buffered peptone water for 20-24 hours at 37°C, followed by standard Rappaport-Vassiliadis and Tetrathionate selective enrichments. Positive samples were then streaked onto XLT4 agar. Presumptive positives were confirmed by PCR for *hilA.*

Results: We found no *S. enterica*. However, we found that only 51.7% of people surveyed changed their shoes after walking around their chickens' area, and 34.5% did not wash their hands after handling eggs. Also, 34.5% snuggled with their birds. Happily, 83% of owners knew chickens could carry *S. enterica* while appearing healthy and 86.2% of people surveyed washed their hands after handling their birds. However, 52% of owners thought their eggs were safer than commercial eggs, and 15% thought raw cookie dough made with their eggs was safer than dough made with commercial eggs.

Significance: These data indicate the prevalence of *S. enterica* in backyard chickens in Vermont is low. However, some owner attitudes and practices put them at risk for contracting *S. enterica* from their birds.

T15-03 Shelf-life Extension of a Belgian Artisanal Burger by Biopreservation

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Introduction: Nowadays people are turning to natural products and are asking food industries to develop products with less or no chemical additives because of the environmental and ecological impacts of these additives.

Purpose: The aim of this study is to extend the shelf life of an artisanal burger by promoting some bacteria of interest naturally present in the product during its shelf life.

Methods: A first step of bacterial screening was performed by storing the product at 4°C (3 days) + 8°C (5 days), under modified atmosphere (MAP: 20% CO_2 + 80% O_2). Potential bioprotective and spoiling bacteria isolated at the end of shelf life were used for challenge testing. Four different batches including control batch, batch inoculate with spoiling bacteria, batch inoculated with bioprotective bacteria and batch inoculated with both spoiling and bioprotective bacteria were studied. Conventional microbiology, qPCR and 16S rDNA metagenomics were conducted to highlight the bacterial diversity, their concentration and their identity. Physico-chemical parameters (A_w, pH, proteins, fat, biogenic amines, aldehydes, and sensory analysis were carried out using ISO methods.

Results: High potential bioprotective bacteria (*Lactobacillus fuchuensis, Lactobacillus sakei, Leuconostoc carnosum, Carnobacterium maltaromaticum, Staphylococcus equorum*) and potential spoiling bacteria (*Pseudomonas lundensis, Pseudomonas fragi, Pseudomonas gesardii, Leuconostoc gelidum, Leuconostoc mesenteroides and Brochothrix thermosphacta*) were identified by metagenetics at the end of shelf life and isolated with conventional microbiology. Sensory analysis combined with metagenetic analysis showed that two different cocktails (*S. equorum* + *C. maltaromaticum*+ *Lb. sakei*and *Lb. fuchensis* + *C. maltaromaticum*+ *Lb. sakei*) of bioprotective bacteria secure the extension of the shelf life from 8 days to 13 days.

Significance: 16S rDNA metagenomic analysis is a breakthrough approach to identify and follow bacterial dynamics. This study allowed the development a new product with long shelf life with less conventional additives and reducing waste due to early spoilage phenomena.

T15-04 *Nigella sativa* and Kefir as Antibiotic Alternatives to Promote Growth and Enhance Broiler Health

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❖ ***Developing Scientist Entrant***

Introduction: The poultry industry has significant challenges of coccidiosis and necrotic enteritis (NE) as acute diseases. NE prevalence can reach 30-50% in broilers in winter with no antibiotic treatment, leading to high mortality and unacceptable growth.

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

Methods: *In vitro* studies were conducted to identify a black seed oil with strong anti-*Clostridium perfringens* (CP) activity. *In vivo* studies consisted of 384 Cobb 500 male broiler chicks in each trial distributed in a randomized block experimental design. The first trial compared three inclusion levels (1, 2, and 5 mL/kg) of black seed oil in feed, with birds challenged with coccidia and CP strain CP6. Trial 2 included black seed oil (2 and 5 mL/kg) in feed and kefir (1:10) in drinking water, challenged with coccidia and CP strain CP4. For both trials, broiler live performance, disease outcomes and CP populations were measured.

Results: Four brands of black seed oil were analyzed and one brand was selected with the highest anti-CP activity. In trial 1, feed conversion for black seed oil-treated groups was higher than antibiotic control, while NE scores for treatment groups were lower than control groups. Black seed oil of 2 mL/kg had no mortality and 5 mL/kg was similar to that of antibiotic control. In trial 2, kefir treatment increased ($P < 0.05$) weight gain and improved feed efficiency. Lesion scores in black seed oil of 5 mL/kg and kefir treatments were not different ($P > 0.05$) from antibiotic control. In conclusion, both black seed oil and kefir improved broiler live performance and reduced the incidence of NE lesion scores and mortality rate.

Significance: *Nigella sativa* and kefir enhanced broiler growth performance and alleviated NE.

T15-05 Foodborne Pathogen Surrogates Reduction Using Antimicrobial Interventions Capable of Reduced Water Use Demand during Beef Harvest

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❖ ***Developing Scientist Entrant***

Introduction: Conservation of food safety intervention-purposed water should not compromise microbiological safety of beef carcasses during harvest. Multiple opportunities exist for the Small and Very Small beef harvesting establishments to reduce water usage during food safety intervention application, but little data exist describing the efficacy of such interventions.

Purpose: This study was conducted to develop and validate the efficacy of food safety interventions that were designed to reduce pathogen surrogates on beef carcasses while lowering food safety-purposed intervention water consumption.

Methods: Hot beef carcass cuts (brisket and shoulder/clod) were inoculated with a gelatin-based slurry containing 6.80 ± 03 log CFU/mL of non-pathogenic *E. coli* strains (ATCC BAA-1427, BAA-1428, BAA-1429, BAA-1430, BAA-1431). After 15 min of attachment, carcass cuts were halved for sampling. Samples were assigned 1 of 5 treatments: a conventional lactic acid spray (LA) (2.5%, 55°C), a lactic acid spray delivered through electrostatic spray system (ESS) (2.5%, 55°C), a conventional hot water spray (HW) (82°C), a recycled hot water spray (RW) (82°C), and an inoculated, untreated control. One hundred mL of each treatment was applied to surfaces of inoculated cuts. Residual hot water was collected, volume recorded, and reheated to at least 82°C before reapplication. Surviving surrogates were enumerated onto 3M® *E.coli/*Coliform Count petrifilms.

Results: Statistical analysis identified treatment type as a significant main effect on resulting data. Least square means for LA (4.0 log CFU/300 cm^2) and ESS (4.1 log CFU/300 cm^2) were statistically lower versus surviving *E. coli* surrogate counts from the control (5.4 log CFU/300 cm^2) and both hot water treatments (HW 5.1 log CFU/300 cm^2 and RW 5.1 CFU/300 cm^2).

Significance: For the Small and Very Small beef harvesting establishments, the use of reclaimed water as beef safety interventions provides equivalent efficacy as conventional, single-pass hot water interventions while reducing water demand for beef safety protection.

T15-06 Virulence Attenuation Effect of Medium- and Long-chain Fatty Acids on *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a significant food-borne pathogen, with multiple genes involved in its virulence. Medium- and long-chain free fatty acids (FAs) may inhibit pathogen growth and decrease virulence gene expression, in a species dependent manner.

Purpose: To investigate the impact of free FAs on expression of *L. monocytogenes* genes, in particular those contributing to virulence, using RNA sequencing.

Methods: Commercial free FAs including C16:1, C18:1, conjugated C18:2, C18:2, and C18:3 and FAs derived from beef fat including total beef fat, beef FA fractions enriched for mono-unsaturated fatty acids (UFAs) or poly-UFAs were used in this study. Log phase *L. monocytogenes* was exposed to FAs for 3 h at their respective minimum inhibitory concentrations. Total RNA was then extracted and subsequently subjected to rRNA depletion. The libraries were sequenced on an Illumina HiSeq4000 platform (100 bp paired-end). Good quality reads (mean 9.53 million; sd 3.89) following quality-based filtering and trimming were mapped to the *L. monocytogenes* EGD-e reference genome assembly. Differential gene expression analyses were conducted using the 'edgeR' Bioconductor package in R. Ten confirmed virulence genes (*prfA*, *mpl*, *InlA*, *InlB*, *inlC*, *plcA*, *plcB*, *actA*, *hly* and *uhpt*) were further analyzed. The experiment was conducted in triplicate.

Results: Principal component analysis (PCA) based on gene counts showed separation of samples by treatment groups, with C16:1, C18:2 and C18:3 (c1), control and mUFAs, and the remaining 4 groups in different clusters. The number of DE genes found (false discovery rate < 0.05 and at least 2.5 fold change) in each treatment group were reflective of clustering by PCA. Of the 10 virulence genes, all but *mpl* were down-regulated in all groups from c1, and 8, 3 and 6, and 0 were down-regulated in cj.C18.2, pUFAs and TBF, and in mUFAs and C18.1, respectively.

Significance: FAs including those from beef may be explored as an alternative to antibiotics against *L. monocytogenes* to combat antimicrobial resistance.

T15-07 Protective Cultures Inhibit *Staphylococcus aureus* Growth and Enterotoxin Production

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❖ ***Developing Scientist Entrant***

Introduction: *Staphylococcus aureus* is a common contaminant in milk products including cheese. Despite efforts to control *S. aureus*, recalls and outbreaks associated with the pathogen continue to occur, highlighting the need for additional interventions.

Purpose: The purpose of this study was to investigate the potential for commercial protective cultures (PC) to control the growth of *S. aureus* and the production of staphylococcal enterotoxins (SE) in growth medium and raw milk.

Methods: Coculture assays were conducted in raw milk using a cocktail of enterotoxigenic *S. aureus* added at ~2 log CFU/mL and stored at 4°C for 24 h. PCs were then added at ~7 log CFU/mL and samples were incubated for one week following a typical cheesemaking time and temperature profile. Enterotoxin production assays were conducted as cocultures in raw milk and BHI using varying concentrations of *S. aureus* and PCs. The production of SEs after 24 h at 37°C was measured by commercial ELISA. All experiments were repeated three times and significance was determined at $P < 0.05$.

Results: *Hafnia alvei* and *Lactococcus lactis* were the most effective in inhibiting *S. aureus* growth in raw milk with counts ~5 log CFU/mL lower than control. Two *Lactobacillus plantarum* cultures also reduced pathogen growth by ~1.5 log CFU/mL compared to control. Several PCs reduced SE production in raw milk and growth medium. Cocultures of *S. aureus* with *Lc. lactis*, *H. alvei* or *Lb. plantarum* in raw milk reduced SE levels by 24.9%, 62.4%, and 76%, respectively, compared to control while pathogen growth was minimally inhibited. *Lc. lactis* also decreased SE production in the absence of PC-mediated inhibition of pathogen growth. Moreover, *Lb. plantarum*, *H. alvei*, and *Carnobacterium* spp. reduced SE production in growth medium.

Significance: These results demonstrate the potential for commercial PCs to not only control *S. aureus* growth, but also SE production in growth medium and raw milk products.

T15-08 *Listeria monocytogenes* in Cheese - a Model to Determine the Concentrations of Undissociated Lactic Acid in Cheese and to Predict Complete Growth Inhibition

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Introduction: Specific microbiological criteria for *Listeria monocytogenes* in RTE foods have been set in regulation (EC) No 2073/2005. For this category of food products it is important to establish whether the food can support growth of *L. monocytogenes* or not. Challenge tests have demonstrated 'no growth' on Gouda cheese, and we established that the major inhibitory factor in this cheese is undissociated lactic acid.

Purpose: The purpose of the current study was to assess whether major factors that inhibit growth of *L. monocytogenes* in Gouda cheese are also determining factors in other types of cheese.

Methods: We developed a model to calculate the concentration of undissociated lactic acid in cheese by taking important product parameters into account (fat content, water content, pH, lactic acid and salt). The growth rates of *L. monocytogenes* in various different cheese types were calculated using a predictive model, taking the factors undissociated lactic acid, temperature, pH and a_w into account (based on available literature data of different cheese types). Experimental growth/no growth data for *L. monocytogenes* for the different cheese types were obtained from the scientific literature. The outcomes of the modelling were compared with the reported growth/no growth.

Results: For 9 out of 10 different cheese types, correct predictions of growth/no growth were obtained: no growth was correctly predicted for Feta, Cheddar and Gouda, and growth was correctly predicted for Ricotta, Queso Fresco, Camembert, high-moisture Mozzarella, Cottage and Blue. Growth of *L. monocytogenes* was not observed in practice upon inoculation of Emmental, whereas growth was predicted when including the above mentioned factors in the models. Other factors that are thought to be important to inhibit growth in this cheese type.

Significance: The results from our study show the importance of undissociated lactic acid, temperature, pH and aw for full inhibition of *L. monocytogenes* in cheese.

T16-01 Role of Plant Type in the Colonization of Mature Fruit by Salmonella

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Introduction: Fresh produce has been implicated in numerous food-borne outbreaks involving *Salmonella enterica*. Because female blossoms are a direct route for pathogens to mature fruit, differences in flower type between commodities may contribute to *Salmonella* colonization.

Purpose: To compare the ability of *Salmonella* to colonize different fruit commodities during pre-harvest blossom inoculation.

Methods: Cucumber, cantaloupe, and tomato plants were grown from commercial seed and maintained in the NCSU BSL-3P greenhouse. *Salmonella* (a cocktail composed of a mixture of 5 of the following serovars Javiana, Montevideo, Newport, Panama, Poona, Saintpaul or Typhimurium) contamination was introduced via blossoms at ca. 3.9-4.5 $\log_{10}$ CFU/blossom. Mature fruits were analyzed for *Salmonella* (surface and inside) by enrichment in accordance with modified FDA-BAM methods. Data were analyzed for prevalence of contamination (surface and inside), and the Pearson Chi-Square test was used to determine significant differences in prevalence of contamination by commodity.

Results: Of the total fruit harvested from *Salmonella*-inoculated blossoms [cucumber (n = 59), cantaloupe (n = 63) or tomato (n = 200)], 81.4% (48/59), 89.0% (56/63), or 14% (28/200) were found colonized and 35.6% (21/59), 73.0% (46/63) or 2.5% (5/200) had *Salmonella* internalized into the fruit, respectively. Prevalence of surface contamination was equivalent when comparing cucurbits (cucumber vs. cantaloupe) (X^2=1.375, P = 0.3094). However, significant differences were observed in internalization between cucurbits (X^2=17.234, $P < 0.0001$). Surface contamination was significantly lower in tomatoes than cucumber (X^2=99.694, $P < 0.0001$) or cantaloupe (X^2=123.603, $P < 0.0001$). *Salmonella* internalization of tomatoes was significantly lower than observed in cucumber (X^2=55.250, $P < 0.0001$) or cantaloupe (X^2=152.403, $P < 0.0001$).

Significance: The ability of *Salmonella* to colonize and internalize fruit during pre-harvest was dependent upon plant species, with higher prevalence in cucurbits as compared to tomatoes.

T16-02 Chlorine Resistance and Sub-lethal Injury of Long-term Survival Phase *Escherichia coli* in In-Vitro Planktonic Cells and Cells Attached to Romaine Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: The use of sanitizers such as chlorine is a common practice in post-harvest washing of fresh produce. The effectiveness of chlorine on *Escherichia coli* cells in stationary phase of growth is well known; however, little is known about the sanitizer efficacy against bacteria in the long-term survival phase.

Purpose: The objective of the study was to evaluate the difference in inactivation of stationary phase (STAT) cells and long-term survival phase (LTS) cells of *Escherichia coli in vitro* and in a lettuce wash model.

Methods: Four lettuce-outbreak strains of *E. coli* (O145, O26, O121, O157:H7) were used as a cocktail, and treated with three different concentrations of free chlorine (0.25 – 40 ppm). The *E. coli* strains were cultured separately in tryptic soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE; 35°C) for 24 h to obtain stationary phase cells and for 21 days in TSBYE (35°C) to obtain LTS cells. Round shaped lettuce leaf coupons (6.15 cm^2) were spot inoculated using 50 µL of STAT and LTS cell inoculum. The cell cultures were treated for 30 s with different chlorine concentrations in triplicate and reaction was neutralized using sodium thiosulfate (5% w/v).

Results: In the *in vitro* model, higher resistance of LTS cells was observed compared to STAT cells for all chlorine concentrations tested ($P < 0.05$). Additionally, the average sublethal injury percentage was more for STAT as compared to the LTS cells at all concentrations ($P < 0.05$). However, statistically insignificant results were observed in the lettuce model ($P > 0.05$) with maximum log reduction of < 2 log CFU/mL and no statistical difference in sublethal injury between the two types of cells ($P > 0.05$).

Significance: Diverse factors including organic content, pathogen load, and produce type may have significantly affected the efficacy of chlorine treatment. Further research is suggested to better understand the resistant behavior of LTS cells in the food supply chain, which may lead to optimizing industrial treatment processes and minimizing food safety risks.

T16-03 The Use of International Genomic Data to Complement Traditional Hypothesis-generation Methods during a Multi-provincial *Salmonella* Enteritidis Outbreak Investigation (Canada, 2019)

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Introduction: In February 2019, Canadian health authorities initiated a national outbreak investigation into a cluster of *Salmonella* Enteritidis cases related by whole genome sequencing (WGS).

Purpose: The investigation aimed to determine the source of the *S.* Enteritidis outbreak and implement control measures.

Methods: A case was defined as a resident of or visitor to Canada with lab-confirmed *Salmonella* infection and an isolate matching the outbreak cluster by WGS. WGS data from case isolates were compared to international databases. Investigators also employed centralized case interviewing, food purchase histories, and food safety investigations.

Results: Eighty-five cases were identified in six provinces. Onset dates ranged from November 6, 2018 to May 10, 2019, case ages ranged from 1 to 88 years (median = 51), and 60% of cases were female. Twenty-two hospitalizations and three deaths were reported. Among cases with available information, 4/64 reported travel to Thailand. International WGS matches included clinical isolates from the United States ($n = 8$) and the United Kingdom ($n = 32$), and non-clinical isolates from the United Kingdom ($n = 4$) and Thailand ($n = 1$). The majority of US and UK cases reported travel to Thailand. The UK non-clinical isolates were products imported from Thailand; the Thai isolate was sampled from a local market. Three case purchase histories revealed Brand X frozen profiteroles and/or éclairs purchased prior to illness. Food safety investigations determined that Brand X products originated in Thailand, and were first imported to Canada in October 2018. Following centralized re-interview, 21/26 (81%) of cases with available information reported consuming profiteroles, éclairs, or both. Brand X frozen profiteroles and éclairs were identified as the source of the outbreak. These products were recalled on April 26, 2019.

Significance: International genomic data complemented traditional outbreak investigation hypothesis-generation approaches and supported the identification of Brand X frozen profiteroles and éclairs from Thailand as the source of a multi-provincial outbreak of *S.* Enteritidis.

T16-04 Use of Molecular Typing in the Investigation of Cases of Cyclosporiasis, 2019

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Introduction: Most cases of gastrointestinal illness caused by the foodborne parasite *Cyclospora cayetanensis* cannot be linked, and improved genotyping approaches could be useful to support outbreak investigations.

Purpose: To assess the utility of a novel genotyping approach by comparing the extent to which it resolved associated cases into the same clusters as epidemiologically-linked cases.

Methods: Cases linked by epidemiologic investigations in 2019 were analyzed by sequencing eight *Cyclospora* loci from fecal specimens; algorithms next assessed the likelihood that any specimen pair was related. Results were visualized (dendrograms/networks) to observe genetically tightly grouped specimens. Genetic clusters were compared to epidemiologically-defined clusters to assess the concordance between these two approaches. We defined concordance as all cases assigned to a cluster epidemiologically that were assigned to a corresponding cluster (the largest corresponding cluster if there was more than one) based on tight genetic linkage. Only samples from epidemiologic clusters with multiple samples genotyped were included.

Results: Specimens from 1,110 cases were genotyped, of which 123 were epidemiologically categorized as being part of one of 31 clusters. Genotyping assigned these 123 specimens to one of eight genetic clusters. Eight samples were excluded from concordance analysis because one sample from these epidemiologic clusters was genotyped. Ninety of the remaining 115 specimens (78.3%) assigned to epidemiologic clusters were assigned to corresponding genetic clusters. Several epidemiologically-defined clusters corresponded to multiple genetic clusters; one epidemiologic cluster was divided into three genetic types.

Significance: It has been historically difficult to identify food vehicles for cyclosporiasis and link cases associated with common food exposures due to the absence of a robust genotyping approach. Our method generally assigned cases into clusters concordant with epidemiologic linkages, and for some epidemiologic clusters, identified sub-sets of cases that were genetically linked, raising the question that some epidemiologic clusters may result from multiple strains.

T16-05 Investigating a *Salmonella* Outbreak: How North Carolina Public Health, Environmental Health and Department of Agriculture Collaborated to Find a Source

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Introduction: Outbreak investigations are evolving to require collaboration among partners and to include more sophisticated techniques to improve efforts to find, investigate and determine the source.

Purpose: An outbreak of *Salmonella* London was detected and investigated after several months of sporadic cases in Nash County North Carolina through collaboration of multiple state and local agencies.

Methods: The case findings were accomplished through retrospective review of *Salmonella* London diagnoses from May through December 2019. The multi-agency team who conducted the investigation included state and local communicable disease nurses, epidemiologists, environmental health specialists and the North Carolina State Laboratory of Public Health; the North Carolina Department of Agriculture and Consumer Services RRT team and their laboratory assisted to conduct environmental swabbing inside the restaurant. Stool cultures were collected and analyzed to complete the investigation and environmental assessments were conducted.

Results: Fifteen illnesses were identified and linked using whole genome sequencing (WGS). Nine out of 73 (12%) environmental samples were found to be positive for *Salmonella* London. Locations positive included food contact surfaces such as the pork chopper, barbecue storage bins and reusable heat-resistant gloves. Remediation included cleaning and sanitizing the equipment and other areas within the establishment, followed by repeat environmental sampling and testing all food service employees via stool culture analysis.

Significance: This outbreak illustrates the importance of WGS and environmental swabbing to assist with detection of outbreaks and their sources. Advances in outbreak detection and investigation methods combined with increased collaboration can lead to an improved understanding of foodborne illness and an increased ability to protect public health.

T16-06 Retrospective Foodborne Illness Cluster Evaluation, Outbreak Investigation, and Interagency Collaboration

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Introduction: In 2019, the Food and Drug Administration's (FDA) Office of Coordinated Outbreak Response and Evaluation (CORE) Signals and Surveillance Team established weekly meetings including partners from FDA, the Centers for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) to evaluate retrospective isolate clusters of interest for potential follow-up investigative activities.

Purpose: We hope to demonstrate the challenging, complex, and resource-intensive nature of retrospective foodborne illness cluster evaluation and related outbreak investigations.

Methods: Traditional outbreak investigations rely on epidemiologic information to identify a possible food vehicle, while traceback and/or sampling is used to confirm a link between illnesses and the vehicle. Retrospective cluster analysis begins with the identification of a possible vehicle, due to the isolation of a foodborne pathogen from product or environmental sample and laboratory analyses, through whole genome sequencing (WGS), and identification of highly related clinical isolates. This is followed by epidemiologic and traceback investigations to confirm their association. In 2019, FDA evaluated Single Nucleotide Polymorphism (SNP) *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp. clusters in the National Center for Biotechnology Information Pathogen Detection database for potential ongoing outbreaks.

Results: Over 100 clusters were evaluated among FDA partners and 26 were selected for sharing with the interagency group. They comprised of 11 *Salmonella* spp.,10 *L. monocytogenes*, and 5 *E. coli* clusters. Four *L. monocytogenes* clusters, 3 *Salmonella,* and 3 *E. coli* clusters were further investigated for supporting epidemiologic information. Four investigations led to regulatory activities and two led to public communications.

Significance: The use of the retrospective cluster pathway is critical for the identification of linkages between contaminated food and illnesses. Additionally, the process allows for the attribution of food vehicles to a small numbers of illnesses, making it key to the FDA's focus on preventing foodborne illness.

T17-01 Addition of Potassium Sulfite Improves Recovery and Detection of Listeria monocytogenes from Garlic Powder

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Introduction: Garlic powder is considered a low-risk matrix for *Listeria monocytogenes* contamination because of its low moisture and proven antimicrobial features. However, recent recalls of low-moisture foods contaminated with *L. monocytogenes* increased testing for this foodborne pathogen in garlic powder. Due to the antimicrobial properties of garlic, the detection is quite challenging.

Purpose: The objective of this study was to evaluate the efficacy of adding potassium sulfite (K_2SO_3) into enrichment media for recovery and detection of *L. monocytogenes* from garlic powder.

Methods: Twenty-five (25) g of garlic powder was homogenized with 2,475 mL media with or without 0.5% K_2SO_3 and inoculated to 10^4, 10^3, and 10^2 CFU *L. monocytogenes*/test portion. Three replicate portions per media and inoculation level were prepared. Two enrichment media and analysis protocols were used. For the FDA BAM cultural method, samples were enriched in BLEB with additives for 24 and 48 hours at 30°C; enrichments were streaked to MOX and ALOA plates and suspect colonies were confirmed using *Listeria* API. For BAX real-time PCR, samples were pre-enriched with Demi-Fraser at 30°C for 24 hours, transferred to MOPS-BLEB and incubated at 35°C for an additional 24 hours. The presence of *L. monocytogenes* was tested using BAX PCR following the manufacturer's instructions. All results were confirmed using FDA BAM cultural method.

Results: The results showed that without K_2SO_3 neither method recovered or detected *L. monocytogenes* in any sample at the level of 3.2 log/test portion. The addition of 0.5% K_2SO_3 to the pre-enrichment media resulted in improved detection of *L. monocytogenes* to 100% for both the molecular and cultural methods in garlic powder at the level of 2.8 log/test portion.

Significance: This study demonstrated that the addition of 0.5% K_2SO_3 to enrichment media can improve the recovery and detection of *Listeria monocytogenes* from garlic powder.

T17-02 Automated Surface-scanning Detection of *Salmonella* Typhimurium on Chili Pepper

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❖ ***Developing Scientist Entrant***

Introduction: Microbial detection principle has relied on phenotypic and/or genotypic methods which are complex, expensive, and labor-intensive. Our research group has developed an automatic, wireless, and on-site phage-based magnetoelastic (ME) biosensor for *S.* Typhimurium detection.

Purpose: The purpose of this study was to develop and employ the ME biosensor combined with a surface-scanning detector for the detection of *S.* Typhimurium on chili pepper.

Methods: A ME platform was diced from METGLAS® 2826 MB alloy and annealed at 220°C for 2 h prior to deposition of Cr and Au. Tailed phage (8 log PFU/mL) previously isolated and purified from a poultry plant was immobilized on ME sensor's surface directly and the phage-immobilized sensors were measured by a network analyzer. Fifty microliters of *S.* Typhimurium (2 to 8 log CFU/mL) was inoculated on the chili pepper's surface. After employing the phage-immobilized sensors on the chili pepper's surface, resonant frequency of each sensor was measured, followed by confirmation of bacterial density and coverage by SEM.

Results: Tailed phage was successfully immobilized on ME sensor's surface with density of 410 ± 10 phages/100 μm^2. As concentration of *S*. Typhimurium increased, resonant frequency shifts of phage-immobilized sensors increased in a dose-response manner with R^2 value of 0.95 ($P < 0.05$). Sensitivity and detection limit of ME biosensors were 854 Hz/log CFU and 2.3 log CFU/mL. Based on SEM images, the number (CFU/100 μm^2) and coverage (%) of *S*. Typhimurium bound on the sensors were calculated as 7.8 ± 0.3 and 1.2 ± 0.1, 11.4 ± 0.7 and 1.7 ± 0.1, 432.2 ± 49. 4 and 64.0 ± 7.3, and 617.7 ± 29.7 and 96.8 ± 14.5 at 2, 4, 6, and 8 log CFU/mL, respectively.

Significance: This study demonstrated that phage-immobilized sensor combined with surface-scanning detector had great potentiality as a novel, automatic, and on-site applicable rapid detection for *S.* Typhimurium on fresh produces.

T17-03 Multiple Detection of Murine Norovirus, *Salmonella* spp., *Shigella* spp., and Shiga Toxin-producing *Escherichia coli* from the Same Fresh Produce Portion

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Introduction: Norovirus is the leading cause of foodborne disease worldwide. Norovirus foodborne outbreaks are commonly caused by the consumption of contaminated shellfish, oysters, fruits and vegetables, and ready to eat foods. Fresh produce has also been linked to enteric pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., and Shiga toxin-producing *E. coli* (STEC). Additionally, the simultaneous occurrence of norovirus and enteric pathogenic bacteria in fresh produce has led to coinfection cases.

Purpose: The purpose of this study was to simultaneously detect by polymerase chain reaction (PCR), norovirus and enteric pathogenic bacteria from the same fresh produce portion.

Methods: Tryptic soy broth and modified buffer peptone water (BPWm), selective agents (vancomycin, novobiocin, green brilliant) at two different concentrations respectively (0.8-8, 0.055-0.55, 0.02-0.2 ppm) and two temperatures (35 and 41.5°C) were evaluated in pure culture and in lettuce, coriander, strawberry and blackberry (120 samples). Primers for *Salmonella* (*InvA*), *Shigella* (*IpaH*) and STEC (*Stx1* and *Stx2*) were designed. Murine norovirus primers from American Type Culture Collection were used. Toxicity of glycine buffer on bacterial pathogens was evaluated (18 samples).

Results: No difference between enrichment treatments was observed ($P > 0.05$). Selected conditions were 41.5°C/BPWm/selective agent mixture at high concentration. *Salmonella* growth was 7.63 to 8.91, *Shigella* 6.81 to 7.76 and STEC 7.43 to 9.27 log CFU/mL. The population reached for the APC was 5.11 to 6.56 log CFU/mL. The sensitivity detection was 10-100 PFU for the murine norovirus and 1-10 CFU for each pathogenic bacteria.

Significance: BPWm with selective agents and modification in temperature promotes the simultaneous growth of *Salmonella, Shigella* and STEC. The multiple PCR technique allows simultaneous detection of viral and bacterial pathogens from the same portion.

T17-04 Application of Magnetic Nanoparticles for the Detection of Pathogenic Microorganisms

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Introduction: Magnetic separation is an efficient method for target enrichment and elimination of inhibitors in the molecular detection systems for pathogenic microorganisms.

Purpose: The purpose of this study was to rapidly and sensitively detect pathogenic microorganism using magnetic nanoparticles and PCR.

Methods: We prepared amino-modified, silica-coated magnetic nanoparticles, and used them to adsorb a trace amount of genomic DNA from pathogens. The complexes of magnetic nanoparticles and the target could be used as DNA templates to be added into PCR directly. Then, we prepared magnetic capture probes by modifying oligonucleotides complementary to target mRNA sequences on the surface of ASMNPs to separate the target mRNA. This method could detect the live pathogens. In addition, we labeled the pig gastric mucosal protein (special receptor for human norovirus) on to the surface of the magnetic nanoparticles to separate and enrich the norovirus.

Results: We used the amino-modified, silica-coated magnetic nanoparticles to adsorb a trace amount of genomic DNA from pathogens in raw milk, and then the complexes of magnetic nanoparticles and DNA were used as DNA templates to be added into PCR directly. For *Salmonella* Enteritidis, *Listeria monocytogenes* and *Staphylococcus aureus*, the detection limits could reach 10-100 CFU/mL. Then, we used the capture probes to separate the target mRNA. The magnetic capture probes were used to separate mRNA from *Salmonella* in artificially contaminated milk samples, and then the complexes were used as templates for RT-qPCR. The detection sensitivity was 10^4 CFU/mL. In addition, we used the PGM-labeled magnetic nanoparticles to separate and enrich norovirus, followed by RT-qPCR. The capability of the detection based on magnetic separation for norovirus was enhanced by three times than the PEG precipitation method.

Significance: Magnetic separation has great potential to rapidly and sensitively separate and enrich pathogenic microorganism.

T17-05 Evaluation of Real-time Nanopore Sequencing for *Salmonella* Serotype Prediction

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Introduction: Whole genome sequencing (WGS) technology has been widely utilized in foodborne pathogen identification, for instance to provide results for more reliable *Salmonella* serotype prediction. Emerging long-read sequencing platform developed by Oxford Nanopore Technologies provides an alternative WGS method to meet the needs of industry. Advantages of the technology include portability, real-time base-calling and long-read sequencing.

Purpose: To explore whether nanopore sequencing could accurately predict *Salmonella* serotypes.

Methods: Thirty-eight *Salmonella* strains representing 34 serotypes were sequenced using R9.4 flow cells for up to two hours. The bioinformatics analysis was performed using pipelines with different assemblers including Canu, Wdbtg2 combined with Racon, or Miniasm combined with Racon. *In silico* serotype prediction programs were carried out using both SeqSero2 and SISTR. The same strains were also sequenced by Illumina Hiseq (shot-gun) as a benchmark for serotype prediction.

Results: The serotypes of all 38 strains were correctly assessed using nanopore sequencing with the *in silico* serotype prediction programs SeqSero2 combined with SISTR. Predictions using data generated after 30 minutes, 45 minutes, one hour, and two hours of nanopore sequencing all matched the prediction results from shot-gun sequencing. The entire process from pure culture to serotype prediction was achieved within one day.

Significance: Our study systematically evaluated the performance of *in silico Salmonella* serotype prediction using nanopore sequencing, and successfully proved the potential of nanopore sequencing as an alternative approach to shot-gun sequencing for *in silico* serotype prediction for *Salmonella*, achieving a more rapid, molecular-based *Salmonella* confirmation and serotype classification. This study also provides an optimized procedure for field application of *Salmonella* serotyping and contamination source tracking.

T17-06 Single Lab Validation Study for Simultaneous Isolation of Norovirus and Hepatitis A Virus from High Fat Dairy Products

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Introduction: Foodborne viruses, such as hepatitis A virus (HAV) and norovirus are among the leading causes of epidemic and sporadic gastroenteritis worldwide. Although multi-ingredient foods are frequently implicated in outbreaks, there is a lack of analytical methods for sample preparation and virus detection in such items.

Purpose: During food testing for foodborne virus contamination, we are frequently challenged as a particular food item (i.e., berries) cannot be individually tested if it is part of a more complex product (i.e., frosting or ice-cream). For this reason, we developed a two-day, sensitive method to isolate viral particles and subsequently virus RNA from raspberry ice cream and strawberry frosting.

Methods: The protocol tested fifty-gram portions of each matrix type that has been co-inoculated with both hepatitis A and norovirus and stored for at least two weeks at -20◦C. The preparation involved initially eluting the virus with a 0.1 M Tris-HCl, 0.05 M glycine, 1% beef extract, pH 9.2 (TGBE) buffer containing 2% polyvinyl pyrrolidone (PVP) and pectinase. The eluate was subsequently clarified with chloroform, and virus particles were concentrated with 10% polyethylene glycol (PEG)/0.3M NaCl overnight. The virus-containing pellet was re-extracted with TGBE and, to further concentrate the sample, the PEG precipitation was repeated. Viral RNA was isolated from the resulting pellet using a commercial kit (RNeasy PowerPlant Kit, Qiagen). Viral genome detection and quantification was performed using an in-house real-time RT-PCR assay and full-length RNA standards.

Results: Low contamination of at least 1000 PFU of a lab adapted HAV strain or 100 genomic copies of either GI.1 and GII.4 clinical norovirus isolates could be simultaneously and consistently detected per 50g of ice cream sample. For frosting, detection was successful at one log lower for all three viruses. Mouse Norovirus that was co-inoculated as a process control virus could be detected in all samples and the Internal Amplification Control used during RT-PCR assay suggested that there was not significant inhibition.

Significance: Having a robust protocol to detect low levels of two separate viruses and from different genogroups in two types of dairy products is the first step towards method standardization that can be used as a critical tool during outbreak investigation and regulatory action.

T18-01 Web-based Game Engages Post-secondary Students in Food Safety and Shifts Perceptions

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Introduction: Electronic games and simulations are increasingly used in education; the impact of a web-based food safety game on student engagement, attitudes and cognition warranted investigation.

Purpose: A game to prevent foodborne illness by mitigating risky practices from production through consumer preparation was evaluated for impact on interest in and understanding of safe food handling practices, perception of food systems, and scientific expertise to achieve a safe food supply.

Methods: Student subjects (n = 141) in an introductory animal and food sciences course utilized the game (average 30 minutes) and anonymously completed pre-gameplay and post-gameplay surveys linked by user-generated identifiers according to institutional approvals for human subjects research. Survey questions addressed food safety attitudes (15 questions), behaviors (7), knowledge (12), perception of game play (8) and demographics (16). Knowledge-based questions were balanced for difficulty for pre- and post-gameplay surveys based on formative assessment studies.

Results: Subjects were first-semester college students (84%); some reported learning food safety in high school (55%) and having changed purchasing or consumption habits (65%) after news of a foodborne illness outbreak. Students reported enjoying the game (91%) with consequent increased awareness of (94%) and interest in food safety (83%) and the food science major (66%), and intent to learn more (86%). Food safety attitudes that shifted significantly (P < 0.05) from pre- to post-gameplay included an increased confidence in the food supply and regulation, decreased trust in families' food preparation practices, increased recognized need for scientific knowledge to produce safe food, and increased recognition that companies employ people responsible for food safety. There was no significant change in correct responses to knowledge-based questions. Students performed better on questions concerning consumer practices than microbiology.

Significance: One-time, short-term use of a web-based game increased student interest in and recognition of food safety as a scientific discipline with potential implications for career development.

T18-02 Competing Interests of Food Safety and Food Waste Reduction – What is the Role of Food Date Labels?

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❖ ***Developing Scientist Entrant***

Introduction: Food date label confusion by consumers may contribute to the waste of otherwise safe, edible food, a growing social, economic and environmental problem.

Purpose: The objective of this research was to determine consumer knowledge regarding three common food date labels and their meanings, and whether knowledge correlated with food waste behaviors.

Methods: A ten-question, online survey was administered to 1,042 adults. The survey measured respondents' use and knowledge of food date labels and their propensity to waste food as defined by disposing of food which would likely still be safe for consumption. Chi-square analysis was used to determine differences in food date label use and knowledge and food waste behaviors between age and gender groups. Chi-square was also used to assess to what extent those with higher food date label knowledge wasted food unnecessarily.

Results: The majority of respondents (81%) reported using food date labels. Only 57%, however, correctly identified what "best by, use by" meant, while 68% and 80% correctly identified the definitions of "expiration date" and "sell by date," respectively. Respondents who correctly defined "best by, use by" were less likely to throw food away if it had passed its "use by" date or was shelf stable without a visible date. Those with greater knowledge of what food date labels meant were also more likely to identify the "expiration date" as the only (date label) reason to throw away a food. Conversely, respondents who knew the "sell by" or "expiration date" were significantly more likely to throw away a food just because it had passed the "use by" date.

Significance: Findings indicate that greater knowledge of what food date labels mean may result in reduced food waste. Consumer education around the least understood food date label, "best by, use by" may help to reduce consumer food waste.

T18-03 Evaluation of the Produce Safety Alliance Grower Training Course: Two Years of Outcomes and Impacts for Small Farms

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Introduction: The Produce Safety Alliance (PSA) Grower Training (GT) was developed to meet the educational requirement in § 112.22(c) of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR).

Purpose: Evaluation data from 18,893 participants attending the PSA GT from September 2016 to September 2018 were analyzed to assess perceived knowledge gain, confidence in implementing practices, instructor effectiveness, sufficiency of the information presented and educational materials provided, and voluntary demographic information.

Methods: A total of 15,715 evaluations from registered domestic PSA GT English and Spanish-language courses held in the first two years were analyzed. Statistical analysis was performed using Pearson Chi-Squared and Friedman's tests to identify significant relationships between farm food safety experience and course sufficiency, and module-specific differences, respectively.

Results: More than 95% of participants indicated that the PSA GT was sufficient to guide them in implementing regulatory requirements, irrespective of farm size. Production Water, Postharvest Water, and Postharvest Handling and Sanitation modules had the highest mean reported knowledge gain on a scale of 1 to 5 (4.54, 4.53, and 4.53, respectively), yet among the lowest confidence in implementation (4.43, 4.43, and 4.48, respectively). Prior to attending the full-day PSA GT, 57.4% (n =10,703) participants had no previous produce safety or Good Agricultural Practices training and 53% (n = 10,707) did not have a written farm food safety plan. Farms with greater than $250,000 in annual produce sales were significantly more likely to have attended a previous food safety training than very small farms (≤$25,000 annual produce sales) ($\chi^2(3, n = 10{,}703)=527.7$, $P = 0.000$).

Significance: Participants gained knowledge from attending the PSA GT, even those who had not previously attended produce safety training. Despite gaining knowledge, growers still have significant concerns about how to implement practices, especially related to agricultural water and sanitation. These results indicate continued need for training and technical assistance to achieve PSR compliance.

T18-04 Empowering Small Manufacturers to Obtain Food Safety Certification by Identifying and Overcoming the Barriers

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Introduction: To enable continued growth and success of the food-industry in Wales, the Welsh Government has recognized there is a need to support small food-manufacturing businesses to obtain food-safety certification.

Purpose: To identify the barriers that exist, and determine the desired support mechanisms, to inform the development of a support-package to assist small food-manufacturing businesses to obtain food-safety certification.

Methods: Firstly, focus-groups (n = 7) were conducted with manufacturers (n = 37), stakeholders (n = 19), and scheme auditors (n = 9) to identify barriers to obtaining food safety certification. Secondly, to determine desired support mechanisms, group discussions (n = 2) were conducted with manufacturers (n = 37) and online-questionnaires (n = 29) were completed. Finally, a support-package was designed, developed, piloted and evaluated with food manufacturers (n = 9).

Results: The barriers for Welsh manufacturers to obtain food-safety certification were related to three key categories; 'knowledge and skills', 'time, cost and resources' and 'access to information'. The desired support mechanisms, perceived necessary to obtain food-safety certification included; funding for training, support for implementing scheme documentation, on-site support by means of mentoring/coaching and pre-audits. Findings informed the development of the support-package, which consisted of six mechanisms: (i) self-assessment tool, (ii) internal systems review, (iii)'audit-ready' workshop, (iv) pre-audit factory inspection, (v) post-audit support and (vi) audit-fee contribution. Welsh manufacturers (n = 62) expressed an interest in joining the support-package, eligible businesses (n = 9) participated in the study. Participation in the support-package took manufacturers 4–10 months to complete. Two-thirds of the businesses completed the program (n = 6). Seven companies underwent an official audit; however, only those that had complete the support-package program (n = 6) obtained food-safety certification.

Significance: This study has successfully identified the barriers and delivered a support-package that has enabled small food-manufacturing businesses to overcome such barriers and obtain food-safety certification. Launch of the support-package may assist in accelerating food sector growth in-line with Welsh Government aspirations.

T18-05 Food Safety Listening Sessions with Local Food Producers

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Introduction: Local food producers (LFP), defined as those having a majority of sales within 275 miles of their operation, are vital contributors to food systems across the country with a diversity of food safety needs that must be met in order to maintain the safety of the food supply.

Purpose: The purpose of this study was to identify and evaluate food safety needs and food safety implementation barriers of LFP.

Methods: A nationwide survey was followed by two listening sessions in each of four regions of the country (Northeast, North Central, Southern, Western), for processors and growers. All listening sessions were transcribed and coded using ATLAS.ti 8, a qualitative data analysis software. Deductive analysis was used based on the research questions and session structure to analyze the responses to specific questions. Inductive analysis was used to identify patterns in additional discussion topics.

Results: There were 81 participants in the listening sessions with some attending both grower (n = 54) and processor (n = 27) sessions. Sixteen growers had developed a farm food safety plan and 16 processors had a recall plan for their operations, but only 8 had tested it. Barriers to adopting food safety practices included a lack of resources including time, funding, and labor. LFP want technical assistance from people they trust who possess the expertise and information they need, but LFP felt access to these individuals is limited. Suggestions for improving resources included tailoring training to specific commodity groups or operation size. Worker training was not prevalent as many participants identified they had non-traditional labor.

Significance: LFP need competent technical assistance and additional educational materials to help them implement food safety practices. These results provide guidance for the development of additional food safety educational resources. Many LFP are exempt from federal regulation but because they supply all markets, meeting their educational needs should be a priority.

T18-06 Using the Triple Helix Mechanism to Support Food Safety Development and Its Impact on Food Safety Systems and Commercial Performance

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Introduction: Increasing demand on food safety controls allied with a reduction in many countries of food safety professionals entering the sector have led to an increased demand from the private sector for support in developing and delivering food safety systems.

Purpose: This feasibility study investigated the effectiveness of developing a sector specific food safety support mechanism based on the triple helix model and knowledge transfer principles.

Methods: The development of the study and model involved 3 food technology centers across Wales to allow delivery across the whole country encompassing both rural and urban areas. The system developed incorporated 3 levels of engagement with the sector dependent on industry partner needs. The triple helix model engaged the knowledge based partners, Welsh Government and over food processing companies.

Results: Over 250 food companies engaged with this innovative model of knowledge transfer. The results/ impacts were monitored by the knowledge transfer organizations and validated by the Managing Directors within the private sector partners. Knowledge based partners mentored food companies in food safety systems which helped successfully achieve 89 food safety accreditations including 48 against GFSI approved schemes. This resulted in 744 jobs being created or safeguarded with 71% of partner companies being based in areas of high unemployment. The financial impact was measured and verified at $132,670,000 as new markets opened up through improved confidence in the companies food safety systems.

Significance: This innovative model of utilizing 2 models (triple helix and knowledge transfer) demonstrates that all 3 partners can successfully engage and develop mutually beneficial outputs. Food safety is the cornerstone and basis of the study and delivery; however, this supports a significant need within the private sector. Government is supportive of such a scheme as it promotes industrial growth whilst assuring product safety within the supply chain. The university partner engaged more closely with industry, developed data over the 5 year period of delivery and produced numerous food safety related academic papers from the data.

T19-01 Modeling Salmonella Inactivation in Flour under Dynamic Heating Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Flour is a vehicle for foodborne illness and there is interest for heat treatments to control pathogens. A previous study verified *Salmonella* can be reduced by 3-4 logs during a treatment at 177°C for 5 minutes, but these results did not agree with estimates calculated from a model for *Salmonella* inactivation in flour. The 3-4 log reductions achieved in flour baked in a home toaster oven were lower than the tens of thousands of log reductions predicted by a published model.

Purpose: This study aimed to estimate *Salmonella* inactivation parameters (D_{ref}, z_T, and z_{aw}) using data from oven baking of flour.

Methods: Flour inoculated with *Salmonella* was spread in a layer on a home toaster oven tray and treated at various temperatures (149, 177, and 204°C) for up to 7 min. Sample temperature was measured during treatment using a thermocouple placed at the center of the layer. Water activity was measured and *Salmonella* enumerated after treatment. Inactivation parameters were modeled as a function of time, product temperature, and product moisture.

Results: In this study, parameters were estimated for *Salmonella* inactivation in flour under dynamic conditions with relative errors below 15%. D_{ref} was 82°C at reference conditions of 80°C and 0.52 a_w. At these same reference conditions, D_{ref} was previously estimated as 2.94 min. The estimate for z_T was greater, at 56.2°C as compared to 15.2°C, indicating greater resistance to temperature changes, and z_{aw} was less, at 0.12, as compared to 0.39, indicating less resistance to water activity changes.

Significance: This study underscores the importance of estimating microbial inactivation parameters under dynamic real-world conditions. Applying model parameters estimated under isothermal conditions at lower temperatures may drastically overestimate the lethality of a dynamic process.

T19-02 Inactivation Kinetics of *Salmonella* spp., Shiga Toxin-producing Escherichia coli (STEC), Listeria *monocytogenes*, and a Surrogate (Pediococcus acidilactici) on Macadamia Nuts, Dried Apricots, and Raisins Following Treatment of Low-temperature, Vacuum-assisted Steam

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne pathogens may survive for extended periods on dry, low-water activity foods. Low-temperature, vacuum-assisted steam pasteurization is an effective post-harvest thermal treatment for the reduction of pathogens on delicate LWAF, including nuts and dried fruits.

Purpose: The objective of this study was to determine and model the inactivations of *Salmonella*, STEC, *L. monocytogenes*, and a possible surrogate (*Pediococcus acidilactici*) on dried apricot halves, whole macadamia nuts, and raisins after treatment with vacuum-steam at three temperatures (62°C, 72°C, or 82°C).

Methods: LWAF were inoculated with pathogens and the non-pathogenic surrogate and treated with low-temperature, vacuum-assisted steam for pre-determined time intervals. Samples were enumerated and bacterial reductions were calculated to evaluate thermal inactivation trends. ANOVA and pairwise comparisons were used to determine treatment significance. Data were fit to the Weibull and Gompertz models and evaluated by AIC and RMSE.

Results: Time and temperature for bacterial reductions were influenced by commodity. Pathogens were reduced by 5 log CFU/g on apricot halves after 20 min at 72°C and after 5 min at 82°C. Nearly 5-log CFU/g reductions occurred on macadamia nuts after 38 min at 72°C (4.6-6.5 log CFU/g) and after 12 min at 82°C (4.9-5.7 log CFU/g). 5-log CFU/g reductions occurred on raisins at lower temperatures of 62°C (20 min) and 72°C (5 min). Pathogen inactivations were not significantly different from one another ($P < 0.05$), but the surrogate was reduced significantly less by the steam than the pathogens. Inactivation kinetics of bacteria were modeled for each pathogen on each food type and temperature. The Weibull model fit bacterial reductions best for raisins and macadamia nuts, while the Gompertz model best described reductions on apricot halves.

Significance: Thermal inactivation kinetic models and 5-log reduction parameters help food processors design and evaluate similar vacuum-assisted steam interventions to comply with FSMA regulations and preventive control plans to improve LWAF safety.

T19-03 Thermal Death Kinetics of *Salmonella* Enteritidis in Peanut Butter and the Effect of Water Activity

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❖ ***Developing Scientist Entrant***

Introduction: Major outbreaks caused by *Salmonella* in peanut butter since 2006 have raised the attention of the food industry and the research community. Measuring the thermal resistance of *Salmonella* in peanut butter is the first step in developing appropriate thermal treatments to ensure the safety of the product. Our recent studies suggest that oil segregated from peanut butter and sample exposure to relatively large headspace in heat treatments might be the cause of some of the reported nonlinear thermal death curves of *Salmonella* in peanut butter and other oil-rich products. There is a need for a systematic study to obtain reliable thermal death kinetics data of *Salmonella* in peanut butter.

Purpose: Systematically study the thermal death time of *Salmonella* in peanut butter corresponding to different temperatures and water activities (a_w).

Methods: Peanut butter samples were inoculated with *Salmonella enterica* Enteritidis PT 30 (*S.* Enteritidis) and conditioned for more than 5 days as a thin layer (5 mm) at 23°C and relative humidities (RH) of 33 and 53%. The chosen RH values corresponded to a_w of 0.33 and 0.53, reflecting the typical range for this product in the market. For the thermal death time measurement, the inoculated samples were treated isothermally in fully filled thin (1.3 mm sample thickness) aluminum cells at 70, 80, 90 and 100°C, each for biological triplication.

Results: Linear semi-log thermal death curves were observed under all circumstances. At each temperature, the *D*-value was significantly lower in the sample of a higher a_w. The log *D*-values reduced linearly with temperature at each a_w condition with *z*-value equals 16.3°C.

Significance: This study suggests that the thermal death of *S.* Enteritidis in peanut butter follows first-order kinetics. Temperature and a_w are the two main factors influencing bacterial thermal resistance in peanut butter.

T19-04 Predicting Pathogen Survival in Soy Sauce-based Acidified Foods by Using Real Food-matrix Data: An Academia-industry Collaboration

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❖ ***Developing Scientist Entrant***

Introduction: Producers of acidified foods that do not receive a full thermal treatment are required to validate the safety of new product formulations through challenge studies. These studies determine the holding time required for a formulation to achieve a 5-log reduction in pathogenic microorganisms. *Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella* are the main pathogens of interest for challenge tests, and manufacturers have collected survival data on numerous products. Analyzing this data may be helpful in predicting the holding times for newly formulated products, as challenge experiments slow down product development and add additional costs.

Purpose: The purpose of this study is to predict 5-log pathogen reduction times for cold-fill-hold soy sauce-based acidified food products to identify critical factors influencing the survival of foodborne pathogens and facilitate new product development.

Methods: Bacterial survival data obtained by challenge experiments for 52 product formulations with 10 factors measured and three pathogens (*L. monocytogenes, Salmonella, E. coli* O157:H7) tested were provided by a commercial producer. Time-dependent survival data were modeled using non-linear regression methods and 5-log reduction times were obtained using inverse estimation. Kaplan-Meier curves and log-rank tests were used to determine the most resistant type of pathogen in the given product set. Various regression models were evaluated regarding their prediction accuracy of 5-log reduction times for new product formulations.

Results: Kaplan-Meier curves and log-rank tests indicated that there is a statistically significant difference between the three pathogens studied ($P < 0.05$) and *L. monocytogenes* has the longest median survival time as 3.52 (95% CI: 2.7-4.17) days in soy sauce-based acidified products. Random forest regression identified pH, Brix, water activity and total alcohol content as the most important product characteristics for the survival of *L. monocytogenes*.

Significance: This study exemplifies an industry-academic collaboration for integration of quantitative methods with actual food-matrix data to address real-life problems faced by the food industry.

T19-05 Development of a Monte Carlo Simulation Model to Predict Pasteurized Fluid Milk Spoilage Due to Post-pasteurization Contamination

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Introduction: Gram-negative bacteria introduced as post-pasteurization contamination (PPC) will result in reduced shelf life for pasteurized fluid milk. A Monte Carlo simulation model is a tool that can be used to understand the bacteria growth behavior and assess interventions to reduce PPC.

Purpose: To improve predictions of pasteurized fluid milk shelf life due to post-pasteurization contamination by developing a Monte Carlo simulation model of milk spoilage due to Gram-negative bacteria introduced as PPC.

Methods: Isolates used for growth characterization were selected to represent the diversity of species that have been previously associated with fluid milk spoilage and dairy processing facilities. A total of 18 isolates were included and grown in skim milk broth at 6°C. Frequency of the selected isolates, initial microbial concentration, and growth parameters were used to develop a Monte Carlo simulation model to predict spoilage of pasteurized fluid milk packaged in ½ gallon containers due to PPC.

Results: Among the different sequence types, lag phase (λ) ranged from 0 to 56.9 hours and maximum growth rate (μ_{max}) ranged from 0.0584 to 0.4635 log CFU/mL/h. Preliminary Monte Carlo simulations indicate that 84% of samples of HTST milk contaminated by PPC will have a cell density greater than 1,000,000 CFU/mL by day 10 of storage at 6°C.

Significance: This study provides an understanding of Gram-negative bacteria growth patterns in fluid milk and can be used to improve shelf life prediction. The model can eventually be used to evaluate spoilage interventions that can should be implemented to reduce PPC.

Poster Abstracts

P1-01 Multi-stress Adaptation of *Lactobacillus plantarum* Enhances Its Survival in Different Food Matrices and in Simulated Gastrointestinal Fluids

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Introduction: Pre-adaptation of probiotics to sub-lethal stress levels is effective in boosting their stability. There is a growing interest in developing non-dairy probiotic products to increase their accessibility to more consumers.

Purpose: This study investigated survival of multi-stress adapted *Lactobacillus plantarum* in yoghurt, carrot and cranberry juices and in simulated gastrointestinal fluids (SGIF).

Methods: Non-stress adapted *L. plantarum* cells, those that were sequentially adapted to acid, bile and temperature (freshly adapted) and those that were adapted and then frozen (old adapted) were inoculated into each food products and then stored at 4°C for six weeks. Subsamples were analyzed weekly for pH, Brix content and for enumeration of viable counts. Additionally, viability of these cells when incorporated into the different food products and then exposed to SGIF was assessed.

Results: There was a significant reduction in viability of *L. plantarum* cells during storage of all juices ($P < 0.05$), however, multi-stress adapted (old and fresh) cells survived better than the non-adapted cells. Carrot juice showed greater *L. plantarum* survival, followed by cranberry juice and then yoghurt. Reduction of pH was more pronounced in juices than in yoghurt. The Brix content of cranberry juice was higher than that of carrot juice. Non-adapted cells were the most vulnerable to gastrointestinal fluids while the freshly adapted cells survived better than their old-adapted counterparts. In SGIF, stress adapted cells that were subjected to long-term low temperature storage experienced significantly diminished viability compared to their freshly adapted counterparts ($P < 0.05$). Carrot juice was the matrix that preserved viability of *L. plantarum* during storage and in SGIF.

Significance: Multi-stress adaptation enhanced survival of *L. plantarum* during storage with no notable differences between the freshly and old-adapted cells. Food products differently affected survival of *L. plantarum*, therefore, care should be taken when choosing probiotic food vehicle.

P1-02 Synergistic Antimicrobial Activities of Essential Oils Against Lactic Acid Bacteria in Organic Hallabong Tangor (*Citrus kiyomi* × *Citrus ponkan*) Juice

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✦ ***Undergraduate Student Award Entrant***

Introduction: The antimicrobial activities of plant-derived essential oils (EOs), which allowed for use in organic foods, against lactic acid bacteria (LAB) have not been intensively studied.

Purpose: This study was done to develop an antimicrobial technique which inhibits the growth of LAB (*Leuconostoc citreum, Leuconostoc mesenteroides, Lactobacillus plantarum*) in organic Hallabong tangor (*Citrus Kiyomi* × *Citrus ponkan*) juice.

Methods: Among 27 kinds of EOs allowed to use in organic foods in Republic of Korea, EOs with relatively strong antimicrobial activities against LAB were screened by an agar well diffusion assay. Next, the minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) of screened EOs against LAB were determined in Hallabong tangor juice. Finally, combinations of EOs eliciting synergistic antimicrobial activities against LAB in Hallabong tangor juice were determined by using a checkerboard assay.

Results: Four types of EOs (cinnamon bark, cinnamon leaf, oregano, and thyme thymol) showed relatively strong antimicrobial activities (inhibition zone ≥ 14.0 mm) against LAB. The MICs and MLCs of those EOs were in the range of 0.0781 - 1.25 µL/mL and 0.0781 - 2.5 µL/mL, respectively. Oregano and thyme thymol EO showed the strongest antimicrobial activities (MICs and MLCs = 0.0781 µL/mL) against *Leu. citreum* and *Leu. mesenteroides*. Finally, it was confirmed that the combination of cinnamon bark and cinnamon leaf EOs showed synergistic antimicrobial activities (fractional inhibitory concentration index = 0.5) against *Leu. citreum* and *Lac. plantarum* in Hallabong tangor juice.

Significance: This is the first study to report the antimicrobial activity of plant-derived EOs against LAB in organic Hallabong tangor juice. The results of this study would provide useful information in developing antimicrobial techniques to extend the shelf life of organic Hallabong tangor juice.

P1-03 Evaluation of Foodborne Pathogens Die-off in Back-sweetened Wine and Hard Cider Models

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Introduction: Back-sweetening wine and hard cider with unpasteurized juice is a common practice. No previous research has been performed to validate pathogen die-off in back-sweetened wine and hard cider models.

Purpose: The purpose of this study is to evaluate the survival of *E. coli*, *Salmonella* and *Listeria* under different combinations of pH and alcohol content in wine and hard cider models.

Methods: Modified grape and apple juice were used as models for wine and hard cider. Grape and apple juice were adjusted to desirable pH (3, 4, 5 for grape juice and 3.2, 3.7, 4.2 for apple juice) and filtered through 0.22 µm filters. Undenatured ethanol was added to reach desirable alcohol content (0%, 7%, 10%, 12%, 14% for grape juice and 0%, 3.2%, 5%, 7%, 8.5% for apple juice). A five-strain cocktail for each pathogen was mixed using overnight culture. Pathogen cocktails were separately inoculated into modified juices, with an initial inoculum of 7-9 log/mL. Samples were plated onto tryptic soy agar at 0 h, 2 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h, 96 h. Plates were incubated at 37°C for 48 hours and plate counts were recorded. Three biological replicates were performed.

Results: In both models, a combination of low pH and high alcohol content resulted in a faster pathogen die-off. Similar trends were observed in all three pathogens. At least a 5-log reduction can be achieved in systems under following conditions ($P < 0.05$): 1. pH 3 or 3.2 in 72 hours; 2. pH 3.7 and 8.5% alcohol in 96 hours; 3. pH 4 and 10% alcohol in 72 hours; 4. pH 5 and 12% alcohol in 96 hours.

Significance: Results from this study could be used for validating pathogen die-off to comply with Juice HACCP 5-log pathogen inactivation requirements.

P1-04 Effect of Heat and Acidic pH on *Salmonella* Resistance in Tomato and Mango Extracts

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Introduction: *Salmonella enterica* can acquire resistance to some factors such as high temperatures, acid pH, among others. This resistance can promote the survival of the pathogen during fruit dehydration process.

Purpose: The main goal of this study was to evaluate the stress caused to *S. enterica* cells by exposure to acidic pH and high temperature in tomato and mango extracts.

Methods: Four rifampicin-resistant strains of *S. enterica* (8 log CFU mL^{-1}) were inoculated in tomato and mango extracts (10%) adjusted to pH 4 and 7. The extracts were exposed to 50°C for two hours. *Salmonella* populations were quantified by surface spread method on trypticase soy agar with rifampicin (200 ppm) (STRA) supplemented with pyruvate (0.1%) and STRA supplemented with NaCl (1%); plates were incubated at 35°C/24 h. Additionally, treated cells were stained using ethidium bromide (EB), propidium iodide (PI) and tetrazolium chloride (CTC), to observe changes in membrane integrity, pump activity and respiratory activity, respectively, followed by flow cytometry analysis.

Results: The plate count method showed percentages of stressed cells ranged from 68 to 92% in tomato extract at pH 4.4, while at pH 7 values from 48 to 72%. In mango extract, at pH 4.2 the percentages of stressed cells were from 54 to 88% and at pH 7 were lower (27 to 71%) (Every percentage correspond to the media of six values). Damage to the integrity of the membrane (PI), an increment in pump activity and a slightly decreased in respiratory activity (CTC) were observed by flow cytometry.

Significance: The results showed that high percentages of cells are able to repair the damage caused by heat and acidic pH which may promote the survival capacity of *Salmonella* cells during the process of dehydration of fruits.

P1-05 Effect of Storage Time and Temperature on the Recovery of Milk and Peanut Residue from Environmental Swabs

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✦ ***Undergraduate Student Award Entrant***

Introduction: Environmental swabs of shared processing equipment are commonly utilized by the food industry during cleaning validation studies. Some of these swabs are sent to third-party laboratories for evaluation. However, the recovery of allergens between the time of swabbing and time of testing has yet to be systematically studied.

Purpose: Commercial ELISAs were evaluated to determine allergen recovery from swabs inoculated with peanut and Non-Fat Dry Milk (NFDM) and then subjected to different holding times and temperatures.

Methods: For each allergen, 100, 50, and 25 ppm peanut flour or NFDM were prepared and each spiked onto Neogen Environmental Swabs which were stored at room temperature (RT), 37, 4, and -20°C for 0, 1, 3, 5, 7, 10, and 14 days. Subsequently, swabs were tested using the Neogen Veratox Peanut and Total Milk ELISAs. The percent recovery for all data points was calculated.

Results: While both allergens were detected by ELISA on day 14 at all four storage temperatures, the percentage recovery decreased from day 0 to 14 with the greatest decrease occurring from day 0 to 1. For swabs spiked with peanut, the greatest decrease was observed at RT and 37°C (3 to 6-fold decrease in recovery). However, only a 2-fold decrease in recovery was observed with peanut swabs stored at 4°C and -20°C. For swabs spiked with NFDM, the percent recoveries decreased between 2 to 3-fold when stored at RT and 37°C and ~2-fold when stored at 4°C and -20°C.

Significance: The apparent recovery of peanut and NFDM decreases when the swabs are stored for extended time at higher temperatures but were minimally affected when stored at 4 or -20°C. These results are useful for testing laboratories and the food industry when transporting and testing swabs; however, further evaluation of additional protein targets and ELISAs is warranted as alternate targets and extractions may differ in recovery.

Poster

P1-06 Airline Food Allergy Risk Communication

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Introduction: Prevention of allergenic diseases is of considerable importance to public health and there is a need for airlines to implement effective allergy policies to reduce the risk of allergen exposure. In aviation, allergy research indicates that common reactions occurring on commercial airlines include those predominately associated with peanuts and tree-nuts. Given the potential severity of food-allergic reactions, there is a critical need for airlines to have food-allergy management control policies and effective risk communication to passengers.

Purpose: This study aimed to investigate airline inclusion of food-allergy control in management policies and risk-based information provision for passengers with food allergies.

Methods: Publicly available data regarding airline food-allergy policies was obtained from airlines' websites (n = 39). Data associated with policy content and at-risk passenger required behavior was categorized using a qualitative approach. Data was thematically analyzed using NVivo10.1.

Results: Findings indicated that 75% of airlines have a policy for passengers' food-allergy. However, the content of food-allergy polices differed in detail, comprehensiveness and strength, this may be due to the nature, size and type of the airline operation system. Overall, 40% of airlines indicated serving nut and peanuts products and 57% of airlines (with food-allergy policies) indicated serving peanut/tree-nut free meals. However, all airlines analyzed reported they cannot guarantee that the food served on board is completely free of peanuts or peanut products or that other passengers do not bring products containing peanuts with them. The same applies to other foodstuff that may cause an allergic reaction, for example, for one airlines, a verified gluten-free meal was also considered '*not suitable for individuals allergic to the wheat related crops.*'

Significance: The findings of this study highlighted positive food-allergy policy inclusions and omissions. Such data may be useful for airlines, caterers and suppliers to improving on-board food allergy management and control and associated passenger safety.

P1-07 Determination of Aflatoxin B1 in Oil Seeds Using Immunomagnetic Solid Phase Extraction

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Introduction: Oil seeds are especially susceptible to aflatoxin B1 (AFB1) contamination if they are stored improperly. The contamination of AFB1 on oil seeds can be further transferred to edible vegetable oil, causing harm to human health.

Purpose: The purpose of this study was to develop a highly sensitive and accurate method of liquid solid extraction (LSE) combined with immune magnetic solid phase extraction (IMSPE) for the determination of AFB1 in oil seeds.

Methods: Antibody grafted magnetic nanoparticles (MNPs) were synthesized, characterized using FT-IR and TEM and used to extract AFB1 in oilseeds. After the extraction, AFB1 was detected and quantified by HPLC-Fluorescence Detection. The extraction method was optimized based on several crucial factors including the low-temperature clean-up duration, volume of MNPs and the vortex duration in IMSPE. Real sample analysis was carried out on five oil seed samples and each sample was tested in triplicates.

Results: Due to the highly selective extraction with the aid of antibody-antigen interaction, the MNPs can capture the AFB1 in the samples highly efficiently. The optimum conditions for extraction were 20 h for freezing, 1 mL of magnetic fluid as adsorbent and 5 min for vortex duration in IMSPE. A calibration curve constructed with spiked samples of known concentrations yielded a correlation coefficient of 0.9946, indicating strong linearity of the proposed method. The limits of detection (LOD) and quantification (LOQ) of the developed method were 0.02 and 0.05 ng·g-1, respectively, suggesting desirable sensitivity of the method. The proposed method also showed satisfactory repeatability, with intra and inter-day recovery rates ranging from 94.92 – 118.95 %.

Significance: The proposed method is highly accurate, sensitive and reliable and is promising in detecting AFB1 contamination in oil seeds and similar specimens.

P1-08 Droplet Digital PCR for Detection of Allergenic Peanut

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Introduction: Peanut is one of the most common and significant food allergens. The Food Allergen Labeling and Consumer Protection Act requires that foods containing it be labeled accordingly; this necessitates highly sensitive and accurate detection methods.

Purpose: The purpose of this work was to extend a previously developed assay for detection of peanut from real time PCR to a droplet digital PCR format.

Methods: Primers and probes targeted three different peanut-specific regions in the chloroplast genome: the matK and rpl16 genes as well as the trnH-psbA spacer region. All targets were evaluated and optimized in singleplex prior to being combined in the triplex assay. A probe-mixing format was used for the triplex assay. Digital PCR was optimized for primer and probe concentrations of all three targets and several aspects of thermal profile, including annealing temperature, annealing time, cycle number, and final extension. Linear range was determined using DNA amounts ranging from 0.0005 - 50,000 pg per reaction. Cross reactivity was tested with a variety of other legumes and tree nuts.

Results: The assay was successfully transferred to the droplet digital PCR format. The triplex assay yielded droplet clusters for all possible combinations of one, two, or three targets, as expected. All clusters were well-differentiated from each other and copy numbers were in agreement for all three targets. The linear range was 0.05 -500 pg total DNA per reaction, corresponding to 5-100,000 copies per well. No cross reactivity was found with any of the tested legumes or tree nuts.

Significance: Peanut-allergic individuals rely on avoidance of peanut-containing foods to prevent the severe and potentially fatal reactions peanut allergy can cause. This necessitates accurate, specific, sensitive detection methods. Digital PCR has greater accuracy than other PCR formats and, unlike real time PCR, provides quantitation directly without the use of a standard curve.

P1-09 Incompatibility Group FIB Plasmid-positive *Salmonella enterica* Serovar Typhimurium Isolates from Food Animal Sources

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Introduction: Plasmids play vital roles in the dissemination of antimicrobial resistance and virulence genes among bacteria. These genes likely play a role in the ability of bacteria to survive in food animal environment and cause infections in humans. *Salmonella enterica*, which is identified as the source of multiple outbreaks linked to contaminated foods, carries plasmids that can allow for gene transfer between different bacteria. Carriage of plasmids can impact fitness costs, which is generally evidenced by alterations in bacterial growth rates.

Purpose: This study was undertaken to evaluate IncFIB-positive *Salmonella* isolates from food animals and to assess antimicrobial resistance and virulence properties of the isolates.

Methods: A total of 74 *Salmonella* isolates were identified that carried IncFIB plasmid origination from chicken (*n* = 35, 47%), turkey (*n* = 22, 30%), cattle (*n* = 10, 14%), swine (*n* = 4, 5.4%), and poultry water (*n* = 3, 4%). Whole genome sequencing (WGS) was completed on all isolates using Illumina MiSeq and conjugation experiments were performed with a sodium azide resistant *Escherichia coli* J53 as the recipient and *Salmonella* strains as the donor cells to determine the transferability of plasmids among bacteria. Growth rates were generated for each transconjugant to quantify the impact of the overall plasmids on the fitness costs and growth dynamics of strains.

Results: Bioinformatics analyses using PlasmidFinder software confirmed that all isolates contained IncFIB plasmids along with multiple other plasmid replicon types. ResFinder and PATRIC results revealed that all strains carried multiple antimicrobial resistance genes and virulence factors. PlasmidFinder assessed 24 transconjugants that carried plasmids containing antimicrobial resistance and virulence genes, which were transferred from the donor cells. Transconjugants that carried plasmids with multiple replicons types often had a higher growth rate compared to *E. coli* J53; the reason for these observations is currently being assessed.

Significance: The results of this study provide data on the contribution of IncFIB plasmids for potentially increased virulence, antimicrobial resistance, and their impact on overall growth rates.

P1-10 Estimation of Variance Associated with Measuring Gluten Content in an Oat Flour Sample

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Introduction: Oat flour is used in gluten-free labeled foods. However, gluten cross-contact can occur in a non-homogeneous manner, potentially affecting gluten quantitation.

Purpose: To determine variance equations for oat flour analysis, which predict total variance (Vt) at a specified gluten concentration (G; ppm) depending on sample size (Ns; grams) and number of aliquots analyzed (Na).

Methods: Ten ~45 kg-lots of sorted oat groats, were spiked with varying amounts of wheat kernels (0-255.7 g) and each thoroughly mixed. Lots were milled into flour using a mill (hammer attachment) and 0.6 mm outlet screen. From each lot, 32 flour samples were collected during milling and each ran-

domly split into two 100 g samples to be used in discrete and composite sampling. A set of 32x100 g samples were mixed together and then split to obtain thirty-two 100 g composite samples. A 5 g sample from each 100 g discrete and composite sample was analyzed for gluten content in duplicate aliquots by R5-ELISA. The Vt was partitioned into variance between samples (Vs) and aliquot tested (Va), and regression analysis was done to establish relation between variance and G.

Results: A linear relation was observed between the mean gluten from the 10 lots analyzed by discrete and composite sampling. For both sampling methods, the variance increased as G increased and Vs>Va. When expressed as a percentage (Vt = 100%), %Vs generally decreased and %Va increased as G increased. Though linear line regression between variance and gluten showed good fit (R^2=0.8534 to 0.9783), the power equation on log-log scale had a similar fit and may be more reliable at high G. The Vt=Vs+Va can be estimated from the regression equations for discrete (Vs=(5/Ns)0.8187$G^{1.1586}$, Va=(1/Na)0.0054$G^{1.7329}$) and composite (Vs=(5/Ns)0.4665$G^{1.0937}$, Va=(1/Na)0.0318$G^{1.3881}$) sampling.

Significance: These variance equations can be used to predict sampling dependent variability in gluten test results.

P1-11 Effects of Emulsifiers on Intestinal Barrier Integrity and Exposure to Food Allergens

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Introduction: Enhancement of food allergic responses could be related to cellular membrane effects from certain emulsifiers; however, it is important to understand interactions among the food matrix components for risk characterization.

Purpose: Explore how emulsifiers [e.g. Polysorbate (P)-80 or Lecithin (LE)] might affect intestinal barrier integrity and transport of allergenic proteins such as known egg allergen ovalbumin (OA).

Methods: We challenged monolayers of Caco-2 cells, an *in vitro* model of human epithelial tight junctions with 0.01, 0.05, 0.1, and 0.5% of P-80 or LE alone, and OA alone (0.1, 1, and 10 mg/ml) or in combination (0.5 mg/ml) with emulsifiers (0.2%). We measured toxicity, cellular viability, Lucifer Yellow (LY) penetration, Trans-Epithelial Electrical Resistance (TEER), and expression of tight junction molecules by gene-expression and immunofluorescence assays. An in-house ELISA determined the rate of transport of OA, with or without emulsifiers, to basolateral media.

Results: All P-80 and OA individual treatments showed minimal effects on cellular viability and toxicity. Concentrations of ≤0.1% LE resulted in cytotoxicity of 0-10% and normal cell proliferation. At 0.5% LE, cytotoxicity increased to 50% with <10% proliferation. When treated with either P-80 or LE at 0.5%, penetration of LY increased significantly; 70-90% over lower concentrations. Although TEER was reduced (~20%) with 0.2% of P-80, using 0.2% LE and 0.5 mg/ml OA had no effect. The expression of tight junction genes and proteins were significantly disrupted in 0.5% LE treated cells compared to 0.2% LE, 0.2% and 0.5% P-80 treated cells. About twice the amount of OA was transported paracellularly in the presence of 0.2% P-80, relative to OA alone or LE treatments suggesting certain emulsifiers can augment allergen absorption process by modulating barrier integrity.

Significance: Our experimental system has potential to assess emulsifier effects on barrier functions and how this may secondarily impact allergen absorption from foods.

Poster

P1-13 Detection of Single Kernel Aflatoxin and Fumonisin Contamination Using Visual Factors Associated with Mycotoxin Contamination through Reflectance Spectroscopy.

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❖ ***Developing Scientist Entrant***

Introduction: Reflectance spectroscopy at Ultra-Violet, and Near-Infrared wavelengths can detect aflatoxin and fumonisin in corn, but is limited by difficulties present in the identification of sufficient contaminated single kernels to generate calibration algorithms.

Purpose: Test of enrichment for contaminated kernels using a calibrating library of visual high-risk kernels and samples of uncontaminated kernels for classification methods through reflectance spectroscopy

Methods: From a commercial corn sample (208 g), previously tested in bulk >50 ppb aflatoxin and >4000 ppb fumonisin, kernels were selected according to visual factors associated with mycotoxin contamination and visual characteristics associated with uncontaminated kernels to generate a reject and accept data set, respectively. Kernels were scanned by passing through a LED-ring spectrometer/optical-sorter based on the reflectance values at nine distinct wavelengths (470 nm-1,550 nm), and utilized to calibrate a Linear Discriminant Analysis (LDA) algorithm. Kernels were mixed together and sorted using the optical sorter algorithm. The accepted sorted sample was tested in bulk, while all single kernels from the rejected sorted sample were tested individually (*n* = 192). ELISA kits were used to measure mycotoxins.

Results: LDA algorithm of reject and accept sample yielded a classification specificity of 0.91, and a sensitivity of 0.83. A total of 170 g were sorted as accept, and 38 g were sorted as reject. The reject sample contained only 1 kernel with aflatoxin (48 ppb), while 34 kernels contaminated with fumonisin (<1000 ppb), median aflatoxin and fumonisin levels were 0.72 ppb and 3,801 ppm, respectively. Accept sample bulk levels of aflatoxin and fumonisin were below the limits of detection.

Significance: A library of kernels with visual factors associated with mycotoxin contamination can be used to generate sorting algorithms capable of detecting fumonisin contaminated single kernels using spectroscopy.

P1-14 Development and Validation of Aflatoxin M1 ELISA Assay for Milk Products

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Introduction: When cows consume contaminated feed, aflatoxin B1 is converted by hydroxylation to aflatoxin M1, which is subsequently secreted in the milk of lactating cows. A monoclonal antibody, which is highly specific to aflatoxin M1, was used to develop aflatoxin M1 ELISA assay for milk products.

Purpose: To develop and validate a sensitive aflatoxin M1 ELISA assay with low cross reactivities to other aflatoxins subtypes and a broad detection range.

Methods: Various milk products, such as raw milk, homogenized milk, skim milk powder and yogurt were validated for detection of aflatoxin M1 by ELISA. Raw milk sample was directly tested after removing fat. However, additive is added in homogenized milk before assay. Yogurt sample is diluted with yogurt diluent, and the dilution factor is applied to calculate aflatoxin concentration in yogurt sample. The assay was designed to cover a wide range of aflatoxin contamination by setting up standards ranging from 5 ppt to 500 ppt.

Results: Cross reactivities to other aflatoxins (B1, B2, G1, G2 and M2) were all less than 0.1%. Raw, homogenized, and skim milk samples were spiked with aflatoxin M1 at 5, 50 and 200 ppt levels and tested by ELISA. Recoveries were 80% - 115%. Different spike levels were applied for yogurt sample due to dilution (20, 50 and 200 ppt), and recoveries were 99% - 112%.

Significance: A competitive ELISA for aflatoxin M1 was developed, which is sensitive, specific and detects a wide range of aflatoxin concentration. Due to its very low cross reactivity to other aflatoxins subtypes found in grains, this assay serves as an excellent method for testing dairy products containing grains.

P1-17 Monitoring and Evaluation of 18 Different Metals in U.S. Meat and Poultry

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Introduction: The U.S. Department of Agriculture (USDA) monitors 18 metals as part of the Food Safety and Inspection Service's mission to ensure the safety of meat and poultry. USDA tested more than 5,500 muscle samples collected 2013-2019 from cattle, pork, goat, poultry and Siluriformes fish (including catfish) for aluminum, arsenic, barium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, strontium, thallium, vanadium, and zinc.

Purpose: To determine whether there is public health concern associated with metals in meat and poultry and to inform future metals testing.

Methods: More than 5,500 muscle samples were analyzed using an ICP-MS/ICP-OES method with the minimum level of applicability ranging from 10 ppb for cadmium to 30 ppm for iron and zinc. The data were evaluated using a tiered, risk-based approach, which compared the test results to screening levels derived from health-based guidance values, such as reference doses, and meat and poultry consumption estimates for various life stages in the U.S. population.

Results: In the first tier of analysis, 10 metals were found to not be of public health concern after comparing the test results to the screening levels. For the remaining 8 metals, the magnitude and frequency of detections and the toxicological guidance values were evaluated further, with the conclusion that they were also not of public health concern in meat and poultry. The conservative chronic screening levels were not consistently exceeded for any metal. For metals such as arsenic, cadmium, lead, and nickel, less than 0.2% of samples exceeded the screening levels. USDA also categorized and prioritized these metals to support future refinements to the metals testing program.

Significance: Through extensive sampling and testing, USDA has determined that the metals concentrations in meat and poultry observed in 2013-2019 are not of public health concern.

P1-19 Hazard Ranking in Smoke-cured Fish in Ghana

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❖ ***Developing Scientist Entrant***

Introduction: Smoke-curing is the principal method of fish preservation in Ghana. However, the method and post-processing handling practices contribute to contamination of the products with several food safety hazards. A context-relevant ranking of the hazards based on their importance for domestic public health is needed for efficient food safety management.

Purpose: The study sought to rank selected food safety hazards in smoke-cured fish in Ghana.

Methods: The levels of selected microbiological hazards (*Salmonella* sp. and *Escherichia coli*), biogenic amines (B-phenylethylamine, putrescine, cadaverine, histamine and tyramine), and polycyclic aromatic hydrocarbons (PAHs - benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) were determined in 80 samples of smoke-cured *Sardinella* sp. and *Sphyraena* sp. collected from markets (n = 20 per species) and processing sites (n = 20 per species). Data obtained were compared with regulatory limits, considering contextual food preparation practices and health impact of the hazards. The hazards were then ranked from 1 (most relevant) to 3 (least relevant).

Results: *Microbiological hazards*: *Salmonella* sp. was not detected in any sample. *E. coli* counts ranged from 1.0 to 2.9 log CFU/g. The typical time-temperature regime of Ghanaian cooking (≥80°C for ≥30 min) will eliminate this hazard. *Biogenic amines*: Levels ranged from 10 to 66.8 mg/kg. The highest level of histamine (the regulatory marker for biogenic amines) was 47.9 mg/kg, four times less than the limit of 200 mg/kg. *PAHs*: Benzo(a)pyrene (BaP) and PAH4 levels ranged from 11 to 69.8 µg/kg and 44 to 564 µg/kg, respectively. Thus, the regulatory limits for BaP (2 µg/kg) and PAH4 (12 µg/kg) were exceeded by up to 35 and 47 times, respectively. Based on these data and considering their ultimate consequence for health, the hazards were ranked as PAHs (1), biogenic amines (2) and microbiological hazards (3).

Significance: The study supports efficient food safety management by highlighting PAHs as the key hazards in smoke-cured fish in Ghana.

P1-20 Removal of Heavy Metal Contaminants from Skin Using Commercially Available Soaps

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Introduction: Heavy metal exposure is a risk in some manufacturing settings. Employees who work in these settings, particularly those handling food, are at higher risk of contaminating food with heavy metals, which can cause illness.

Purpose: This study evaluated the ability of nine commercially available soaps to remove heavy metal contaminants from skin.

Methods: Eight commercially available soaps manufactured by GOJO Industries and Esca Tech, Inc. were tested. Arsenic, cadmium, lead, mercury, and nickel solutions were prepared by dissolving each metal in a solution of nitric acid (5%). Skin biopsy samples (1 in^2) were contaminated using 250 µL of each metal solution (equivalent to 1,000 ppm) and allowed to air dry. Soap (0.5 ml) was applied to the contaminated skin sample and gently massaged using a sterile nitrile gloved hand for 20 seconds in a circular motion to simulate a handwash. Skin samples were rinsed with deionized water (60 mL) and transferred to a vessel containing nitric acid (20 mL; 10.0%) and mixed for 5 minutes to remove any remaining metals. The remaining 20 mL solution was analyzed via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Treated skin samples not subject to a soap treatment served as controls. All treatments were repeated 5 times.

Results: Seven soaps significantly reduced metal contamination compared to untreated controls ($P < 0.05$). Average percent removal (all contaminants combined) ranged from 27 to 74%, with CRT Healthy Soap having the highest percent removal. Type of metal contaminant significantly impacted removal performance ($P < 0.05$). Lead and mercury were the most difficult to remove, with all soaps averaging 40% removal of these metals.

Significance: Food handlers that experience occupational exposure to metal contaminants, especially lead and mercury, should use soap formulations effective at removing these contaminants to reduce risk of contamination of food during meal preparation.

P1-21 Validating and Verifying Allergen Cleaning: A Case Study on Traces of Milk, Soy and Egg Allergens in a Processed Poultry Manufacturing Facility

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Introduction: Allergen preventive controls can be achieved by preventing allergen cross-contact and accurate allergen labeling of the finished goods. The validation process should demonstrate that the cleaning procedure and other preventive controls reduce the allergen to a level deemed to be acceptable.

Purpose: To evaluate cleaning validation and control of allergen cross-contact in ready-to-eat (RTE) poultry production.

Methods: Six types of RTE poultry products (two classified as allergen-containing products including milk, soy and egg, and others as non-allergen products) were examined. The production line of an allergen-containing product was run parallel to the non-allergen product. A worst-case allergen cross-contact scenario was determined. Food contact surfaces, equipment, apron, and gloves were swabbed and finished products were sampled. The presence of food allergens was assessed using both Lateral Flow Devices (LFD) (108 samples) and ELISA (200 samples).

Results: Detection limit of LFD for milk, soy and egg are 3, 2 and 0.5 ppm, respectively. Limit of quantitation (LOQ) of ELISA for milk and egg are 1 and 0.5 ppm, respectively. Prevalence of milk and soy on the food contact surfaces or environment after cleaning were 0/54 and 2/21, respectively; however, allergen cross-contact to the non-allergen production line/products was not detected. For the worst-case scenario, cross-contact of egg was detected in three types of non-allergen RTE products probably due to ineffective physical barrier between the production lines (2/5 or 40%), staff unawareness of allergen cross-contact (1/2 or 50%) and insufficient cleaning (6/24 or 25%).

Significance: Using LFD and ELISA methods can give insight into the validation of a cleaning/process for food contact surfaces and products. In addition, detection of allergens enable a food processor to label the products appropriately.

P1-22 Detection and Monitoring of 16 PFAS in Beef

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Introduction: As part of its mission to ensure the safety of meat, poultry, and egg products, the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA) collects samples at slaughter or processing and tests them for a variety of veterinary drugs, pesticides, and environmental contaminants. Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals, some of which are very persistent in the environment, that have been used in many industries around the world.

Purpose: To develop and implement the capacity to monitor the U.S. beef supply for PFAS compounds as part of the National Residue Program (NRP).

Methods: USDA recently developed a method that screens, confirms, and quantifies 16 PFAS compounds in beef muscle and blood plasma, using methanolic extraction followed by UPLC-MS-MS (Agilent 1290 Infinity II UPLC with AB Sciex QTrap 6500+ mass spectrometer). It is applicable to bovine muscle and plasma and includes the following 16 PFAS analytes: PFBS, PFDA, PFDoA, PFDS, PFHpA, PFHxA, PFHxDA, PFHxS, PFNA, PFOA, PFODA, PFOS, PFPeA, PFTeA, PFTriA, and PFUnA. The analytical range in muscle is 0.50-125 ng/g for all analytes, except PFHxDA (1.25-125 ng/g). The method was also extended to plasma.

Results: On October 1, 2019, USDA began using this method on muscle samples collected at slaughter as part of the NRP from production classes including dairy cows, beef cows, steers, heifers, and veal. The method was successfully implemented in the USDA/FSIS Midwestern Laboratory, where about 100-150 beef muscle samples are being tested each month. Data from the first several months of testing will be presented.

Significance: USDA can rapidly and reliably test beef for PFAS, which are a class of persistent environmental contaminants that have garnered significant public attention. This helps the Agency to identify a public health concern, if it arises, and informs potential future monitoring programs of other USDA-regulated food products.

P1-24 Performance Verification of an ELISA-based Assay and a Rapid Lateral Flow Immunoassay for Specific Quantification and Detection of Egg White Protein in Food Matrices, Clean-in-Place (CIP) Rinse Water and Environmental Samples

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Introduction: Egg protein is a common food allergen, particularly for children. Products containing eggs may be processed utilizing shared equipment, increasing the risk of cross-contact contamination. Cleaning verification and food testing are important elements for assessing allergen control management. Thus, effective tools for food allergen analysis are required.

Purpose: To evaluate the performance of two specific protein immunoassays for qualitative and quantitative detection of egg white proteins in food, CIP and environmental samples.

Methods: An ELISA method and a rapid lateral flow (LF) immunoassay were evaluated for cross-reactivity and capability for quantitative and qualitative detection of egg proteins from different sources. Foods (n = 25) were spiked with egg to determine the protein recovery and detection after sample extraction. Cross-reactivity was assessed in 24 different commodities. Recovery was also assessed in CIP rinse water (n = 3) and swabs (n = 3). For LF method the probability of detection was determined in water and food samples and for ELISA accuracy and linearity were also evaluated.

Results: Egg protein standard was used to construct a 4-parameter logistic curve which allows quantification of egg protein in the range of 0.5-13.5 ppm with a precision of <10% CV. The ELISA method showed linearity in samples containing raw and processed egg including products labeled as egg yolk. Cross-reactivity was not detected in the evaluated food commodities with both methods. The probability of detection by LF was 0.5 ppm of egg white protein in food. Recovery of egg proteins by ELISA was above 80% and detection of egg proteins at 0.5 ppm was achieved by LF in all spiked foods.

Significance: Egg white protein specific ELISA and lateral flow tests are reliable analytical methods for specific quantification and detection respectively of egg white proteins in a variety of matrices in the food industry.

P1-25 Verification Study to Assess the Detection of Food Allergens in Swabs and Clean-in-Place (CIP) Rinse Water Utilizing Rapid Lateral Flow Immunoassays in the Presence of Commercial Sanitizers

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Introduction: Cross-contact allergen contamination is one of the causes for food allergen recalls. Cleaning food-contact surfaces for allergens is considered a preventive control to minimize the risk of cross-contact. Thus cleaning verification should be assessed with analytical methods to assess cleaning effectiveness.

Purpose: To verify the performance of a rapid lateral flow immunoassay for allergenic foods to detect specific food proteins in stainless-steal surfaces and CIP rinse water including their performance in the presence of commercial sanitizers.

Methods: Solutions of 13 known allergenic foods (AF) were spiked on stainless-steel coupons and water to determine detection of trace levels of food proteins (0.5 to 5 ppm depending on the AF) from swabs and CIP utilizing rapid immunoassays (*n* = 3/AF/sample). Detection of specific food protein from allergenic foods in swabs and CIP was also assessed in the presence of chlorine, peracetic acid, quaternary salts and four other commercial sanitizers (*n* = 3/AF/sanitizer/sample), non-sanitizer controls were included (*n* = 2/AF/sample). Samples were analyzed with specific immunoassays following manufacturer's instructions for 3M™ Allergen Protein Rapid Kits.

Results: Detection of proteins from AF in swabs and water was achieved at the limit of detection (LOD) claimed for the 13 rapid immunoassay tests. Detection of proteins from AF in swabs was not affected at 200 ppm of chlorine, quaternary salts or peracetic acids, nor by commercial sanitizer solutions diluted to the supplier's recommended concentration for use. Detection of egg protein in the presence of 20 ppm of chlorine was not achieved at the expected LOD (0.5 ppm) but at ≥3 ppm of egg protein. Two ppm of fish protein in quaternary salts solution was detected only when quaternary salt concentration was reduced to 20 ppm.

Significance: Specific rapid lateral flow immunoassays evaluated in this study, can be utilized as verification methods to assess specific protein cleaning in CIP final rinse water and equipment surfaces.

P1-26 Temporal Co-occurrence of Antimicrobial Class Residue in Tissue and Antimicrobial Sensitivity Profile from Cecal Content Strains

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Introduction: Antimicrobial resistance surveillance in food animals is important in strengthening efforts to improve antibiotic stewardship.

Purpose: A paired sampling task combining the National Antimicrobial Resistance Monitoring System (NARMS) and the National Residue Program (NRP) – RESNARMS – was created to determine potential links between the antimicrobial resistance profiles of bacterial strains isolated from cecal content, and drug residues present in the kidney tissue samples collected from the same carcass.

Methods: Carcasses were sampled from June 2017 through May 2018. Cecal and kidney samples (2,477 samples) from dairy cows and young chickens were collected by FSIS in-plant veterinarians following the guidance provided in FSIS Notice 23-171. Residues in animal kidney tissue samples were analyzed at FSIS laboratories using NRP methods routinely described in FSIS Chemistry Laboratory Guidebook. The target bacteria (*Salmonella* sp., *Campylobacter* sp., *Enterococcus* sp., and generic *E. coli*), were recovered and subjected to antimicrobial sensitivity testing as described in the NARMS Manual of Laboratory Methods.

Results: Of the 2,477 samples collected from 282 establishments, 1,570 (63.4%) were from dairy cows and 907 (36.6%) were from young chickens. Pan-susceptibility (no resistance) was identified in 388 (62%) of *Salmonella* isolates, 500 (61%) generic *E. coli* isolates, 322 (39%) *Campylobacter* isolates, and 50 (8%) *Enterococcus* isolates. Only four matched pairs had both the presence of drug residues in the tissue and an antimicrobial resistant strain concurrently present in the same carcass. Of those, only one sample showed a concurrent presence of a resistance profile for Sulfonamides (generic *E. coli*) and similar drug class residue (Sulfadimethoxine).

Significance: This suggests that during this period there is no correlation between the antimicrobial resistance profiles of bacterial strains isolated from cecal content and drug residues present in carcass.

P1-27 Extraction Efficacy of Three Different Extraction Buffers in Solubilizing Proteins from Nine Commercially Important Fish Species

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❖ ***Developing Scientist Entrant***

Introduction: Fish protein is an important protein source for humans; however, it may cause severe IgE-mediated food allergies in sensitive individuals. Protein solubility often governs protein preparation and utilization for protein analysis methods. The selection of an appropriate extraction buffer is critical for efficient fish protein solubilization but it has yet to be evaluated.

Purpose: The objective of this study was to compare the extraction efficacy of three different extraction buffers in solubilizing proteins from nine selected fish species and to find robust cross-species analytical protein targets in selected fish that are extractable in an ELISA compatible buffer.

Methods: Proteins from nine fish species (Cod, Pollock, Herring, Salmon, Tuna, Skate, Tilapia, Grouper, and Halibut) were extracted in triplicate using Urea buffer (pH 8.8), Phosphate Buffered Saline (PBS, pH 7.4), and PBS with sodium sulfite (PBS-S, pH 7.4), respectively, under similar extraction conditions. Electrophoresis (SDS-PAGE, reducing conditions), protein determination (2D-Quant) and DDA LC-MS methods were used for qualitative and quantitative assessment of protein extracts.

Results: Qualitative differences in fish protein electrophoretic profiles were observed and are dependent on the extraction buffer and specific fish type. Low molecular weight proteins (~5-25 kDa) were extracted with apparent equal efficiency with PBS and PBS-S buffers. Among the three extraction buffers tested, Urea buffer, as expected, was found to be the most effective extraction buffer in extracting proteins from all nine fish species as determined by the 2D-Quant protein assay. The extraction efficiency of Urea buffer was at least 6-fold higher than that of PBS or PBS-S buffer. For each extraction buffer, protein extraction efficiency varied among different fish species. Quantitative comparisons of selected allergens were assessed via label-free MS quantitation.

Significance: The results indicate that the type of extraction buffer has a strong influence on fish protein solubility and is critical for fish protein preparation and utilization.

P1-28 Detection of Food Adulterants Using Multi-spectral Imaging

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Introduction: Multi-spectral imaging was used to determine the percentage of ingredients vs. adulterant in test food matrices (flour, milk powder, and sugar).

Purpose: To apply multi-spectral imaging for instantaneous and non-destructive analysis capable of identifying non-conforming product among model low-water activity foods.

Methods: Multi-spectral imaging system consisting of a high-resolution camera capable of image capture at wavelength bands ranging from ultraviolet to near infrared light was used to identify the presence of adulterants in common food ingredients. Pure samples of flour, milk powder, and sugar were analyzed in duplicate using multi-spectral imaging for a total of 6 images. Pure samples (controls) were compared using normalized canonical discriminant analysis (nCDA) to identify the optimum combination of wavelength bands for differentiating between samples using the Rayleigh quotient and to produce a CDA value for each pixel. The system was then trained with the correct combination of bands from the nCDA analysis to identify each pure sample. Next, 6 blind mixtures of each of the 3 ingredients at varying concentrations were imaged.

Results: A color image of the mixed samples did not reveal any differences, nor were differences detectable by the naked eye. However, by observing the entire spectra, repeatable differences were detected in the mixed samples using nCDA analysis. Additionally, multi-spectral imaging was able to determine the percentage of each of the ingredients in the blind mixed sample with an accuracy of ± 5%.

Significance: Multi-spectral imaging is an emerging technology and effective tool for detecting food adulterants in a fast, non-destructive, and versatile way. The development of methods for analyzing low-water activity ingredients will enhance global food safety.

P1-29 Development of a Microfluidic Paper-based Analytical Device to Detect Allergens in Food Samples

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Introduction: The prevalence of food allergies increased significantly in the past decades, and undeclared food allergens account for the most number of recalls in North America. Rapid, portable, easy-to-use, and low-cost devices are highly demanded by the government, industry, and consumers to monitor food allergens.

Purpose: This study aims to develop a cellulose paper-based lateral flow immunoassay that can detect protein allergens in real food samples.

Methods: Ovalbumin was selected as the target to detect egg content. First, gold nanoparticles were synthesized with a modified citrate reduction method and were conjugated to anti-ovalbumin antibodies. Then, antibody pairs were screened to select a recognizing (gold-labelled) antibody and a capturing antibody. The modification of Whatman Grade 1 filter paper was optimized with different recipes, followed by optimization of patterns to increase the effective volume of the sample liquid. The optimized devices were used to test standard ovalbumin solutions (0 – 100 ppm), egg-white-spiked cake mix (0 - 2%), and commercial food products (muffin, mayonnaise, etc.). Tests on each sample are at least triplicated. Only 15 min is need for each test, including sample preparation.

Results: The modification of paper substrate with No. 33 recipe successfully achieved the complete release of conjugated and the elimination of background signal due to non-specific binding of antibodies to cellulose paper. The developed device could detect as low as 1 ppm ovalbumin in standard solutions. As low as 0.01% (w/w) egg white spiked in cake mix could be detected with sample preparation. Egg content in commercial products with heat processing was also detected.

Significance: This study expanded the potential of cellulose filter paper as a novel platform for chemical hazard detection, especially large molecules (e.g., protein allergens). This study may serve as a template to develop more paper-based devices for the detection of chemical hazards and food allergens.

P1-31 Effect of Probiotic Bacteria on Fungal Growth and Mycotoxin Production by *Aspergillus* spp.

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Introduction: Aflatoxins (AF) and ochratoxin A (OTA) are highly toxic metabolites mainly produced by *Aspergillus spp.* Aflatoxins and OTA have been found in various foods, such as cereals, coffee and nuts. They may post nephrotoxic, immunotoxic, neurotoxic and teratogenic effects on both humans and animals. Previous studies showed the ability of lactic acid bacteria (LAB) to reduce fungal growth. However, the extent of LAB to reduce or remove mycotoxin might be different depending upon the different strains.

Purpose: This study aimed to evaluate the influence of probiotic strains from commercial probiotic supplements on fungal growth and total aflatoxin production by *A. flavus* and *A. parasiticus*.

Methods: Nine strains of probiotics were isolated from commercial probiotic supplements. (1) Each probiotic strain (OD_{540nm} 1.1, 1 mL) was co-cultured with AFB1 and OTA standard 100 mg/kg in De Man, Rogosa and Sharpe media at 37°C for 24 h. (2) Spore suspensions (OD_{540nm} 0.25, 0.1 mL) prepared from four *Aspergillus spp. strains* (*A. flavus*, *A. parasiticus*, *A. fresenii* and *A. sulphureus*) were spread on MRS agar. The different probiotic was spot inoculated (10 µL) on plates and incubated at 37°C for 10 days. Inhibition zone and toxin production were measured. Total AF and OTA concentrations were analyzed by high-performance liquid chromatography.

Results: *Lactobacillus plantarum* 299V, *L. rhamnosus* GG, and *L. acidophilus* LA-14 strains have the antifungal ability on the MRS agar plate. The inhibition zone varied by the probiotic strains. *L. acidophilus* LA-14 also has the highest binding ability to reduce AF during 24 hours fermentation; the aflatoxin degradation rate was 47%. However, *L. rhamnosus* GG was less efficient on AF binding ability.

Significance: Potential strains of LAB from commercial probiotic supplements, which have the detoxification ability of probiotics to bind or utilize AF and OTA, were screened. The antifungal ability of the LAB strains to eliminate AF and OTA production was investigated.

P1-33 Evaluation of *Listeria monocytogenes* Composite Enrichment with and without Food Matrix

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❖ ***Developing Scientist Entrant***

Introduction: Environmental sampling in a food production plant is routinely conducted using swabbing devices to verify cleaning procedures and determine presence of target organisms, such as *Listeria monocytogenes*. Sample enrichment compositing may be conducted to reduce testing costs.

Purpose: To assess the U.S. FDA Compliance Document enrichment method for the detection of *L. monocytogenes* with and without food matrix, and to assess the number of enriched samples which may be composited without loss of sensitivity.

Methods: Romaine lettuce wash (RLW) was prepared by homogenizing one head of Romaine lettuce with 1 L of BPB. *L. monocytogenes* was inoculated into UVM broth at 7.20 ± 0.18 CFU/225 mL with (n = 30) or without (n = 45) RLW with a native microbiota population of 4.25 ± 0.17 log CFU/225 mL. UVM was incubated at 30°C for 24 h. For secondary enrichment, 0.1 mL from UVM was inoculated into 10, 20, 30, 50, and 80 mL Fraser broth representing positive:negative compositing ratios of 1:0, 1:1, 1:2, 1:4, and 1:7, respectively. Results were statistically analyzed via Fisher's Exact Test (α = 0.05).

Results: *L. monocytogenes* was detected in 30/30 (100%) and 34/45 (76%) enrichment samples with and without RLW, respectively. When UVM enrichments were composited at 1:1, 1:2, 1:4, and 1:7 without RLW, *L. monocytogenes* was detected in 36, 36, 33, and 33 samples, respectively. Although *L. monocytogenes* was detected in fewer samples when compositing increased from 1:2 to 1:4, this difference was not statistically different; thus, no loss in sensitivity was observed. *L. monocytogenes* was detected in all 30 samples with RLW at all compositing ratios.

Significance: Results suggest that compositing *L. monocytogenes* from culture in ratios of up to 1:7 may be appropriate for detecting the pathogen from enrichment and that the native microflora present in a food matrix background may impact the detection of *L. monocytogenes*.

P1-34 Comparative Study between 3M™ Petrifilm™ Aerobic Count Plate and Conventional Agar Method for Setting Expiration Date of Ready-to-Eat Food Sold by Japan's Convenience Store

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Introduction: Many types of RTE foods are sold at convenience stores in Japan, and their manufacturers conduct aerobic plate count test to RTE foods stored at low temperature for setting expiration date. Verification test is required for using Petrifilm™ in test of setting expiration date for RTE foods. However, there are few Petrifilm™ verification test data of this type of samples.

Purpose: To evaluate 3M™ Petrifilm™ Aerobic Count Plate (AC plate) in comparison to Standard method agar (SMA) for aerobic plate count in various types of RTE foods stored at low temperature.

Methods: Test samples are 25 types of RTE foods, a total sample is 50 samples and these samples were provided by RTE food manufacturer. Two samples were prepared for each type of food, one was stored at 10°C for 24 h, and one was stored at 10°C for 48 h. After storage for a scheduled period, each sample was homogenized and diluted with saline, each serial dilution was inoculated to AC plate and SMA (n = 2), incubated at 35°C for 48 h. Appeared colonies were measured as aerobic plate count. This study was conducted by Incorporated Foundation Tokyo Kenbikyo-in (ISO17025 certificated 3rd party laboratory).

Results: Test results of AC plate and SMA showed high correlation coefficient (R^2 = 0.90). Mean of difference of two methods is 0.10 log CFU/g. Samples with over 1.0 log CFU/g difference in two methods was only 3 samples out of 50 samples in this study. Of these 3 samples, 2 samples are a small number of bacterial count (<300/g) and could be considered to be because of variations that could normally occur.

Significance: It was confirmed that an appropriate test result could be obtained by AC plate even in various type of RTE food stored at low temperature for setting expiration date by these data.

P1-35 A Novel Chromogenic Detection System for the Isolation of *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii*

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Introduction: Various cultural methods have been employed to isolate and detect foodborne pathogenic *Arcobacter* spp.; however, false-positive isolates may interfere with *Arcobacter* isolation using current methods.

Purpose: This study developed and tested a selective enrichment broth and selective/differential plating medium that incorporated an improved inhibition system to reduce false-positives and a chromogenic substrate to detect C-2 esterase activity in *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*.

Methods: The detection system comprised of NRJ-*Arcobacter* selective enrichment broth (NRJ-ASEB) and NRJ-*Arcobacter* Chromogenic Plating Medium (NRJ-ACPM) was evaluated for inclusivity and exclusivity using 46 *Arcobacter* and 39 non-*Arcobacter* strains. *Arcobacter* strains were grown in *Brucella* broth under aerobic conditions at 30°C for 48 h. Non-*Arcobacter* strains were grown in non-selective broth at 35°C for 24 to 48 h. After incubation, strains were serially diluted to 10^3-10^4 CFU/mL and 0.1 mL was inoculated in 10 mL of NRJ-ASEB. Tubes were incubated aerobically at 30°C and enumerated on non-selective after 48 h. Concurrently, the microbial strains were tested qualitatively on NRJ-ACPM by the K-streak method and incubated aerobically at 30°C for 48 to 72 h. Tests were repeated three times in triplicate.

Results: *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* were enumerated to a final concentration of 10^7-10^8 CFU/mL after 48 h incubation in NRJ-ASEB. Furthermore, NRJ-ASEB inhibited growth of non-*Arcobacter* strains. *Arcobacter* isolates produced flat to convex, salmon colonies with diameters of 1.0 to 2.0 mm on NRJ-ACPM after 72 h. Overall, the efficacy NRJ-ACPM resulted in 97.8% inclusivity and 92.3% exclusivity for the strains tested.

Significance: The novel chromogenic detection system utilized a chromogenic substrate to detect C-2 esterase activity in *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* with an enhance inhibition system to reduce false-positives and false-negatives. Future direction will compare the chromogenic detection system to current methods when isolating *Arcobacter* spp. in poultry.

P1-36 Comparisons of Diluting Solvents to Enhance the Vaporization of Essential Oils

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✦ ***Undergraduate Student Award Entrant***

Introduction: Although there are many studies for the antibacterial activities of plant origin essential oil vapors (EO vapors), the optimum diluents for the vaporization of EOs have not been reported.

Purpose: This study was done to determine the optimal dilution solvent of liquid EOs for the generation of EO vapors.

Methods: Among 97 EO vapors tested, EO vapors with relatively strong inhibitory activities against *Staphylococcus aureus* were screened by a vapor diffusion assay. The screened EOs were diluted in 4 types of organic solvents (dimethyl sulfoxide, ethyl acetate, ethyl ether, ethanol) and 6 kinds of plant oils (almond, apricot kernel, avocado, jojoba, sunflower, walnut oil) and minimal inhibitory concentrations (MICs) of EOs against *Staphylococcus aureus* in each diluents were determined.

Results: Among 97 EO vapors, 3 EO vapors (Cinnamon bark, oregano, thyme thymol) showed relatively strong antibacterial activity against *S. aureus*. It was observed that, when ethanol was used as a diluent, 3 EO vapors (Cinnamon bark, oregano, thyme thymol) showed the lowest MIC (0.1563 mL/mL, 0.0781 mL/mL, 0.0781 mL/mL, respectively). The second lowest MIC was measured when Dimethyl sulfoxide was used as a diluent.

Significance: This study is the first attempt to identify the optimal diluent in generating EO vapor from liquid EO. These results would provide basic information in developing a decontamination strategy using EO vapors.

P1-37 Mammalian Cell-based Immunoassay for Detection of Viable *Salmonella enterica* Serovar Enteritidis from Poultry Products

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❖ ***Developing Scientist Entrant***

Introduction: Current *Salmonella* culture-based methods detect viable cells but usually take 24-72 h to complete, while culture-independent nucleic acid-based and immunological methods are unable to verify the viability of the target organism.

Purpose: We developed a mammalian cell-based immunoassay (MaCIA) for detection of viable *S.* Enteritidis from contaminated poultry products to overcome current limitations.

Methods: Fixed human ileocecal adenocarcinoma cell line, HCT-8, was used as the platform to capture only viable *S.* Enteritidis cells. Captured *S.* Enteritidis cells were detected in 4 h using a combination of *S.* Enteritidis-specific monoclonal antibody (mAb-2F11), anti-mouse HRP conjugated antibody and a colorimetric substrate. A panel of 6 strains of *S.* Enteritidis, 12 strains of other *Salmonella* serovars and 7 strains of non-*Salmonella* spp. at 10^7 CFU/mL was used to confirm the ability of MaCIA to distinguish viable *S.* Enteritidis from the dead ones, other *Salmonella* serovars, and non-*Salmonella* spp.

Results: The limit of detection (LOD) of MaCIA was estimated to be 10^6 CFU/mL after 30-min of incubation of artificially inoculated chicken samples, however, the LOD was 10 CFU/mL when 8-h on-cell enrichment was used. Unlike other cell-based sensors using live cells, fixed cell-based MaCIA platform provides longer shelf life (30 days), and a stable cell monolayer for improved assay performance for the onsite point-of-care application.

Significance: The intestinal cell-based MaCIA platform has great potential to be applied for the detection of other enteric foodborne pathogens using an appropriate antibody combination.

P1-38 Assessing the Ability of Acid Treatment and Plating on Selective and Non-selective Differential Agar Plates to Improve the Recovery of *Shigella* and Enteroinvasive *Escherichia coli* (EIEC) Post Enrichment.

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Introduction: *Shigella* methods for foods uses antibiotics that kill Gram-positive bacteria during enrichment. Recovery on MacConkey agar plates (MAC) relies more on differentiation of Lactose fermentation than selection.

Purpose: Evaluate a 5-min treatment in 0.2M KCl pH 2.2 and plating on MAC or on a differential non-selective Tryptic Soy Agar (TSA) developed in house, FDA Lactose Green TSA (LGTSA), as an improvement in *Shigella* and EIEC recovery post enrichment.

Methods: Pure cultures representing 39 *Shigella*, 60 EIEC and 29 putative bacterial competitors from produce were acid treated for 5 minutes then serially diluted onto TSA or MAC and counted after incubation at 35°C to measure acid susceptibility and the level of cell injury. A selection of 6 *Shigella* and EIEC strains showing different response to 0.2M KCl pH 2.2 were then evaluated, in pure cultures and mixed with enrichment broth from produce types, for recovery on TSA or LGTSA and MAC, with or without acid physiological enrichment.

Results: Following acid treatment and plating on selective MAC, 36/39 of *Shigella* and 55/60 EIEC strains were moderately affected or resistant (< 2 log-kill), compared to 15/29 competitors. TSA was milder; all *Shigella,* 57/60 EIEC were resistant (< 1 log-kill) and 3 EIEC were moderately affected (between 1 and 2 log-kill). A total of 15/29 competitors were resistant to acid and 7/29 strains were moderate. All strains tested on LGTSA behaved like on TSA after acid treatment, with additional differentiation between Lac^+/Lac^- and H_2S^+/H_2S^-. A resistant *Shigella* strain was tested in mixed cultures at ratios 1/82 and 1/112. After acid treatment, the final ratio on TSA was 1.2/1 for both, and was 46/1 and 18/1, respectively, on MAC.

Significance: Acid treatment can enrich for resistant strains on MAC, and TSA to a lower extent. Acid physiological enrichment is being evaluated in mixed cultures with recovery on LGTSA and MAC, using strains that were the most affected by acid in pure cultures.

P1-39 Analysis of Five Methods for the Concentration of Genetic Material from the Apple Peel

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❖ ***Developing Scientist Entrant***

Introduction: Controlled atmosphere cold storage used for apples delays fruit ripening and decay; however, some fungal species survive and grow on the fruit surface, causing rot. As these organisms decay the fruit surface, it may leave the fruit at increased risk of colonization by saprophytic foodborne pathogens such as *Listeria monocytogenes*. Microbiome analysis will provide insights to how microbial populations shift throughout cold storage (up to twelve months), but DNA extraction methods must be optimized which are reproducible and allow for high-quality downstream analysis.

Purpose: To identify a reproducible method to concentrate and analyze fungal and bacterial DNA from fresh apple surfaces.

Methods: Microbial species were collected from the apple peel (n = 45) using one of five methods: 1) a 250 mg sample of excised surface material, 2) swabbing of a 25 cm^2 area of the apple surface, 3) submersion in a 250 mL 1X Tris-EDTA (TE), 4) method 3 with 2% Tween 80, or 5) filtration of a wash solution containing 250 mL 1X TE. The filter was then submerged in a wash solution containing 25 mL 1X TE + 2% Tween 80. All wash solutions were sonicated for a minimum of 5 min and centrifuged, from which the pellet was resuspended in 250 µL 1X TE before DNA extraction. DNA was extracted from all samples using a ZymoBIOMICS DNA/RNA Miniprep Kit and quantified using the Qubit 4 Fluorometer.

Results: The surface excision method isolated the highest average quantity of DNA (662 pg/µL), followed by surfactant sonication (615), sonication (25), filtration (5), and surface swabbing (<1). Surface excision was also the method most likely to capture higher quantities of apple DNA during collection.

Significance: Surfactant sonication could provide the greatest opportunity for isolating low concentrations of microbial species from fresh apple surfaces with a low risk of host DNA contamination.

P1-40 Process Validation of Hepatitis A Virus Inactivation in Spinach Using *Staphylococcus carnosus* CS 300 Grown with 20% Glycerol at 42ºC

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❖ ***Developing Scientist Entrant***

Introduction: Hepatitis A virus (HAV) is more heat resistant ($D_{65°C}$=1.73 min in buffer) than most non-sporeforming bacteria. Validation of HAV inactivation in microwavable food is necessary to ensure food safety. *Staphylococcus carnosus* CS300 has increased thermal resistance after growth in media containing 20% glycerol at 42°C. Therefore, it is a potential HAV surrogate for heat inactivation studies.

Purpose: The objective of the study was to compare the thermal inactivation kinetics of *S. carnosus* CS300 grown with 20% glycerol at 42°C to HAV in frozen spinach, for process validation.

Methods: Frozen cut-leaf spinach homogenized in sterile phosphate buffered saline was inoculated with ~7 log colony forming units (CFU) of *S. carnosus* CS300 grown at 42°C in Tryptic Soy Broth containing 20% glycerol. After 2-h bacterial attachment, the mix was aliquoted into 2-ml sterile glass vials and heated at 58, 60, 64, and 68°C, with come-up times of 151±7, 131±19, 132±9, and 112± 8s, respectively. Heat-treated suspensions were serially diluted and surface spread-plated in duplicate on Tryptic Soy Agar plates for enumeration. Data from triplicate experiments were statistically analyzed using mixed model analysis of variance and Tukey's adjustment.

Results: Linear model D-values of *S. carnosus* CS300 in spinach at 58, 60, 64 and 68°C were 69.44 ± 8.02, 20.72 ± 0.70, 3.67 ± 0.39, and 1.24 ± 0.10 min, respectively, with a Z-value of 5.77°C. Reported D-values of HAV at 56 to 72°C in spinach ranged from 8.43 to 0.91 min. At 64°C, an estimated 4.14-log *S. carnosus* reduction is needed for equivalent 6-log HAV reduction and an estimated 6.15 log *S. carnosus* is needed at 68°C.

Significance: These results indicate that *S. carnosus* CS 300 grown with 20% glycerol at 42°C shows potential as a HAV surrogate for validation of pasteurization (at 64 and 68°C) of microwavable frozen spinach.

P1-41 Immunodetection of Meat Adulterants

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❖ ***Developing Scientist Entrant***

Introduction: It is estimated that food adulteration costs the world economy around $49 billion annually. Around 10% of the foods produced in the United States were adulterated. From 1980 to 2013, the leading reported type of fraudulent foods was animal products including meat and meat products (7%).

Purpose: In order to (1) reduce the risk of intentional or unintentional contamination of foods, (2) better comply with food regulations, and (3) decrease economic loss to the food industry caused by recall, it is necessary to develop reliable and robust methods for the detection of different food adulterants.

Methods: Different monoclonal antibodies (mAb) specific to two target analytes (i.e., mammalian skeletal troponin and porcine hemoglobulin) were developed using the hybridoma technique. Their properties, such as epitopes and species/tissue-selectivity, were characterized using fluorescent and/or chemiluminescent immunoblotting. Three mAb-based enzyme-linked immunosorbent assays (ELISA) were developed and validated for the detection of mammalian meats and porcine blood residues in foods, respectively.

Results: First, the optimized sandwich ELISA was specific to heated mammalian troponin and could detect as low as 1% (g/g) of heated mammalian meats adulterated in poultry meats. Second, an indirect competitive ELISA was established for the quantification of porcine hemoglobin spiked in heated (100°C/15 min) meat products with excellent sensitivity (limit of detection: 0.5 ppm). Finally, a sandwich ELISA specific to porcine hemoglobin was established to quantify porcine hemoglobin residue in unheated animal meat.

Significance: Overall, these immunoassays have high species/tissue-selectivity, low limit of detections, high precision, and reproducibility with low inter- and intra-coefficient of variances, and a wide working range. The established immunoassays have the potential to fight food fraud, comply with food regulations, and decrease food recalls, which may open up new diagnostic methods for the food industry and the food regulatory authorities.

P1-42 Evaluation of a Microbial ATP Bioluminescence-based Method As a Rapid Detection System for Testing Commercial Sterility in Ultra High Temperature (UHT) Pasteurized Milk

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Introduction: UHT milk may be transported and released without refrigeration and it is critical to ensure that commercial sterilization is achieved. In China, commercial sterility in UHT milk is commonly evaluated utilizing pH and/or agar and requires 10-day sample incubation. The use of rapid methods for commercial sterility test can provide 2-3 day results which enables faster release of UHT milk to the food industry.

Purpose: To evaluate a microbial ATP-bioluminescent-based method as a rapid test for commercial sterility in UHT milk comparing to traditional agar.

Methods: UHT milk containers (*n* = 26 containers/microorganism/spike level) were inoculated with *Bacillus subtilis, Staphylococcus aureus, E. coli* or *Geobacillus stearothermophilus* with 3 spike levels (range of 10-1,000 CFU/container). Containers were incubated for 48 h and 72 h at 36°C for all mesophiles and at 55°C for *Geobacillus* spiked containers. Microbial detection was performed on agar and with the ATP-bioluminescent method. Containers were also incubated with the four microorganisms in a range of 10^1-10^8 CFU/mL (*n* = 4/microorganism/spike level) to determine the lowest concentration that provided a differential signal with the ATP-bioluminescent method. To determine basal ATP levels of non-contaminated containers, samples were also incubated under the same conditions as the spiked containers. Non-incubated containers were also evaluated (N = 20 containers/treatment). A growth curve of *Geobacillus* in UHT milk was also constructed.

Results: Basal ATP levels in non-spiked UHT milk were established to enable differentiation from contaminated UHT milk. Contamination was detected in all spiked samples with *B. subtilis*, *S. aureus* and *E. coli* and the bioluminescent method with 100% accuracy with the agar results. Detection of these microorganisms occurred in a range of 10^3-10^4 CFU/mL after incubation. *G. stearothermophilus* was not detected, but growth curve showed die off after 6 h of initial inoculation in milk.

Significance: The microbial ATP-bioluminescent method offers a rapid test to evaluate commercial sterility in UHT milk.

Poster

P1-43 Impact of Gas Nanobubbles on the Efficacy of Commonly Used Antimicrobials in the Food Industry

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Introduction: Nanobubbles (NB) are defined as fine bubbles with diameter varying from 20 to 200 nm with distinctive surfactant properties because of their small size. NB are proven to be effective in irrigation systems, fisheries, wastewater treatments and dentistry.

Purpose: To study the efficacy of commonly used antimicrobials (AM) in food industry by incorporating gas NB.

Methods: This study was conducted as completely randomized block design with three replications as blocks. Air, CO_2 and N_2 were used to generate NB in water. Lactic acid, citric acid, peracetic acid, and chlorine were used to make NB-AM solutions and were tested against *Escherichia coli* O157:H7 (EC) and *Listeria monocyto*genes (LM). AM mixed with water without gas NB represented controls. Nine-milliliter AM solutions (with or without NB) were taken into individual test tubes and inoculated with 1 mL of respective microbial inoculum. After holding time of 1.5 and 3 min, 1 mL of sample was neutralized using Dey-Engley neutralizing broth and plated on brain heart infusion agar. pH and redox potential of AM solutions were measured before and after addition of microbial inoculum.

Results: For EC, Cl_2 solutions (4.3 logs) were most effective; whereas, for LM, PAA solutions were most effective (2.4 logs). AM-CO_2 solutions for 3 min were most effective for both EC and LM. As a function of AM × gas, CO_2-Cl_2 solutions was most effective (5.2 logs) for EC, and CO_2-PAA was most effective (4.4 logs) for LM. Both PAA- and Cl_2-NB solutions were more effective at 3 min compared to 1.5 min treatment time. Other treatments did not have any significant effects on EC and LM reductions with NB incorporation.

Significance: This study demonstrated that efficacy of various antimicrobials can be increased by incorporating CO_2 NB. Further, NB incorporation in AM should be tested against other pathogens in different food matrices.

P1-44 Detection of *Listeria monocytogenes* in Mixed Environmental Sponge Swab Enrichment Cultures Using the biomérieux VIDAS® Lis Assay or USDA and FDA Reference Methods

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Introduction: Environmental testing for *Listeria* is a vital food safety program. Individual sponge swabs are collected from multiple sites to take action when screening is positive, but this requires more time and cost. Creating mixed samples post-enrichment could address both while allowing traceback for a positive mixed set. Importantly, savings reinvested in the environmental monitoring program would further enhance pathogen surveillance.

Purpose: To assess the impact on *L. monocytogenes* detection when enrichments from pathogen-inoculated sponges are mixed with enrichments from four competitor inoculated sponges.

Methods: Sponge swabs hydrated with DE neutralizing broth were co-inoculated at a fractional level with approximately 1.0 CFU *L. monocytogenes* and approximately 10 CFU *Enterococcus faecium* (competitor) (n = 20). Sponges (n = 80) were inoculated with 10 CFU *E. faecium* and enrichments from each were mixed with the co-inoculated enrichments in a 1:5 ratio. Positive (5 CFU/sponge, n = 5) and negative (uninoculated, n = 5) sponges were included. Inoculated sponges were refrigerated 18 h to simulate shipping conditions prior to enrichment and screened using the VIDAS®LIS immunoassay or USDA and FDA reference methods. All sample enrichments were streaked to isolation media and the plates examined for typical colonies.

Results: Individual sponge enrichments demonstrated comparable recovery to the five-sponge mixed enrichments when tested by immunoassay and reference methods. The proportion of fractional positives was 10/20 (POD 0.50) for individual and mixed USDA and immunoassay enrichments, 8/20 (POD 0.40) for individual FDA enrichments, and 6/20 (POD 0.30) for mixed FDA enrichments. The Probability of Detection (POD) was calculated. No significant difference was observed between individual sponge enrichments and secondary mixed sponge enrichments when tested by any of the methods evaluated. All confidence intervals (95%) contained 0. All five high inoculated sponges tested positive (POD 1.00) and all five negative sponges tested negative (POD 0.00).

Significance: Five-sponge mixed enrichments provide a comparable recovery to testing sponges individually.

P1-45 Independent Evaluation of the Real-time BAX® PCR Assay for *Listeria monocytogenes* in Food Samples for Health Canada Compendium Inclusion

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Introduction: Detection of *L. monocytogenes* in food samples is of increasing importance highlighted by rising numbers/severity of food safety recalls and outbreaks in recent years. This study, performed by the University of Guelph's Agriculture and Food Laboratory (Ontario, Canada) aimed to evaluate a real-time PCR method's ability to detect the pathogen.

Purpose: This study assessed a real-time PCR assay for screening *L. monocytogenes*. Assay sensitivity/specificity, level of detection, inclusivity and exclusivity were assessed in pure culture and in food enrichments relative to the MFHPB-30 (Microbiology Food Health Protection Branch; *Isolation of Listeria monocytogenes and other Listeria spp. from foods and environmental samples*), the Health Canada Compendium reference method.

Methods: Fifty target and 30 non-target strains of *L. monocytogenes* were tested during inclusivity/exclusivity studies. The inclusivity portion tested 10 cells/225 mL of 24 LEB Complete; the exclusivity portion tested pure cultures at ≥10^5 CFU/ml. Lysates of artificially spiked and naturally contaminated food enrichments from 15 food types across 5 food categories (n = 675) in Actero™ Elite Listeria Enrichment Media were prepared. Test kit results were compared to independently spiked or naturally contaminated samples, enriched per MFHPB-30.

Results: The assays were 100% inclusive for all *L. monocytogenes* tested; exclusivity was 100% against closely/ distantly related genera. Sensitivity studies showed equal or better performance, compared to Health Canada Compendium method, in all 5 sample categories with Relative Sensitivity of 100% and a Relative Specificity of 98.3%.

Significance: The real-time PCR assay for *L. monocytogenes* exhibits increased assay sensitivity and faster/easier sample preparation and cycling times than endpoint PCR detection. This new assay allows for rapid time-to-results for the testing of food samples, while preserving the ease, accuracy and dependability of the BAX® System.

P1-46 A Novel Optical Biosensor Based on Target-induced Immunomagnetic Beads Aggregation for Label-free and Portable Detection of Enrofloxacin

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Introduction: Antibiotic residues in poultry could lead to the development of resistant bacteria, posing a risk to human health. Enrofloxacin (ENR) is a kind of synthetic antibiotic that has been used in the poultry industry in China. Since conventional methods for ENR detection, such as HPLC are complicated and time-consuming, new methods are needed for rapid detection with portable devices for in-field application.

Purpose: The objective of this study was to develop a novel biosensor based on target-induced immunomagnetic beads (IMBs) aggregation for one-step and portable detection of ENR in poultry products.

Methods: IMBs were prepared by conjugating antibodies to the surface of magnetic beads based on a carbodiimide method, and used for both sample separation and signal generation. After reaction with ENR, initially well-dispersed IMBs were aggregated in a concentration-dependent manner. Two detection strategies were developed for signal outputs: 1) 200 µL of the mixture of IMBs and IMBs-ENR aggregates were magnetically separated for 20 s, and the turbidity of the collected supernatant was measured for ENR quantitation; and 2) 50 µL of the mixture of IMBs and IMBs-ENR aggregates was loaded into a specially designed syringe and automatically filtered through a nitrocellulose membrane, and the gray scales of the trapped IMBs on the membrane were measured to determine the concentration of ENR.

Results: The proposed method allowed sensitive detection of ENR in less than 15 min without any labels, and exhibited a satisfactory limit of detection of 0.91 ng mL^{-1}, which is better than or comparable with most of reported immunoassays with complicated signal amplification. Recoveries in the range of 74.9-121.8% demonstrated its ability to detect ENR in chicken samples.

Significance: The developed biosensor is rapid, label-free and portable for in-field detection of ENR and has the potential for applications to poultry supply chain to improve food safety.

P1-48 BAX® System SalQuant from Farm to Final Product: What's Your Number?

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Introduction: Throughout the food industry there's a need to develop a holistic management system to address various levels of *Salmonella* from farm to final products. Prevalence testing for *Salmonella* is the quickest and most affordable method of monitoring; however, the initial contamination location or verification of corrective actions are difficult to observe with only prevalence sampling.

Purpose: Utilization of *Salmonella* enumeration will provide a reliable system to track and address contamination throughout food production.

Methods: Linear fit equations utilizing BAX Cycle Threshold (CT) values were developed to estimate pre-enrichment levels of *Salmonella* in live production (poultry boot swabs, ceca, whole viscera packs), the processing environment (poultry chicken skin, whole bird and parts rinsates), and final products (ground chicken, turkey, beef; pork trim). Multiple enrichment parameters were evaluated with linear fit equations tested by entering CT values into equations and comparing the known spike log levels to SalQuant estimated log levels with 95% confidence intervals surrounding the best fit line.

Results: All matrices from farm to final products were successfully quantified at varying log levels utilizing BAX® System SalQuant application of BAX® System Real-Time assay for *Salmonella*. Enrichment parameters for matrices included pre-warmed 42°C BAX MP media with antibiotic and incubation at 42°C. Specific incubation times for enrichment varied between matrices based upon meeting acceptable statistical parameters. R^2 values ranged from 0.82-0.98 and log RMSE ranging from 0.14-0.57 were observed. Various matrices were compared to conventional enumerative methods with no statistical difference between populations ($P < 0.05$).

Significance: SalQuant provides accurate and precise estimations of pre-enrichment log *Salmonella* with wider enumerable ranges compared to conventional enumerative methods. Implementation of this method provides the industry with a route to rapid, reliable, data-driven food safety decision by utilizing numerical data for incoming farm *Salmonella* levels, intervention efficacy during processing, and final levels in finished products.

P1-49 Evaluation of a Loop-mediated Isothermal Amplification (LAMP) - Bioluminescent Assay for *Salmonella* Detection in Boot Swabs from Brazilian Poultry Industry

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Introduction: Poultry products are recognized as a recurrent vehicle for *Salmonella* outbreaks. In the poultry industry one of the measures to maintain quality control and speed up decision-making is to monitor *Salmonella* incidence from farm through boot swabs. Rapid and accurate *Salmonella* detection in primary production samples is critical for effective monitoring to prevent product contamination.

Purpose: To evaluate a Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay (AOAC 2016.01 and NF 3M 01/16-11/16) for detection of *Salmonella* in litter poultry bedding boot swabs as compared to ISO 6579-1:2017 by determining specificity, sensitivity, and accuracy.

Methods: A total of 136 boot swab samples collected from poultry farms were artificially contaminated with *S.* Typhimurium ($n = 26$) and *S.* Gallinarum ($n = 110$) with low (1-5 CFU/25g, $n = 46$ for *S.* Gallinarum and $n = 12$ for *S.* Typhimurium) to high (5-50 CFU/25g, $n = 61$ for *S.* Gallinarum and $n = 11$ for *S.* Typhimurium) populations. Some samples ($n = 13$) were also inoculated with *Proteus* spp. as an interferent organism. All samples were enriched 1:10 in BPW-ISO at 37°C for 20-24 h and analyzed with the LAMP-bioluminescent assay and ISO 6579 method. Sensitivity, specificity and accuracy, were determined.

Results: The alternate method had no false positives and low (0.86%) false negative rate. The candidate assay was able to detect both *S.* Typhimurium and *S.* Gallinarum in boot swabs and an interferent organism at high level (about 4000 CFU/sample) did not affect the detection of *Salmonella*. Compared to the traditional method, accuracy and specificity of the LAMP-bioluminescent assay was 99.3% and 100%, respectively, for the boot swabs. This was further confirmed by POD analysis which did not show any significant difference between the alternate and culture confirmation method.

Significance: The enrichment in BPW-ISO allowed growth of *Salmonella* in the boot swabs enabling rapid and automated detection of *Salmonella* by the LAMP-Bioluminescent assay. The alternative LAMP-Bioluminescent method offers a rapid and reliable method to screen boot swabs for *Salmonella* contamination.

P1-50 Evaluation of a Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay for *Campylobacter* Detection in Cooked Breast Chicken from the Brazilian Poultry Industry

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Introduction: *Campylobacter* is globally recognized as a major cause of foodborne infections in humans. *Campylobacter* is a significant problem in the poultry industry throughout the world. Regulations are evolving to monitor the incidence and implement effective prevention measures to control *Campylobacter* in poultry. *Campylobacter* is a fastidious microaerophilic organism needing special media and conditions for growth. This has necessitated easy monitoring solutions for laboratory analysis to accurately detect *Campylobacter* to avoid false-negative results.

Purpose: To determine the specificity, sensitivity and accuracy of *Campylobacter* LAMP-bioluminescent Assay (AOAC PTM 111803) coupled to a ready-to-use enrichment broth for detection in cooked chicken breast as compared to ISO 10272-1:2017 method.

Methods: A total of 40 cooked breast chicken samples collected from poultry industry were artificially contaminated with *C. jejuni* (about 4 CFU/25 g; *n* = 10); *C. jejuni* and *E. coli* as interferent (about 4 CFU/25 g and 113 CFU/25g, respectively; n=10) and non-contaminated samples (*n* = 20). All samples were enriched 1:10 in 3M™ Campylobacter Enrichment Broth (CEB) at 41.5°C for 24-28 h and analyzed with the LAMP-bioluminescent assay and ISO 10272-1:2017 method. Sensitivity, specificity and accuracy, were determined.

Results: The alternate assay was able to detect *Campylobacter jejuni* in cooked chicken breasts. Compared to the traditional method, sensitivity, specificity and accuracy of the LAMP-bioluminescent assay was 100%. The alternate method had no false positives or false negatives. The POD analysis between the LAMP method and culture confirmation did not show any significant difference at a 95% confidence interval.

Significance: The ready-to-use enrichment medium allowed growth of *Campylobacter* under aerobic conditions in cooked chicken and enabled LAMP-bioluminescent assay to provide a reliable and specific detection of this pathogen. The easy-to-use RTU enrichment broth coupled to LAMP-bioluminescent assay provides next-day results that can be easily adopted by most food testing laboratories.

P1-51 Performance of Rapid Enumeration Methods for Indicators in Brazilian Concentrated Juices

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Introduction: In Brazil, juices are among the highest averaged consumed drinks. In 2017, 511,385 liters of concentrated juices were produced, with 2.46 liters consumed *per capita*. Concentrated juice is defined as a drink submitted to physical process for water removal, increasing in at least 50% its soluble solids. This high sugar content, along with acidic pH, limits microbial development to certain groups such as lactic acid bacteria (LAB) and yeast and molds, but these factors also hinder microorganism recovery in microbiological analysis, necessitating a differentiated sample preparation, focusing on neutralization.

Purpose: This study aimed to verify correlation among aerobic, LAB and yeast and molds alternative and ISO methods.

Methods: Five types of concentrated juices were evaluated and divided into 67 samples (10 g each). Lemon (pH 0.63), orange (pH 2.88), grape (pH 2.88), mango (pH 3.89) and apple (pH 3.05) had their pH measured again under several dilutions with single and double strength BPW. Applying BPW double strength, lemon juice was brought to neutrality in 1:100 and 1:50 to all others. All samples were contaminated with 3 log CFU/g of *E. coli*, *Leuconostoc* sp. and *Candida albicans*. Samples were naturally contaminated with gram-positive rods. Samples were analyzed with aerobic (25 samples), LAB (20 samples) and rapid yeast and molds (22 samples) 3M™ Petrifilm™ Plates methods and their respective ISO method. A paired t-test was conducted to determinate statistical differences ($P < 0.05$).

Results: There was no statistical difference between the alternative and ISO methods to enumerate aerobic, LAB and yeast and mold counts in concentrated juices. *P*-values obtained were 0.248, 0.0679 and 0.440, respectively.

Significance: The alternative methods enabled reliable enumeration of mesophilic aerobes, yeast and molds and LAB in acidic concentrated juice samples compared to their respective ISO methods.

P1-52 MALDI-TOF MS Analysis for Simultaneous Discrimination of Cereulide-producing *Bacillus cereus* and Psychrotolerant *Bacillus cereus* Group from Other *B. cereus* Group

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Introduction: Cereulide-producing *Bacillus cereus* strains can cause foodborne illnesses with vomiting, and psychrotolerant *Bacillus cereus* group such as *Bacillus mycoides* and *Bacillus weihenstephanensis* can grow rapidly at 7°C and thus are industrial concerns due to their ability to cause spoilage of refrigerated food.

Purpose: To establish a novel, rapid and simple method for simultaneous discrimination of cereulide-producing *B. cereus* and psychrotolerant *B. cereus* group from other *B. cereus* group.

Methods: The cereulide-producing ability of 35 *B. cereus* group strains was examined by PCR based on the cereulide synthetase gene and confirmed by LC/MS analysis, also the psychrotolerance of these strains was examined by a growth test at 7°C. To determine biomarkers to discriminate psychrotolerant *B. cereus* group by MALDI-TOF MS, genes in the *S10-spc-alpha* operon, which encodes ribosomal proteins, were sequenced and the theoretical masses of the ribosomal proteins were calculated. The actual masses of the ribosomal proteins were then assigned based on MALDI-TOF MS results. In addition, potassium adducts of cereulide were identified by MALDI-TOF MS analysis. All test strains were analyzed by MALDI-TOF MS running in positive mode.

Results: To discriminate psychrotolerant species, four biomarkers were selected. Among 35 test strains, 5 strains had cereulide productivity and 12 strains grew at 7°C. Simultaneous detection of both the four biomarkers and cereulide by MALDI-TOF MS analysis could successfully discriminate 5 cereulide-producing *B. cereus* strains and 12 psychrotolerant *B. cereus* group from other *B. cereus* group.

Significance: The results suggest that this simple and rapid MALDI-TOF MS method allows simultaneous discrimination of both cereulide-producing *B. cereus* and psychrotolerant *B. cereus* group from other *B. cereus* group, and thus may be a valuable tool for ensuring food safety.

P1-53 Evaluation of Rapid *Cronobacter* and *Salmonella* Detection in Powder Infant Formula and Related Matrices Using Loop-mediated Isothermal Amplification (LAMP)–Bioluminescent Assay Compared with the GB Methods

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Introduction: *Cronobacter* and *Salmonella* have emerged as major pathogens of concern in powdered infant formula (PIF). China PIF industry is growing and the consumers are concerned about potential risk of these pathogens in PIF. The PIF producers need rapid, easy to use and specific detection of *Cronobacter* and *Salmonella* for monitoring of raw materials, process environment and finished products for better risk control.

Purpose: To determine the specificity and sensitivity of LAMP assays compared to GB 4789.4-2016 (National Food Safety Standard Food microbiological examination: *Salmonella* spp.) GB 4789.4-2016 (*Salmonella* spp.) and GB 4789.40-2016 (National Food Safety Standard Food microbiological examination: *Cronobacter* spp.) methods.

Methods: Fifty-eight natural samples (37 PIF and 21 raw material samples), 93 artificially contaminated samples (1 to 10 CFU for 64 PIF samples, 0.1 to 10 CFU for 29 raw material samples) and 19 each of PIF and environmental samples inoculated with *Cronobacter*, *Salmonella* and *E. coli* were enriched in BPW ISO at 37℃ for 18 hours and analyzed with respective LAMP assays and GB method. Specificity, sensitivity and accuracy were determined. For inoculation with multiple organisms, 10 CFU each or 100 CFU each of *Cronobacter* and *Salmonella* were used and for some samples (n = 4), 100 CFU of *E. coli* was used as an interferent organism.

Results: The LAMP assays detected *Cronobacter* and *Salmonella* in all artificially contaminated samples and both methods were in agreement with the GB method**.** Both specificity and sensitivity of the *Cronobacter* LAMP assay was 100% and for Salmonella LAMP assay the specificity and sensitivity 89.66% and 100%, respectively. No significant differences were observed among all the 185 samples (95% confidence interval).

Significance: For the different PIF matrices tested, the LAMP assays were in agreement with the culture based GB methods. The LAMP assays are a reliable method for the rapid and specific detection of *Cronobacter* and *Salmonella* in PIF matrices and provide next-day results for the PIF industry.

P1-54 Evaluation of a Loop-mediated Isothermal Amplification (LAMP)-Bioluminescent Assay for *Salmonella* Detection in Yogurt and Yogurt-based Drinks as Compared to the GB Method

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Introduction: The *Lactobacillus* concentration of yogurt and yogurt-based dairy drinks in China is more than 10^7 CFU/mL and the pH is around 4.0. Pathogens such as *Salmonella* are hard to recover from these matrices and optimization of culture conditions is needed for accurate detection of *Salmonella* in these products.

Purpose: To optimize growth conditions for *Salmonella* in low pH yogurt and yogurt-based drinks and compare the LAMP assay and the traditional GB 4789.4-2016 method (National Food Safety Standard Food microbiological examination: *Salmonella* spp.) for *Salmonella* detection in these matrices.

Methods: In a paired study, LAMP assay and GB method were compared using 1:10 and 1:20 dilution of the matrices in BPW-ISO. In addition, enrichments were incubated and evaluated at 24 h, 26 h, 28 h and 30 h of incubation. Naturally contaminated (n = 8) or artificially contaminated [approximately 1 CFU *Salmonella* and 10 CFU *E.coli* per 25 g (n = 5) and 10 CFU *Salmonella* and 10 CFU *E. coli* per 25 g (n = 5)] samples were enriched in BPW-ISO for 24-30 h at 41.5°C and analyzed by the LAMP and the GB method.

Results: *Salmonella* failed to grow to detectable levels in 1:10 enrichments even at high levels of artificial contamination (100 to 1,000 CFU/25 g). The pH was reduced to <5.0 after enrichment. Both LAMP assay and GB method were not able to detect *Salmonella* with 1:10 enrichments in 16 out of 20 samples. However, with 1:20 dilution, *Salmonella* grew to detectable levels at low spike levels (1 to 10 CFU/25g) in all samples and detected by both methods. Both sensitivity and specificity of the LAMP assay was 100% as compared to the GB method. Based on POD analysis, there was no significant difference between the alternate and culture method with modified protocol.

Significance: Higher dilution of matrices in BPW-ISO for low pH yogurt and yogurt-based drinks enables growth of *Salmonella* facilitating detection by both the LAMP assay and the GB method. The alternative LAMP assay enables reliable and rapid detection of *Salmonella* providing next day results.

P1-55 Rapid Detection of STEC and *Salmonella* in Beef and Poultry Matrices Using Loop-mediated Isothermal Amplification (LAMP)–Bioluminescent Assays

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Introduction: Cattle have been identified as important reservoirs for STEC and *Salmonella*. The hides, hooves, and gastrointestinal tracts of cattle can contain these pathogens resulting in contamination of raw products. Several outbreaks of *Salmonella*, a common poultry adulterant, have also been traced to beef products.

Purpose: To compare the LAMP-Bioluminescent assays for detection of *Salmonella* and STEC in beef and poultry matrices to the USDA FSIS MLG reference methods. Dual enrichments for STEC and *Salmonella* were also evaluated by the LAMP methods.

Methods: STEC: Detection of STEC in 325 g each of raw ground beef (n = 15), beef trim (n = 15), poultry parts (n = 26) inoculated with a cocktail of three STEC isolates was compared in a paired study (mTSB) and unpaired study (BPW-ISO) using a LAMP and MLG PCR assay. STEC and *Salmonella*: Detection of STEC and *Salmonella* in 325 g each of raw ground beef (n = 15), beef trim (n = 15), poultry parts (n = 26) inoculated with a cocktail of three STEC and three *Salmonella* isolates was conducted after enrichment in BPW-ISO at 42°C for 15 hours using LAMP assay and culture confirmed.

Results: Detection of STEC from the LAMP method were in agreement with the reference method for both the paired and unpaired analysis. The POD analysis between the two methods did not show any significant difference at a 95% confidence interval. In dual enrichment samples, the LAMP assays were able to detect both STEC and *Salmonella* in all inoculated samples and were in agreement with culture confirmation.

Significance: The STEC LAMP-bioluminescent method provides a rapid and specific approach for the detection of STEC in beef and poultry matrices. In addition, analysis of both STEC and *Salmonella* using same enrichment provides next-day results with one workflow. The faster turnaround time of results enables release of products to market faster, especially for products with limited shelf life.

P1-56 Comparative Evaluation of the 3M™ Molecular Detection Assay 2 – STEC Gene Screen for the Detection of STEC in a Variety of Matrices

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Introduction: Shiga toxin genes (*stx1, stx2*) and the intimin gene (*eae*) from shiga toxin-producing *Escherichia coli* (STEC) can cause human illness and disease through severe diarrhea and hemolytic uremic syndrome (HUS) following the consumption of contaminated food products and water. Molecular detection of *stx*1 and/or *stx*2 along with the *eae* gene is commonly used for screening STEC in food samples.

Purpose: To compare the performance of a LAMP-Bioluminescent gene screen method for the detection of STEC in comparison to the reference gene screen method MLG 5C.00 for ground beef and beef trim and BAM Chapter 4A for spinach.

Methods: In an unpaired study**,** two matrices (ground beef and beef trim, *n* = 30 for each matrix) by the LAMP method and the MLG 5C.00 and one matrix (spinach, *n* = 30) by the LAMP method and BAM Chapter 4A were compared. Artificially contaminated samples, 1-3 CFU per 375 g (ground beef and trim) and 200 g (spinach) were enriched in BPW-ISO (LAMP) and mTSB (MLG) or mBPWp with ACV (BAM). Respective enrichments were analyzed by the LAMP method and the reference method and culture confirmed.

Results: The LAMP assay detected all the 50 STEC isolates tested and did not detect any of the 40 strains from the exclusivity panel. Both the sensitivity and specificity of the alternate method was 100%. There were no false positive and false negative results with the alternate method. Based on POD analysis, no significant differences were observed between presumptive and confirmed results for the LAMP method or between the LAMP and reference method results for 375-g test portions of fresh raw ground beef, beef trim and 200 g spinach. For beef trim (14/20), ground beef (8/20), and spinach (16/20) fractional positives were obtained and confirmed.

Significance: For all matrices evaluated, the 3M™ Molecular Detection Assay 2 - STEC Gene Screen was equivalent to the reference methods for the rapid detection of STEC. Hence, LAMP assay is a reliable method for the rapid and specific detection of STEC from fresh raw ground beef, beef trim and spinach.

P1-57 Automated System for Pathogen Detection Using Loop Mediated Isothermal Amplification (LAMP)–Bioluminescence Detection

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Introduction: A key component of pathogen testing is the skill of the technician running the tests. Accurately and repeatedly tracking samples, pipetting correct volumes, and faithfully manipulating a test's consumables are critical to a laboratory's overall reporting accuracy. As a lab's test volume and technician turnover increase, maintaining the quality of these key steps can be strained.

Purpose: Development of an automated pipetting and lysis workstation for the LAMP-bioluminescent detection technology for foodborne pathogens.

Methods: An automated liquid handling workstation was programmed for setting up LAMP-bioluminescent assays from enriched food matrices. Basic test performance (temperature, timing, pipetting volume for the LAMP-bioluminescent assays) of automated and manual operations were compared using pure cultures (*n* = 40) for *Salmonella*, *E. coli, Listeria* spp. and *L. monocytogenes*, inherently difficult to pipette enriched food matrices such as 1:4 poultry and beef samples, various cereals and viscous milk powder enrichments, and different dairy products such as cream and cheese (*n* = 35 total) as well as a range of 60 environmental samples from various food manufacturing environments.

Results: For pure culture testing, the samples were diluted to a fractional response and a POD analysis was performed. Manual and automated were statistically equivalent. For testing if the system can pipette difficult to pipette samples and real-world environmental samples, the panel of high-particulate foods were post-enrichment spiked at ~10^5 CFU/mL. For these samples, 100% concordance was obtained between the manual and automated systems.

Significance: The automated system effectively performed the tasks that a manual operator would have performed while avoiding pipetting errors and also providing a detailed auditable log of every task performed.

P1-58 Evaluation of a Loop-mediated Isothermal Amplification (LAMP)–Bioluminescent Assay for *Salmonella* Detection in Ice Cream as Compared to the GB Method

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Introduction: Consumption of food contaminated with *Salmonella* increases the risk of foodborne illness. Although *Salmonella* is generally associated with poultry products, several *Salmonella* food outbreaks have been associated with produce and processed foods. Use of DNA-based methods for pathogen detection allows processed food producers to release product faster as results are available sooner than traditional methods.

Purpose: To compare the traditional method GB 4789.4-2016 (National Food Safety Standard Food microbiological examination: *Salmonella* spp.) and the LAMP assay for detection of *Salmonella* in ice cream products.

Methods: Six natural samples and sixty artificially-contaminated samples of ice cream were compared by the LAMP and the GB methods. Ice cream samples were spiked with approximately 1 CFU/25 g (*n* = 20), 10 CFU/25 g (*n* = 10), and 100 CFU/25 g (*n* = 6) of *Salmonella*. Some ice cream samples were also spiked with approximately 1 CFU/25g (n=6) and 10 CFU/25 g (*n* = 6) of an interferent organism, *E.coli*. In addition, mixed strains of *E.coli* and *Salmonella* were also spiked at approximately 10 CFU *E.coli*/25 g and 1 CFU *Salmonella*/25g (*n* = 6) and 100 CFU *E.coli*/25 g and 10 CFU *Salmonella*/25 g (*n* = 6). The samplers were enriched in BPW-ISO for 18-24 hours at 36 ± 1°C. The enrichments were analyzed by the LAMP and the GB method. The results were used to determine sensitivity and specificity.

Results: LAMP assay had sensitivity and specificity of 100% for ice cream products as compared to GB method. All LAMP results were in agreement with GB culture results. Using POD analysis, no significant differences (95% confidence interval) were observed for the ice cream products between the LAMP method and the reference GB method.

Significance: The alternative *Salmonella* LAMP-bioluminescent assay offers a reliable, specific and rapid approach for the detection of *Salmonella* in ice cream products providing a next-day result to evaluate the microbiological quality and safety of ice cream.

P1-59 Evaluation of Loop-mediated Isothermal Amplification (LAMP)–Bioluminescent Assays for Pathogen Detection in Food Matrices from Mexican Super Market

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Introduction: Consumption of contaminated food with pathogens such as *Salmonella* and *Listeria monocytogenes* can be fatal to humans. Rapid, sensitive and specific detection of foodborne pathogens enables better control of the risks associated with foodborne outbreaks. The LAMP-bioluminescent assays use isothermal DNA amplification and bioluminescence for detection enabling reliable and specific detection of pathogens.

Purpose: To evaluate LAMP-bioluminescent assays for the detection of *Salmonella* and *L. monocytogenes* in different matrices.

Methods: Samples were obtained from a local supermarket in Chihuahua México and used for *Salmonella* (pasteurized egg, ground beef, chicken pieces and smoked bacon) and *L. monocytogenes* (raw egg, ground beef, chicken pieces, beef steak, smoked bacon and ready-to-eat food) testing. In addition, poultry, meat and pork carcasses were sampled with sponges and used for testing. Twenty samples were evaluated for each matrix-pathogen combination. Ten samples were inoculated with *Salmonella* Abaetetuba or *L. monocytogenes* (10 CFU/sample) and ten samples with an interferent organism (100 CFU/sample; *Klebsiella aerogenes* for *Salmonella* assay and *L. innocua* for the *L. monocytogenes* assay). Samples were enriched per FSIS/MLG protocol 4.10 (*Salmonella*) and 8.11 (*L. monocytogenes*) and analyzed by the LAMP assays. Each sample was evaluated by two different technicians and culture confirmed.

Results: All samples inoculated with *Salmonella* were detected by the *Salmonella* LAMP assay and culture, while the interferent microorganism spiked samples were negative by the LAMP assay. All samples inoculated with *L. monocytogenes* were detected by the *L. monocytogenes* LAMP assay and culture, except for smoked bacon which presented a negative result by the LAMP assay, and confirmed as negative by culture. All the interferent microorganism spiked samples were negative by the LAMP assay. Based on POD analysis there was no significant difference between presumptive and confirmed results. The sensitivity and specificity of the assays were 100%.

Significance: LAMP assays can be used as a rapid, accurate and reliable tool for foodborne pathogen detection in the matrices evaluated.

P1-60 Development of a Test Method to Evaluate the Inhibitory Properties of Swabbing Materials

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Introduction: Microbiological environmental sampling plans involving swab devices play a crucial role in ensuring sanitary conditions of food manufacturing plants and identifying bacterial contamination. Yet, studies show that swab devices may contain inhibitory compounds that compromise their accuracy and sensitivity. Testing raw materials used in the manufacture of environmental swab devices is key to verifying appropriateness of the materials and ensuring fit-for-purpose swab devices.

Purpose: Develop a test method based on extraction as a means to ensure that swab raw materials are free of inhibitory compounds.

Methods: Environmental swab materials (28.9-cm^2 cellulose sponge, 24.2-cm^2 polyurethane sponge, 522.6-cm^2 cloth) were autoclaved at 121°C for 15 min in presence of 20 mL tryptic soy yeast extract broth (TSYEB) to extract any inhibitory compounds. *Listeria monocytogenes* ATCC 7644 was inoculated in 10-mL aliquots of the TSYEB extracts (10-100 CFU per tube). An inoculum in TSYEB was used as viability control. The cultures were incubated at 35°C and viable colonies were recovered at 7 h and 24 h on tryptic soy yeast extract agar. Log values were compared using ANOVA with post-hoc Tukey-Kramer test.

Results: Average *L. monocytogenes* counts recovered after exposure to bacteriostatic cellulose sponge extracts were significantly lower than the inoculum control counts (Δlog=2.0±0.2 at 7 h and 5.4±0.6 at 24 h, n = 30-50, $P < 0.05$). Contrarily, known biocide-free materials (cellulose sponge, polyurethane sponge and cloth) demonstrated average recoveries similar to the inoculum (Δlog<0.2, n = 30-50, $P < 0.05$), confirming the ability to discern materials with and without inhibitory properties. One particular material showed significant inhibitory properties at 7 h but not at 24 h, suggesting a mild bacteriostatic effect. The method is rugged, with no significant differences obtained on different days and by different analysts (n = 3, $P < 0.05$).

Significance: The method provides a simple and efficient test for the development and testing of environmental swab devices.

P1-61 Comparison of Sampling Devices for Detection of *Listeria monocytogenes* from Stainless Steel and Plastic Surfaces

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Introduction: Swabs, sponges, and wipes may be used as environmental sampling devices to detect *Listeria* and other bacteria. The utility and efficacy of these devices based on surface material and food matrix presence is not well understood.

Purpose: To compare the limit of detection (LOD) of *Listeria monocytogenes* from stainless steel (SS) and high-density polyethylene (HDPE) surfaces using various environmental sampling devices.

Methods: SS and HDPE surfaces (8 in^2) were dot-inoculated with *L. monocytogenes* diluted in BPB at 1.34-5.11 log CFU/surface or with thawed outbreak-associated ice cream contaminated in-process at 1.00-1.60 log CFU/surface. After drying 24 h, surfaces were swabbed with 1 of 7 devices: foam swab, flocked swab, cellulose sponge, cellulose sponge without handle, polyurethane sponge, cleanroom wipe, and microfiber wipe. Devices were enriched in UVM broth for 24 h followed by secondary enrichment in Fraser broth and detection of *L. monocytogenes* on MOX agar. For each device, matrix, and surface combination, 10-30 replicate samples were tested, and data were compared at $LOD_{95\%}$.

Results: Without a food matrix, the LODs for *L. monocytogenes* from SS and HDPE were 2.33-2.84 and 4.59-5.37 log CFU/surface, respectively, except for the flocked swab, which resulted in the highest LODs for both surfaces at 2.89 and 6.31 log CFU/surface, respectively. The LODs for all devices in the presence of ice cream matrix decreased to 1.58-2.85 and 1.75-2.99 log CFU/surface on the SS and HDPE surfaces, respectively. Overall, the microfiber wipe was more effective at detecting *L. monocytogenes* from HDPE regardless of matrix presence. From SS, the polyurethane sponge and the microfiber wipe were most effective without and with matrix, respectively.

Significance: All environmental sampling devices had lower LODs for *L. monocytogenes* on stainless steel compared to HDPE. Results suggest that sampling device efficacy is affected by surface type and food matrix presence.

P1-62 Performance Evaluation of a Loop-Mediated Isothermal Amplification (LAMP)–Bioluminescent Assay for Rapid Detection of *Salmonella* spp. in Boot Swabs, Feces and Visceral Flour from Brazilian Poultry Industry

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Introduction: Brazil is one of the major global poultry producers. To maintain control of this intense production, the poultry industry adopts measures from farm to food processing to control contamination, including the monitoring of *Salmonella* spp. in boot swabs, visceral flour and poultry feces. Rapid and accurate *Salmonella* detection in these matrices is critical to increase food safety and protect consumers.

Purpose: To determine the specificity, sensitivity and accuracy of a Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay for boot swabs, feces and poultry organs compared to ISO reference method 6579-1:2017.

Methods: In a paired study, 99 samples (59 boot swab, 30 feces and 10 visceral flour) from a local farm were artificially contaminated with *S.* Typhimurium (34 boot swabs, 30 fecal samples and 10 visceral flour samples) and *S.* Gallinarum (25 boot swabs) with low (1-5 CFU/25g) to high (50-60 CFU/25g) populations, pre-enriched 1:10 in BPW ISO at 36°C for 20-24 h and analyzed with LAMP-bioluminescent assay and ISO 6579. Sensitivity, specificity, and accuracy were determined.

Results: The alternate LAMP assay was able to detect both *S.* Typhimurium and *S*. Gallinarum in the matrices tested. Compared to the traditional method, the overall sensitivity, specificity and accuracy of the LAMP Bioluminescent Assay was 100.00%, 93.33% and 97,98%, respectively. The LAMP-Bioluminescent assay had a false positive rate of 2.2% and no false negatives. The POD analysis between the LAMP method and culture confirmation did not show any significant difference at a 95% confidence interval for all the matrices tested.

Significance: The alternative LAMP-Bioluminescent molecular method enabled reliable, rapid and automated detection of *Salmonella* spp. in boot swabs, feces and visceral flour. The easy-to-use LAMP-bioluminescent method offers poultry producers a rapid method to screen primary production samples.

P1-63 Acid Treatments for Improved Detection and Isolation of *E. coli* O157:H7 from Mung Bean Sprout Irrigation Water

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Introduction: Detection and isolation of *E. coli* O157:H7 from sprout irrigation water can be difficult due to the high background microflora associated with this matrix.

Purpose: This study optimized enrichment procedures and evaluated post enrichment treatments for isolation of *E. coli* O157:H7 from artificially contaminated mung bean sprout irrigation water.

Methods: Spent mung bean sprout irrigation water was inoculated with *E. coli* O157:H7 at low (0.017 CFU/mL) and high levels (0.17 CFU/mL), and stored at refrigerated (4°C) for 72 h. Three enrichment procedures were evaluated: (1) BAM modified buffered peptone water + pyruvate (mBPWp) for 5 h at 37°C, followed by addition of acriflavine (A), cefsulodin (C), vancomycin (V) and further incubated at 42°C; (2) mBPWp with CV held at 42°C with shaking and (3) mBPWp with CV held at 42°C without shaking.

Enriched samples were streaked directly, diluted and plated or treated with Dynabeads MAX *E. coli* O157 for immunomagnetic separation (IMS) then streaked onto TC-SMAC, R&F *E. coli* O157 Chromogenic medium and Chromagar O157 for cultural recovery of the pathogen from the artificially contaminated samples. Acid treated samples (broth and IMS) were also culturally isolated. Real-time PCR detection of *E. coli* O157:H7 was done on the ABI 7500 Fast platform, screening for *stx*1, *stx*2 and O157*wzy* gene targets.

Results: Isolation of *E. coli* O157:H7 was difficult from the enrichments due to excessive growth of competing organisms on the selective plates. Acid treatment of the enrichments improved recovery of the inoculated pathogen. Higher numbers of isolates were recovered on the acid treated samples (both after direct plating and after IMS) from the CV+42ºC enrichments with or without shaking than from BAM enrichments.

Significance: This study demonstrates the use of optimized enrichment procedures and the utility of an acid treatment for effective isolation of *E. coli* O157:H7 from sprout irrigation water samples.

P1-64 Improved Detection Efficiency with Modified Enrichment Broth and qPCR with *Iap* Primer and Tm Value for *Listeria monocytogenes* in Golden Needle Mushroom

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Introduction: *Listeria monocytogenes* became a significant foodborne pathogen in recent decades, and the pathogen was recently detected in a mushroom. To detect *L. monocytogenes*, conventional methods take more than 2 days. Thus, qPCR can be used to reduce the time, but it has low sensitivity to detect the pathogen after enrichment.

Purpose: This study compared the detection efficiencies of qPCR with *iap* primer and Tm value to determine *L. monocytogenes* presence after enrichment in the modified enrichment medium with conventional culture methods for *L. monocytogenes* in golden needle mushrooms.

Methods: From August 2019 to September 2019, 35 golden needle mushroom samples were collected from retail stores. Twenty-five gram portions of golden needle mushroom were placed aseptically in sample bags. Fifty milliliters of Listeria enrichment broth (LEB) plus 0.1% ferric citrate (LEB+FC) were added into the sample bags and enriched at 30°C for 3 h. Three-milliliter aliquots of the enrichment cultures were used for DNA extraction, and analyzed by qPCR with *iap* primers. As a result of the melting curve analysis, a sample having a Tm value of 84±0.5℃ was determined as positive. The identical samples were also analyzed with the conventional culture method, which was enrichment in LEB and isolation of colony on PALCAM agar, followed by colony identification by 16S rRNA sequencing.

Results: Of 35 samples, 13 (37.1%) samples were *L. monocytogenes* positive by the conventional culture method, but the qPCR methods identified 26 samples (74.3%) as *L. monocytogenes* positive in golden needle mushrooms, which were determined with the *iap* primers and Tm value.

Significance: These results indicate that the qPCR method with the *iap* primers and Tm value to determine the presence after enrichment with LEB+FC can save time and improve the detection sensitivity for *L. monocytogenes* in golden needle mushroom, compared to the conventional culture method.

P1-66 The Use of a Novel Selective Supplement in the Rapid Recovery and Detection of Pathogenic Gram-Negative Organisms from Challenging Food Matrices

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Introduction: Greater food safety, particularly identifying contamination of challenging spices, cocoa-based or raw beef matrices, is required. Through development of a novel supplement, selective recovery of pathogenic Gram negative organisms and in turn shortened presumptive result times are achieved in these difficult matrices without impacting specificity, sensitivity and precision.

Purpose: Preferential recovery of *Salmonella* spp. and *Escherichia coli* O157:H7 from multiple enrichment broths following addition of a novel selective supplement. Detection occurs from 25 – 375 g samples through a single enrichment step with a negative or presumptive positive result within 12 – 14 or 24 h for raw beef or challenging matrices, respectively.

Methods: Un-paired matrix studies comparing test to reference protocols were carried out. 25 – 375 g samples, either un-inoculated or artificially inoculated (1 – 158 CFU/portion) with 13 different *Salmonella* strains and an *E. coli* O157:H7 strain, were enriched with 3 – 9 parts supplemented broth for 10 – 12 or 20 – 22 h at 41.5°C, respectively, for raw beef and challenging matrix samples. All enriched test and reference method samples were confirmed according to BAM Chapter 5 and USDA MLG 5.09. protocols.

Results: A total of 321 spices/cocoa-based and 83 raw beef samples were tested with 176/321 and 69/83 samples containing *Salmonella* and *E. coli* O157:H7 by the BAM Chapter 5 and MLG 5.09. reference methods, respectively. A total of 181/321 and 67/83 samples were test ELISA positive with subsequent cultural confirmation and no false positive results. POD analysis between respective test and reference methods indicated no significant difference at $P < 0.05$ (-0.06 – 0.09 and -0.14 – 0.09 confidence interval of respective dPODs).

Significance: Development of a novel supplement that selectively recovers pathogenic Gram negative organisms and shortens time to a presumptive result from challenging matrices.

P1-67 Application of a High-throughput Targeted Amplicon Sequencing Approach for Detection of Foodborne Pathogens from Produce Samples

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Introduction: Next-generation sequencing (NGS) approaches provide resolution and sensitivity for both detection and identification of foodborne pathogens. Technical challenges remain in detecting low levels and unculturable pathogens in contaminated food using either whole genome or metagenome sequencing methods.

Purpose: We assessed the limit of detection and efficacy of the targeted amplicon sequencing (TAS) approach for detection and identification of bacterial (Shiga toxin-producing *Escherichia coli* [STEC]), spiked in romaine lettuce and RNA viral pathogens, (hepatitis A virus [HAV]), added to berry extracts.

Methods: Three different inoculum levels (3 to 300 CFU) of STEC were used. Cells were harvested at 0 h, 4.5 h and 20 h for DNA isolation. For HAV, RNA was extracted from berries following the BAM method. Different concentrations (ranging from 3 to 300,000 copies) of an in-house HAV transcript, were then added to the extract, followed with random pre-amplification. Libraries were prepared for shotgun metagenomics and TAS, and sequenced using the Illumina MiSeq Platform. An in-house bioinformatics pipeline was used for the identification and quantification of the targeted pathogens from the sequence reads datasets.

Results: For *E. coli*, reads were detected from both shotgun metagenomics as well as amplicon libraries at high spike levels. At low spike levels, the amplicon libraries were better at detecting the pathogen to the species level and detecting the pathogenic markers such as *stx2A*, *eaeA* and *ehxA*. For the detection of HAV, the results from both methods were comparable with strain-level detection at the lowest spike level tested.

Significance: A targeted approach for detecting low levels of pathogens provides a rapid and effective method for FDA to identify both foodborne bacterial and viral pathogens such as *E. coli* and hepatitis A virus. This technique in parallel with currently used methods like PCR may augment the detection of such foodborne pathogens in produce samples implicated in outbreaks.

P1-69 Evaluations of Lactose Broth and Three Buffered Pre-enrichment Broths for Use in the Bacteriological Analytical Manual *Salmonella* Culture Method for the Analysis of Low Microbial Load/Low-moisture Foods

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Introduction: Variable effectiveness among lactose broth (LB) and buffered pre-enrichment media for recovery of *Salmonella* from low microbial load/low moisture foods (LMF) has complicated the search for a common pre-enrichment medium for the analysis of LMF using the *Bacteriological Analytical Manual* (BAM) *Salmonella* culture method.

Purpose: To compare *Salmonella* detection among LB and 3 buffered pre-enrichment broths {Universal Pre-enrichment Broth (UPB), Buffered Peptone Water (BPW), and modified Buffered Peptone Water (mBPW)} for analysis of LMF with the BAM *Salmonella* culture method.

Methods: Recoveries for *S*. Reading, *S*. Irumu, and *S*. Bareilly from dry egg noodles, walnut pieces, and collagen hydrolysate, respectively, were evaluated for LB, UPB, BPW, and mBPW preenrichment by their pairwise comparisons for *Salmonella* detection following analysis of 20 test portions of an LMF for each pre-enrichment medium (Fisher's exact test)($P < 0.05$).

Results: *S*. Reading was detected in dry egg noodles with LB (1 positive test portion), UPB (8), BPW (4), and mBPW (0). UPB was significantly more effective than LB for those test portions ($P < 0.05$). For walnut pieces, no significant differences in the detection of *S.* Irumu among the pre-enrichment broths was observed ($P > 0.05$). For collagen hydrosylate, LB was significantly more effective than BPW (LB, 6 positive test portions; UPB, 1; BPW, 0; mBPW, 1) ($P < 0.05$).

Significance: Significant differences for *Salmonella* detection among LB and buffered preenrichments for analysis of these LMF showed LB was less effective than UPB for the analysis of dried egg noodles, yet significantly more effective than BPW for *Salmonella* detection with collagen hydrolysate ($P < 0.05$). No significant differences among the preenrichments occurred for analysis of walnut pieces ($P > 0.05$). Further research with additional matrices will be needed to determine if a single pre-enrichment broth will be appropriate for all LMF.

P1-70 Detection of *Campylobacter jejuni* in Water Using Dead-End Ultrafiltration and Its Application for Field Testing

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Introduction: Several *Campylobacter* species cause human gastroenteritis worldwide, with the infectious dose being as few as 500 bacteria. Human campylobacteriosis is usually associated with the consumption of raw milk, contaminated water, poultry, and seafood. Additionally, *Campylobacter* species may be transferred from contaminated irrigation water to ready-to-eat fresh produce. Therefore, it is essential to develop a sensitive method to detect *Campylobacter* in water.

Purpose: To establish the limit of detection for *Campylobacter* species using dead-end ultrafiltration (DEUF), by spiking 10 L of double distilled water with different concentrations of *Campylobacter jejuni* (*C. jejuni*).

Methods: Double distilled water (10 L), spiked with different concentrations of freshly cultured *C. jejuni*, was pumped through the Rexreed-25S hollow fiber filter that was pretreated with sterile 5% fetal bovine serum. After filtration, the filters were stored at 4°C for at least 3 days to mimic shipping temperature and time. The bacteria were recovered from the filters by backflushing with 500 mL of elution buffer, containing 0.5% Tween 80, 0.01% sodium polyphosphate and 0.001% Antifoam Y-30. The eluate (100 mL) was enriched in 2X Bolton broth under microaerobic conditions at 37°C for 48 h and observed for the presence of *Campylobacter* by phase contrast microscopy. Enriched eluates were plated on mCCDA plates and the colonies were screened for motility and *Campylobacter*-like morphology.

Results: We were able to detect 10 CFU *C. jejuni* in 10 L water using DEUF after the filters were stored in the refrigerator for up to 3 days. Our future experiments will include other human pathogenic *Campylobacter* species.

Significance: Our results show that DEUF can be effectively used to filter large volumes of agricultural water samples and allow for the efficient recovery of as few as 10 CFU *Campylobacter* from 10 L of water.

P1-71 Environmental Indicators for Norovirus and Hepatitis A in the Agricultural Environment: A Systematic Review

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Introduction: Monitoring for the presence of pathogens, that pose a risk to consumers, in the agricultural environment is difficult. Thus, detection of indicators of fecal contamination is used as a proxy for the potential presence of human pathogens such as norovirus and hepatitis A.

Purpose: The objective of this systematic review was to determine whether the presence of human norovirus or hepatitis A was associated with microbial indicators in agricultural samples including fresh produce, equipment and hands.

Methods: A systematic literature search of four databases (EMBASE, PubMed, Web of Science, and Agricola) between 1994-2016 was conducted by three independent reviewers. After article selection and data extraction, individual indicator/pathogen relationships (n = 36 relationships) from 15 articles were assessed using Cohen's Kappa coefficient. Kappa coefficients were combined across studies using a weighted average based on sample size.

Results: Sample types included surface water, irrigation water, and hand or produce rinses. Indicator microbes-pathogens assayed and analyzed included human adenovirus (norovirus, hepatitis A), *E. coli* (norovirus, hepatitis A), F-specific coliphage (norovirus) and total (norovirus) and fecal (hepatitis A) coliforms. The proportion of agreement was poor, using Landis and Koch's criterion, between norovirus and adenovirus (0.09 95% CI -0.05, 0.23, n = 16,) *E. coli* (0.27 95% CI -0.08, 0.48, n = 11,) F-specific coliphage (0.07 95% CI -0.07, 0.21, n = 2) and total coliforms (0.01, 95% CI -0.01, 0.02, n = 2). The proportion of agreement was also poor between hepatitis A and adenovirus (-0.05 95% CI -0.11, 0.02, n = 3), fecal coliforms (0, n = 1) and *E. coli* (0, n = 1).

Significance: Based on these limited results, common indicator bacteria are not useful in predicting the presence of norovirus and hepatitis A virus in the agricultural environment.

P1-72 ISO 16140-2 (2016) Performance Assessment of a New Protocol for Iq-Check® *Cronobacter* spp. and RAPID'Sakazakii for the Detection of *Cronobacter* spp. in 375 g Samples of Infant Formula, Infant Cereals with and without Probiotics

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Introduction: *Cronobacter* species form a group of Gram-negative bacilli which may cause lethal illness in infants. Both methods iQ-Check® *Cronobacter* spp. and RAPID'Sakazakii were previously "NF-Validation" certified for the detection of *Cronobacter* spp. in infant formula and infant cereals with and without probiotics including ingredients and environmental samples (iQ-Check® only). The previous validations were limited to 30 g sample size.

Purpose: Two independent ISO 16140-2:2016 studies (iQ-Check® and RAPID'Sakazakii) were conducted to extend the scope to 375 g samples with a new enrichment protocol along with ISO 22964:2017 as reference method.

Methods: For each study, at least 30 positive and 30 negative samples were tested for sensitivity assessment. Both studies shared the same new enrichment protocol dedicated to 375 g samples: 1:4 dilution in pre-warmed (37°C) buffered peptone water with PIF supplement prior to 18 h incubation at 37 ± 1°C. For iQ-Check® study, DNA extractions were performed with "Easy Extraction" protocol. The use of Free DNA Removal Solution (FDRS) and Application Protocol File (APF) Fast were also tested independently. For RAPID'Sakazakii study, 10 µL of enrichment were plated onto RAPID'Sakazakii agar and incubated 22 h at 44 ± 1°C. Relative Level of Detection (RLOD) and inclusivity were also evaluated.

Results: The sensitivity of the new enrichment protocol ranged between 90.9% to 100% (iQ-Check® study, depending on the sample type and application of FDRS and/or APF Fast) or was equal to 100% (RAPID'Sakazakii study) whereas it ranged between 50.0% to 84.6% for the reference method. No false positive results were observed. The use of FDRS and/or APF Fast did not significantly change the sensitivity. For both studies, the number of discordant results and RLOD meet the acceptability limits for the 375 g sample size category and for combined categories.

Significance: The new protocol is reliable for the detection of *Cronobacter* spp. in 375 g samples. The use of APF Fast with the iQ-Check® method allows a significant reduction in time-to-result.

P1-73 Assessment of a Real-time PCR Method for the Detection of Shiga Toxin-producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are important enteric pathogens worldwide, causing diarrhea with or without blood visibly present and hemolytic uremic syndrome. Cattle and other ruminants are the natural reservoir of STEC. The sources of these infections are most commonly foods that are intended to be eaten raw or part-cooked (including meat, vegetables and unpasteurized dairy).

Purpose: The purpose of this study was to evaluate the performance of the SureTect *Escherichia coli* O157:H7 and STEC PCR assays for screening and identification of STEC from three different food categories: meat, vegetables and fruits, and unpasteurized dairy.

Methods: An unpaired study was conducted versus the ISO/TS 13136:2012 reference method according to the technical rules of the ISO 16140-2 standard. A first set of samples of twenty-five grams were artificially contaminated with STEC at a low level (1 to 5 CFU/sample) in order to achieve half of the sensitivity study design. All samples were enriched (8 hours for meat, vegetables and fruits products, 16 hours for milk and dairy products), then tested with the SureTect PCR assay. Positive PCR results were confirmed using plating techniques specific to each method.

Results: For each category and for all categories combined, the number of positive deviations was generally higher than the negative deviations. This indicates a skew in performance in favor of the alternative method for the meat and vegetables categories, and equivalent performance for dairy.

Significance: The data suggest equivalent performances but better practicability for the alternative method, for which the present study is the first step towards validation and compliance with European food safety regulation.

P1-74 ISO 16140-2 (2016) Performance Assessment of a Shorter Protocol for Iq-Check® Solutions for the Detection of *Listeria* spp. and *L. monocytogenes* in Production Environmental Samples

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Introduction: Rapid and reliable detection of *Listeria* spp. and *L. monocytogenes* in the food manufacturing environment is a current challenge towards food safety. In such environments, the presence of free DNA released after sanitation or heat processing can lead to false positive issues. A new protocol, including Free DNA Removal Solution (FDRS) and Application Protocol File (APF) Fast is proposed to shorten the "NF-Validation" certified iQ-Check® workflows.

Purpose: Two independent ISO 16140-2:2016 studies (*Listeria* spp. and *L. monocytogenes*) were conducted to assess the performances of the new protocol for "Production environmental samples" category along with ISO 11290-1:2017 as a reference method.

Methods: For each study, 30 to 37 positive and 30 negative samples, including process water, surface samples, dusts and residues, were tested for sensitivity assessment of the new protocol. Samples were diluted in LSB broth and incubated 18 h at 30 ± 1°C. DNA extractions were performed with Easy II protocol with and without FDRS protocol. Amplification was performed with and without APF Fast. Relative Level of Detection (RLOD) and inclusivity were also evaluated.

Results: The sensitivity ranged between 64.3% to 100% (*Listeria* spp. study) or between 55.6% to 90.9% (*L. monocytogenes* study). Interestingly, the FDRS protocol did not significantly change the sensitivity but reduced the false positive ratio from 13.3% to 3.3% (*Listeria* spp. study) or from 6.7% to 3.3% (*L. monocytogenes* study). APF Fast did not significantly affect the performances. For both studies, the number of discordant results and RLOD meet the acceptability limits for the "production environmental samples" category and for combined categories with and without FDRS and/or APF Fast.

Significance: The new protocol is reliable for the detection of *Listeria* spp. and *L. monocytogenes* and allows significant reduction of time-to-result. The use of the FDRS can reduce the false-positive ratio of samples from the food manufacturing environment. Both validation certificates were extended in October 2019.

P1-75 ISO 16140-2 (2016) Method Comparison and Interlaboratory Study of GENE-up® EHEC Method for the Detection of Shiga Toxin-producing *Escherichia coli* (STEC) and STEC from O26, O103, O111, O145 and O157 Serogroups in Raw Meat, Raw Milk and Raw Milk Cheeses

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a serious foodborne hazard of increasing concern to food safety authorities due to significant outbreaks, mainly involving the O26, O103, O111, O145 and O157 serogroups (Top 5). The protocol described in ISO/TS 13136:2012 (reference method) for the detection of these pathogens remains fastidious and time-consuming. Validated alternative methods are still expected by the food industry.

Purpose: An independent study compared an alternative method for the detection of STEC in raw meat (except poultry), raw milk, and raw milk cheese to the reference method for ISO 16140-2:2016 MicroVal validation. The study included two independent validation schemes, allowing interpretation with STEC or Top5 STEC as target microorganisms.

Methods: The alternative method includes 4 different protocols with incubation times down to 8 h (meat 25 g sample size), 10 h (meat 375 g sample size) and 18 h (meat and dairy 25 g sample size). DNA extraction was performed with immuno-based or bead-beating methods prior to PCR screening with different combinations of assays. The comparative study to ISO/TS reference method and the interlaboratory study involving 17 collaborators were performed in accordance to requirements of the ISO 16140-2 standard.

Results: Sensitivity study: Depending on the protocols, extractions and validation schemes, 156 to 282 samples were analyzed with both methods, providing 96 to 128 positive results. Over 2,604 PCR performed from enrichment broths, 12 inhibitions only were observed. The number of discordant results meet the acceptability limits for each category, protocol and for combined categories. RLOD values obtained for 5 matrix/strain pairs (0.663 to 1.794) indicated equivalent levels of detection for both methods. The 61 target-strains were detected, and no cross reaction was observed. Interlaboratory study results were satisfying.

Significance: Equivalent performances but better practicability were observed for the alternative method and supported the certification delivered by MicroVal in December 2019.

P1-76 ISO 16140-2 (2016) Method Comparison of TEMPO® CAM Method for the Enumeration of Thermotolerant *Campylobacter* spp. in Raw Poultry and Ready-to-Cook Poultry Products

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Introduction: *Campylobacter* spp. are gram negative, pathogenic bacteria frequently found in poultry and poultry products and represent public health concerns. TEMPO® CAM is an alternative method designed to enumerate thermotolerant *Campylobacter* spp. in poultry products and environmental samples within 2 days and without a confirmation step.

Purpose: An independent study compared the alternative method to ISO 10272-2:2017 (reference method) according to ISO 16140-2:2016 standard for NF-Validation (Afnor) in raw poultry and ready-to-cook poultry products.

Methods: The alternative method uses cards which reproduce the most probable number (MPN) principle. Raw and non-processed poultry meat, neck skin and seasoned poultry samples were serially diluted in buffered peptone water (BPW) prior to inoculation of a specific culture medium. Cards were filled with inoculated medium and incubated under microaerobic atmosphere 44 h at 41.5°C ± 1°C. Reading of the cards was performed with TEMPO® Reader. The possibility to store the cards for 48 h at 5°C ± 3°C was tested. The validation scheme included the assessment of relative trueness, accuracy profiles, quantification limits, inclusivity and exclusivity.

Results: For the relative trueness study, 33 samples were analyzed providing 17 interpretable results with 29.4% naturally contaminated samples. The mean difference between both methods counts was 0.24 log CFU/g. Performances remained unchanged after 48 h storage at 5°C. For the accuracy profile study, chicken breast samples were contaminated with C. *jejuni* with three contamination levels and five test portions/level. The lower and upper β-ETI were comprised within +/- 0.5 log. For inclusivity, 50 target strains were successfully enumerated by the alternative method and gave generally higher enumeration than the reference method due to a better recovery. No cross-reaction was observed except for one non-target strain over the 30 tested.

Significance: The alternative method is reliable for the enumeration of thermotolerant *Campylobacter* spp. and offers more practicability to the user and a shorter time-to-result than the reference method.

P1-77 Direct Metatranscriptome RNA-Seq and Multiplex RT-PCR Amplicon Sequencing on Nanopore MinION – Promising Strategies for Multiplex Identification of Viable Pathogens in Food

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❖ ***Developing Scientist Entrant***

Introduction: Despite the recent developments in detection platforms of viable pathogenic bacteria, multiplex identification of viable pathogens in food remains a major challenge.

Purpose: A novel strategy is developed through direct metatranscriptome RNA-seq and multiplex RT-PCR amplicon sequencing on Nanopore MinION to achieve real-time multiplex identification of viable pathogens in food.

Methods: This study reports an optimized universal sample extraction and library preparation protocol applicable to both Gram-positive and Gram-negative pathogenic bacteria, demonstrated using a cocktail culture of *E. coli* O157: H7, *Salmonella enteritidis*, and *Listeria monocytogenes*, which were selected based on their impact on economic loss or prevalence in recent outbreaks. Further evaluation and validation confirmed the accuracy of direct metatranscriptome RNA-seq and multiplex RT-PCR amplicon sequencing using Sanger sequencing and selective media. The study also included a comparison of different bioinformatic pipelines for metatranscriptomic and amplicon genomic analysis.

Results: Bioinformatic pipelines of MEGAN without rRNA mapping showed the highest accuracy of multiplex identification using the metatranscriptomic sequencing. EPI2ME also demonstrated high accuracy using multiplex RT-PCR amplicon sequencing. In addition, a systemic comparison was drawn between the sequencing of the direct metatranscriptome RNA-seq and RT-PCR amplicons. Both methods are comparable in accuracy and time. The RT-PCR amplicon sequencing has higher sensitivity, but the metatranscriptome sequencing excels in read length and dealing with the complex microbiome and non-bacterial transcriptome backgrounds.

Significance: To the best of our knowledge, this is the first report of the metatranscriptome sequencing of cocktail microbial RNAs on this emerging platform. The information pertained in this study could be important for future revelatory research, including predicting antibiotic resistance, elucidating host-pathogen interaction, prognosing disease progression, and investigating microbial ecology, etc.

P1-78 Validation of the 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plate for the Enumeration of Coliform in a Variety of Foods Against the Canadian Reference Method (MFHPB-31)

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Introduction: The 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plate is an alternative selective and differential medium system containing an indicator of glucuronidase activity, and a tetrazolium indicator that facilitates colony enumeration. This alternative method can be used for enumeration of coliforms, visualized as red colonies, in food and beverage industries.

Purpose: The objective of this study was to evaluate the performance of the alternative method against the Compendium of Analytical Methods MFHPB-31 in a variety of food matrices for inclusion as a Laboratory Procedure (MFLP).

Methods: The alternative and comparative reference method (MFHPB-31) were analyzed by testing 5 food categories (Raw poultry, Raw meat, Heat-processed Dairy, Fermented or Dried Ready-to-Eat meat products, Pet Food). Six inoculum levels, ranging from uncontaminated to 6 log CFU/g, were used 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 the comparison of the two methods, for most food items ($\alpha = 0.05$). On average, considering all food items, the bias value is -0.084, which is within the acceptability criteria.

Significance: The 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plate met the Canadian requirements of the MMC. This method offers the capability of enumerating coliforms in foods after 18 to 24 hours of incubation, reducing presumptive reporting times.

P1-79 Evaluation of the BAX® System Real-Time PCR Assays for the Detection of E. coli O157:H7 and STEC O121 from Stainless Steel Surfaces

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Introduction: Shiga toxin-producing *E. coli* (STEC) are typically not considered a significant source of contamination in the production environment since these organisms do not persist and multiply to substantial levels. Nevertheless, if equipment is not properly cleaned and sanitized, cross-contamination can occur from food contact surfaces to raw and finished product.

Purpose: A validation was conducted to evaluate the performance of the BAX® System method compared to the USDA reference method for detecting STEC organisms from stainless steel surfaces.

Methods: In two independent studies, unpaired 4" x 4" stainless steel test areas were inoculated with a low and high level of either *E. coli* O157:H7 or STEC O121 and a non-target competitor strain. The inoculum was dried for 16-24 hours, collected by a sponge and then held at 4°C for 24 hours. Test method sponges were enriched in 90 mL of pre-warmed (35°C) BPW and incubated at 35°C for 15-24 hours. Reference method sponges were enriched in 50 mL of mTSB and incubated at 42°C for 15-24 hours. All samples were tested by PCR and culture confirmed following the USDA MLG 5C.00.

Results: For *E. coli* O157:H7, real-time PCR returned 19/20 positives for low-spiked samples enriched in BPW compared to 15/20 positives for the reference method enrichment. For STEC O121, real-time PCR returned 9/20 positives for low-spiked samples enriched in BPW compared to 6/20 positives for the reference method enrichment. All PCR results were in 100% agreement to culture. For the method comparison, no significant difference was indicated using POD.

Significance: This study demonstrates the ability of the BAX® System to accurately detect *E. coli* O157:H7 and STEC O121 from stainless steel surfaces statistically equivalent to the reference method.

P1-80 Flow Cytometry Detection Studies with Plant-based and Alternative Beverage Drinks

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Introduction: Today's consumer passion for variety as well as 'healthier' options has introduced an onslaught of alternative ready-to-drink (RTD) beverage options both in size and flavor.

Purpose: These unique flavors present different microbial challenges and should be evaluated for compatibility and challenged with organisms of interest to ensure the detection method is fit-for-purpose.

Methods: A variety of plant-based drink options which included Soy, Almond, Walnut, Coconut and Oat were spiked with low levels (<100 CFU/container) of *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Candida* or *Lactobacillus*. Individual containers ranging in size from 12.7 to 96 oz were inoculated and incubated at 30-32°C and tested at different day intervals (1 – 5 days). Replicates were prepared to capture reproducibility and record any limitations on organism growth and recovery. Flow Cytometry was compared to traditional plating and drop in pH which is standard in the industry.

Results: The diversity of the products evaluated required a universal sample preparation step which included a heating and centrifugation step. All 50 product only replicates reported negative by Flow Cytometry. Walnut Milk (32 oz) was the least supportive of growth with no organisms detected even after 5 and 10 days incubation by either Flow Cytometry or traditional plating. Coconut beverages generally required > 2 days before growth could be detected even in the smaller 18 oz volume. Bacterial contamination was detected by Flow Cytometry in Almond and Soy beverages as early as 1 day across all bacterial types. Oat milk contaminated with *Bacillus* and *Staphylococcus* were Flow Cytometry positive in a day but required a second day for *Pseudomonas* detection due to slower growth.

Significance: The different spiking studies demonstrated the efficacy (fit-for-purpose) of Flow Cytometry for screening of potential contamination in plant-based beverages and was consistent with direct plating. A drop in pH did not always accompany bacterial contamination.

P1-81 The Evaluation of the Application of a Smartphone with Colony Distinguishing and Counting AI

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Introduction: Culture-based microbiological analysis carried out by food companies, requires significant work for quality control. Colony counting is a time-consuming process, with possible variability in counted colony numbers among technicians. The use of a smartphone colony counting application could save time and assist in the correct counting of colonies.

Purpose: Nissui Pharmaceutical Co., Ltd. has developed a smartphone application named @BactLAB, which is a colony counter for a prepared media CompactDry "Nissui." The application @BactLAB contains Artificial Intelligence (AI), whose function is distinguishing and counting colonies on CompactDry "Nissui" and shows the number of colonies on the smartphone display. The purpose was to determine how well @BactLAB counts colonies on CompactDry "Nissui" TC.

Methods: The colony distinguishing and counting AI used in @BactLAB was developed with more than 12,000 graphics data of colonies grown on cultured CompactDry "Nissui" TC. Three hundred twenty samples from cooked meat and poultry, dairy, environmental, fish, fresh produce, pet food, and raw meat and poultry were tested with CompactDry "Nissui" TC (30°C, 48 h incubation), and the resulting colonies were counted by AI and a skilled technician. Two data sets were done on regression analysis and Bland Altman analysis following the relative trueness in ISO 16140-2:2016.

Results: The data show very good agreement between @BactLAB and manual count. Regression analysis shows "log CFU of manual count = -0.02106+0.9958 log CFU of @BactLAB count, R^2 value of 0.979." Bland Altman analysis shows the two counting methods at the 95% confidence level (309/320).

Significance: @BactLAB shows the colony numbers on CompactDry "Nissui" TC in a simple and user-friendly way. It supports time saving and getting correct colony numbers for food companies. Acknowledgement of support: Hitachi Solutions, Ltd for AI development (joint patent pending); Campden BRI for Evaluation

P1-82 Use of Proficiency Test Data to Evaluate Method Performance for Sulfite Analysis in Dried Fruits

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Introduction: Sulfites are added to food as a preservative to prevent spoilage and color changes during storage. However, a small population experience allergic reaction when consume high-level sulfite-containing products. FDA requires companies to label foods with sulfite level greater than 10 ppm. A reliable method for quantitation of sulfite is important.

Purpose: To use proficiency test (PT) data to evaluate the performance of a recommended LC-MS/MS method for quantitation of sulfite in dried fruits to meet FDA labeling requirement

Methods: A total of eight blind coded dried fruit samples (5 pineapple, 3 cantaloupe) were tested by 16 Food Emergency Response Network laboratories. Results reported from 12 laboratories that used the recommended method were statistically analyzed using the ISO 13528:2015 protocol, and the repeatabilities/reproducibilities were compared to that of the original multilaboratory validation study. The mean values obtained for each sample set were compared to the corresponding values obtained from the laboratory that used an AOAC method (Monier-Williams).

Results: The mean concentrations of sulfite (as SO_2) obtained from the laboratories that used the LC-MS/MS method (n = 12) were 57.8 and 192.9 mg/kg for sulfited cantaloupe and sulfited pineapple. The corresponding relative repeatabilities were 7.2 and 2.6%; and relative reproducibilities were 17.6 and 20.8%, respectively. The results of repeatability and reproducibility calculated using the PT data were comparable to that of the multilaboratory study (n = 11). The sulfite concentrations obtained by the laboratory that used the AOAC method were 69.3 and 197.1 mg/kg for sulfited cantaloupe and sulfited pineapple, respectively. No false positive was reported on the un-sulfited samples. The Limits of Reporting reported for the LC-MS/MS method were typically less than < 0.5 mg/kg, significantly lower than that (10 mg/kg) of the AOAC method.

Significance: With proper design, data obtained from a PT study can be used to evaluate a method performance, in addition to assessing laboratory performance.

P1-83 Improvement of *Cronobacter sakazakii* and *Salmonella* spp. Detection in Powdered Infant Formula

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Introduction: Probiotics, mainly *Bifidobacterium* and *Lactobacillus* species, are widely used for the supplementation of powdered infant formula in order to promote a healthy microbiome in newborns. However, they constitute a competitive flora that could interfere with the growth of *Salmonella* spp. and *Cronobacter sakazakii* thus deteriorating the performance of detection methods.

Purpose: The objective was to test a new selective compound for its efficiency to inhibit the growth of probiotics and improve the growth of *Salmonella* spp. and *C. sakazakii* for the analysis of 375 g samples of powdered infant formula.

Methods: The study was performed on 17 matrices distributed among infant formula, infant cereal and raw materials. Portions of 375 g were artificially contaminated with stressed *Salmonella* spp. or *C. sakazakii* strains (< 10 CFU/sample). The enrichment step was performed in 1,125 mL of pre-warmed Buffered Peptone Water (dilution ratio 1:4) supplemented with either the new selective compound, novobiocin or vancomycin. The samples were incubated at 37 ± 1°C for 18-26 h before analysis with chromogenic agar and PCR based detection methods. The results were compared to the ISO 6579:2017 and ISO 22964:2017 reference methods.

Results: The new selective compound showed greater inhibition of probiotics than vancomycin. Overall results indicated that detection of *Salmonella* spp. and *C. sakazakii* occurred earlier in the presence of the new selective compound. Mean PCR Cq values were significantly lower (P < 0.05) when the enrichment was performed in the presence of the new selective compound compared to vancomycin and novobiocin. This was correlated with a greater density of characteristic colonies on chromogenic agar.

Significance: These data show that the new selective compound allows a better detection of both *C. sakazakii* and *Salmonella* spp. in powdered infant formula by reducing the growth of probiotics.

P1-84 Validation of the EnviroX Assay for the Detection of *Listeria*, *Listeria monocytogenes* and *Salmonella* in Environmental Surface Samples

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Introduction: The PathogenDx Envrio^{x} is a novel, multiplexed diagnostic technology that uses PCR coupled to DNA microarray to detect dozens of organisms simultaneously from food, water and environmental samples without the need for enrichment. The advent of this technology allows the user to detect and speciate multiple organisms in under 8 hours allowing same shift turnaround.

Purpose: The purpose of this study was to validate the Enviro^{x} Environmental Screening Assay according to the AOAC INTERNATIONAL *Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, Appendix J*.

Methods: The *Performance Tested Method*^{SM} evaluation was performed in two parts by the method developer and independently through Q Laboratories. The study included the evaluation of the Enviro^{x} assay for the detection of *Listeria, Listeria monocytogenes* and *Salmonella* species (inclusivity/exclusivity study), four different matrices (stainless steel, sealed concrete, rubber, and plastic), a consistency/stability study, instrument variation study and robustness testing. The reference method for the matrix studies was the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM) Chapter 5 for *Salmonella* and BAM Chapter 10 for *Listeria* and *Listeria monocytogenes*.

Results: According to AOAC Appendix J, statistical analysis was performed using Probability of Detection (POD) statistical model. Fractional positive results for the low inoculum level, (25-75% positives) was achieved for each of the matrices tested, indicating no statistically significant difference was observed between the candidate and reference method. Results from additional PTM requirements (robustness, stability, inclusivity/exclusivity) indicated no adverse performance of the method.

Significance: The *Performance Tested Method*^{SM} evaluation concluded that the Enviro^{x} assay is able to detect *Listeria, Listeria monocytogenes* and *Salmonella* species on multiple surfaces in accordance with AOAC standards.

P1-85 Validation of the 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plate for the Enumeration of *Escherichia coli* in a Variety of Foods Against the Canadian Reference Method (MFHPB-27)

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Introduction: The 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plate is an alternative selective and differential medium system containing an indicator of glucuronidase activity, and tetrazolium indicator, which facilitates colony enumeration. This alternative method can be used for enumeration of *E. coli*, visualized as blue colonies with and without gas, in food and beverage industries.

Purpose: The objective of this study was to evaluate the performance of the alternative method against MFHPB-27 (Compendium of Analytical Methods), in a variety of food matrices for inclusion as a Laboratory Procedure (MFLP).

Methods: The alternative and comparative reference method (MFHPB-27) were analyzed by testing 5 food categories (Raw poultry, Raw meat, Heat-processed Dairy, Fermented or Dried Ready-to-Eat meat products, Pet Food). Six inoculum levels, ranging from uncontaminated to 6 log CFU/g, were used 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 was 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 was 0.041, which is within acceptability criteria.

Significance: The 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plate met the Canadian requirements of the MMC. This method offers the capability of detecting *E. coli* in foods after 18 to 24 hours of incubation, reducing presumptive reporting times.

P1-86 Evaluation of 3M Rapid Yeast & Mold (RYM) Petrifilm Testing Method for Yeast in Salad Dressings and Acid-Formulated Sauces

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Introduction: Yeast contamination in salad dressings and acid-formulated sauces is a concern, particularly in terms of food spoilage which can cause finished product packaging bloating and resulting in substantial financial losses of product each year.

Purpose: The purpose of this study was to evaluate use of the 3M RYM Petrifilm testing method for yeast in salad dressings and acid-formulated sauces.

Methods: Salad dressings and acid-formulated sauces were inoculated separately with a strain of *Zygosaccharomyces bailii* and a cocktail of *Candida* spp. at target levels of 10^2 and 10^5 CFU/g. Inoculated samples were then analyzed in duplicate using 3M RYM Petrifilm, acidified PDA, and PDA with antibiotics. All sampling plates were incubated at 25°C for up to 5 days. In addition, samples analyzed using the 3M RYM Petrifilm were also incubated at 28°C. Observation of the colonies on plates was performed and documented each day.

Results: No statistical differences were observed between samples analyzed by the 3M RYM Petrifilm, acidified PDA, and PDA with antibiotics at either 25°C or 28°C. However, there was a 1-log difference in counts observed between enumeration of colonies at 48 h and 72 h on the 3M RYM Petrifilm. The 3M RYM Petrifilm incubated at 28°C provided a better visibility of the colonies than at 25°C.

Significance: The study indicates that the 3M RYM Petrifilm method is appropriate for yeast enumeration in salad dressings and acid-formulated sauces. The data suggest incubation of samples at 28°C for a minimum of 72 h is recommended.

P1-88 Characterization of *Campylobacter* Flagellin Protein Specific Monoclonal Antibodies and Evaluation of Their Binding Affinities Using Surface Plasmon Resonance

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter* infections are responsible for foodborne illness in humans resulting in enormous disease burden worldwide. Antigen-antibody based methods such as Enzyme-Linked Immunosorbent Assay (ELISA) and Surface Plasmon Resonance (SPR) are sensitive methods for detection of *Campylobacter.* However, poor characterization of flagellin protein is the issue which needs to be addressed properly. *Campylobacter* consists of two major surface proteins, outer membrane protein and flagella protein, which are highly variable and often targeted for immunogenic sites.

Purpose: The objectives of this study were to evaluate specific epitopes of flagellin protein of *Campylobacter* by using a panel of monoclonal antibodies and to determine the binding kinetics between flagellin protein and the monoclonal antibodies.

Methods: Flagellin protein was extracted from three strains of *Campylobacter jejuni* (BAA-1153, ATCC-49943 and ATCC-33560) grown in the microaerophilic condition in CCDA media. A set of monoclonal antibodies (MAbs 5A6, 5B5, 5D7, 5E4, and 6H12) were tested with flagellin protein using ELISA and SPR. Sandwich ELISAs were used initially for screening of monoclonal antibodies. The antibodies having interactions with the flagellin protein were further verified by SPR sandwich assay.

Results: Paired comparisons of monoclonal antibodies by SPR sandwich assay showed that MAbs 5B5 and 6H12 were capable of binding to flagellin protein captured by MAb 5A6, indicating these antibodies have distinct epitopes. In contrast, MAbs 5D7 and 5E4 did not show any paired binding. Binding kinetics of monoclonal antibodies with flagellin protein was obtained by evaluating association and dissociation events using SPR. Affinities (K_D) of the monoclonal antibodies to flagellin protein were between 9.6 and 97.7 nM. The differences in the binding affinities of a particular antibody to different strains of *Campylobacter* may be attributed to the variations in flagellin protein among serovars.

Significance: These findings provide crucial information on antibody binding affinity to flagellin protein with potential of developing highly specific and sensitive assays for the detection of *Campylobacter*.

P1-89 Assurance® GDS PCR Testing Approach: A Comprehensive Portfolio for STEC Testing in Beef

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Introduction: Raw meat products, such as beef trim and ground beef, are sources of Shiga toxin-producing *Escherichia coli* outbreaks. *E. coli* O157:H7 (EHEC) is considered an adulterant by USDA, and the beef industry requires rapid screening methods to genetically identify EHEC. Recent multistate outbreaks of Top 6 STEC in beef are prompting regulators and Industry to move toward Top 7 STEC testing. The Assurance® GDS Top STEC testing portfolio provides a practical solution, with the accuracy and time to results necessary to meet today's beef testing challenges.

Purpose: To provide complete, accurate and fast STEC detection assays for beef testing.

Methods: A total of 120 375-g samples consisting of raw ground beef, raw beef trim, frozen finely textured beef and carcass sampling cloths were tested for EHEC using Assurance® GDS for *E. coli* O157:H7 Tq (IMS-rtPCR) primary screening method. Potential positive samples were subsequently tested using the Assurance® GDS EHEC ID for *E. coli* O157:H7 Tq to genetically identify EHEC. Similarly, 60 375-g samples were tested for Top 6 STEC using the Assurance® GDS for MPX Top 7 STEC. Presumptive positive samples were further tested using Assurance® GDS MPX ID for Top STEC to determine Top STEC serogroups. Additionally, EHEC ID and MPX ID were used to confirm isolated colonies from presumptive positive samples. Inclusivity and exclusivity were tested with panels of EHEC, STEC and potential cross-reacting bacteria.

Results: GDS EHEC and STEC testing assays offer same day results, including serogroup identification, in as little as 14 hours with an average of 97.3% sensitivity and 100% specificity. Further, all inclusivity strains, and no exclusivity strains, were identified, demonstrating 100% specificity.

Significance: The Assurance® GDS STEC testing portfolio provides reliable, cost effective and fast detection of STEC from beef. The use of a single enrichment broth allows streamlined lab workflows and reduces costs.

P1-90 Application of bioMérieux D-COUNT® as the Rapid Solution for Commercial Sterility Test in Coconut Products

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Introduction: The bioMérieux D-COUNT® system combines fully automated florescent viable cell labelling and digital flow cytometry into a powerfully system that can detect and count individual cells directly without the need for cell growth. It has been applied for the rapid testing of micro-organisms in dairy and beverage products; however, its suitability and analytical performance of coconut products are unknown.

Purpose: The objective of this study is to determine the suitability and analytical performance of D-COUNT system for the commercial sterility test in coconut products.

Methods: Fifteen coconut water, coconut milk and cream products were analyzed for commercial sterility test using D-COUNT system and ISO 4832-1:2013 or ISO 21527-1:2008. Thirteen samples were analyzed without contamination and 29 samples were analyzed with contaminations from 5 different microorganisms (*Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus licheniformis*, *Staphylococcus aureus*, or *Saccharomyces cerevisiae*) using both D-COUNT and cultural methods to determine whether or not products interfere with the detection of microorganisms.

Results: All thirty-five samples tested on D-COUNT showed negative results, which indicates the method suitability of these product for commercial sterility test. When compared the test results of products with or without artificial contaminations, D-COUNT provided 93.1% positive agreement and 100% negative agreement, which yield a relative trueness of 95.2%. This demonstrated that the tested product materials have no adverse effect on the ability of D-COUNT reagents to efficiently label and detect viable microorganisms. The analytical performances of D-COUNT system and associated reagents are equivalent to cultural methods compared.

Significance: The D-COUNT system provides a rapid microbial detection solution that enables coconut industrial producers to release final products 3 to 5 days earlier compared with cultural method. Additionally, this rapid solution will help producers reduce cost by reducing quarantine time, decreasing inventory costs, and minimizing the risks and costs associated with potential in-process contamination.

P1-91 Impact of the Quality of Buffered Peptone Water on the Detection of *Salmonella* spp. in Food

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Introduction: *Salmonella* can be a challenging target for food safety depending on considered strains and food matrices. It has been suggested that the quality of Buffered Peptone Water (BPW) can influence *Salmonella* recovery based on the detection protocol used (time, selectivity). Thus, the use of a poor quality BPW may be associated to a lack of sensitivity of the detection method.

Purpose: The purpose of this study was to evaluate the impact of the BPW quality on the detection of *Salmonella* strains in ISO and alternative protocols.

Methods: A set of 8 commercial BPWs were studied. *Salmonella* strains like *S.* Enteritidis ATCC 13076, *S.* Typhimurium ATCC 14028, *S.* Gallinarum DSM 13674 were submitted to heat or chemical stress. Low inoculums (< 10 CFU / sample) were used to artificially contaminate 25 g of food samples. The ISO 6579 reference method and the validated RAPID'*Salmonella* short protocol were applied and the *Salmonella* growth density obtained on the agar plates was compared. Growth kinetics of pure cultures were also tested with Bioscreen C to monitor the bacterial recovery in terms of lag phase, duration and maximal final OD.

Results: With the ISO complex protocol, the performance differences were leveled out and all the BPWs gave a similar detection level. With the shortened protocol, a significant gap between *Salmonella* recovery rates was observed, ranking from no to high detection for a same sample. Those results were explained through the growth kinetics experiments. The BPWs with the poorest *Salmonella* detection were associated with the longest lag phases and the lowest OD values.

Significance: These data suggest that end users should not neglect BPW quality. Indeed, it can significantly impact *Salmonella* detection based on the nature of the protocol employed in particular for fast time-to-result protocols, highly injured strains or low a CFU number in food matrices.

P1-92 Evaluation of STEC Detection from 25 g and 375 g Beef Samples with a PCR Method Workflow vs. ISO and USDA Reference Methods

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Introduction: Cattle are natural reservoirs for Shiga toxin-producing *Escherichia coli* (STEC) and their feces may contaminate food product during slaughter and processing. Raw or undercooked beef products pose a risk to consumers if a robust screening and identification method is not applied to this product group.

Purpose: The purpose of this study was to evaluate the performance of the SureTect *Escherichia coli* O157:H7 and STEC PCR assays (alternative method) for screening and identification of STEC from 25 g and 375 g beef samples.

Methods: Samples were artificially contaminated with a low level (1-5 CFU/sample) of STEC and enriched (8 hours for 25 g, <10 hours for 375 g) in Buffered Peptone Water before testing with the alternative method. A total of 87 samples were tested, including spiked and unspiked samples. An unpaired study was conducted versus the ISO/TS 13136:2012 (25 g) and USDA/FSIS MLG 5C.00 (375 g) reference methods. Positive PCR results were confirmed using plating techniques specific to each method.

Results: The number of total deviations of the alternative method to ISO/TS 13136:2012 (negative deviations – positive deviations) was -6 for beef indicating a skew in performance in favor of the alternative method. Comparable performance was seen between the 375 g alternative method and USDA/FSIS MLG reference method.

Significance: The data indicate that the SureTect workflow is a practical alternative to the reference methods.

P1-93 A Comparison of Two Commercially Available PCR Detection Assays for *Vibrio* from Seafood Samples

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Introduction: Bivalve mollusks are natural reservoirs of *Vibrio* species and, if eaten raw or undercooked, carry the risk of causing foodborne disease. A reliable detection method is a valuable tool to ensure that seafood, including mollusks, are safe to eat.

Purpose: A study was conducted to compare the performance of the SureTect Vibrio PCR Assay (candidate method) at detecting *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* from mollusks to the Hygiena™ BAX® System Real-Time PCR Assay (alternative method) for *Vibrio.*

Methods: Eighty-eight mollusk and crustacean samples were artificially contaminated with 2 CFU/25 g of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus.* Candidate samples were incubated for 8 hours in prewarmed single-strength Alkaline Peptone Water before running on the candidate PCR Assay using the Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System and the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System. Confirmations were performed by streaking onto TCBS medium and performing traditional confirmation methods. Alternative enrichments were tested according to the manufacturer's protocol.

Results: The results showed that the candidate method had superior performance when detecting the different *Vibrio* targets compared to the alternative method, achieving a total of 31 positive deviations. The candidate method achieved a time to result of <10 hours, compared to 20-22 hours for the alternative method.

Significance: The candidate PCR Assay provides customers with a fast and accurate result in less than 10 hours, with the PCR Assay having better sensitivity than the alternative method. The candidate PCR Assay also enables customers to easily differentiate target *Vibrio* strains from co-infected samples.

P1-94 Performance Comparison of the Two Multiplex PCR Assays for Detection of *Campylobacter* from Poultry Samples

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Introduction: *Campylobacter* is the most common cause of diarrheal disease worldwide, usually as a result of the consumption of contaminated poultry meat. The Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR Assay (candidate method) was designed to rapidly and accurately detect and differentiate *Campylobacter jejuni*, *C. coli* and *C. lari* from poultry samples in blood-free, aerobic enrichment.

Purpose: The purpose of this study was to compare the performance of the alternative method to the Hygiena™ BAX® System Real-Time Campylobacter Assay (alternative method).

Methods: Performance of the candidate method was compared to the alternative method in an unpaired study (n = 93); the alternative protocol uses gas jars during enrichment while the candidate protocol uses air-excluded homogenizer bags. Poultry samples including chicken carcass rinses, raw poultry meat with skin, and ready-to-reheat products were tested. Both untreated and artificially contaminated (1 - 10 CFU/sample) samples were tested.

Results: The candidate method detected and confirmed 10% more positive samples from raw poultry meat and 19% more positive samples from ready-to-reheat poultry meat than the alternative method. The alternative method failed to identify any positive samples from chicken carcass rinses, while 69% of samples were identified and confirmed as positive using the candidate method.

Significance: The data indicates that the SureTect Campylobacter PCR Assay has superior performance to the equivalent BAX Assay, making it more reliable for routine poultry testing.

P1-95 Testing the Inclusivity and Exclusivity of Two PCR Assays for the Detection of *Vibrio* Species

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Introduction: The majority of foodborne illnesses linked to *Vibrio* spp. are caused by three pathogenic strains: *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*. It is essential to be able to differentiate these strains to allow correct identification and to reduce the risk of outbreaks and track sources of contamination.

Purpose: A study was conducted to compare the performance of the SureTect Vibrio PCR Assay (candidate method) at detecting and differentiating *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* from non-target strains compared to the current Hygiena™ BAX® System Real-Time PCR Assay for *Vibrio* (alternative method).

Methods: A diverse panel of *Vibrio* isolates and non-target organisms were tested on the candidate PCR Assay using Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System and the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System. Inclusivity strains were grown in prewarmed

single strength Alkaline Peptone Water for 8 hours then diluted to approximately 5x10^5 CFU/mL before testing. Exclusivity strains were grown overnight in Tryptone Soya Broth and PCR conducted with the turbid cultures.

Results: Inclusivity results showed that the candidate PCR Assay had superior performance, achieving 100% inclusivity for target strains, compared to the alternative PCR Assay which achieved 70%. Both the candidate and alternative PCR Assays correctly excluded 100% of non-target strains.

Significance: This study shows that the new candidate PCR Assay can provide confidence to customers to correctly identify and differentiate between target *Vibrio* strains without compromising on inclusivity of target or exclusivity of non-target strains.

P1-96 Evaluation of STEC Isolation from Food Samples Using Chromogenic Coliform Agar

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Introduction: Isolation media such as CT-SMAC are effective for the detection of *Escherichia coli* O157:H7 but there are many challenges concerning the detection of non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC). There are few different biochemical traits between isolates of *E. coli* that can be utilized in plating methods to rapidly identify presence of non-O157:H7 STEC which makes STEC confirmation challenging.

Purpose: The purpose of this study was to evaluate the performance of Thermo Scientific™ Oxoid™ Chromogenic Coliform Agar (CCA) for detection of non-O157:H7 STEC, including from meat, vegetables, fruit and dairy samples.

Methods: Thirty-eight non-O157:H7 STEC were grown on CCA, TBX, and CHROMagar STEC to test inclusivity of each medium. Food sample enrichments, including artificially contaminated with a low level (1-5 CFU/sample) of STEC, were streaked directly onto the same agars for detection. Where direct streaking failed to isolate presumptive positive colonies, an IMS method was employed based upon results from a PCR screen for present serogroups.

Results: CCA displayed positive growth for 100% of non-O157:H7 isolates and displayed typical morphology of *E. coli* for 94.7% (36/38). CHROMagar failed to support growth for 14/38 isolates and displayed reduced growth for a further five. When isolating from food matrices, CCA displayed advantages over other media types with vegetables, fruit and unpasteurized dairy whereas CHROMagar showed advantages in selectivity when testing with meat.

Significance: The data indicates that CCA is a suitable alternative agar to previously identified options in confirmation workflows.

P1-98 Evaluation of the Post-Enrichment Process Times for Commercial *E. coli* O157:H7 Molecular Detection Systems

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Introduction: The high sensitivity of DNA-based rapid pathogen detection systems has significantly improved total time to results (TTR). However, production for food commodities such as ground beef and produce still remains a time-sensitive endeavor, such that seemingly small differences in time can have significant impact on the overall operation.

Purpose: This study compared the post-enrichment process time for five commercial *E. coli* O157 Including H7 molecular detection systems.

Methods: Simulated 325 g + 975 mL post-enrichment samples (*n* = 8) were processed through A: BAX; B: GENE-UP; C: GDS; D: iQCheck; and E: MDS, as per the respective package inserts with time (seconds) of each step recorded for two independent users, repeated 3 times. Steps were classified as manual or machine time for steps within three categories: lysis (template preparation), PCR (reaction set up), and instrument (software interaction plus machine run-time). For the time estimation of TTR for a full instrument load (72 to 96 samples), the manual times were projected by a factor of 11 (A, B, D, E) or 8 (C) based on required controls and thermocycler capacity, while the machine time remained the same.

Results: The post-incubation TTR were 11,952; 15,110; 16,141; 15,515; and 26,185 sec, for B, E, A, C, and D, respectively. Most systems spent the least amount of time on the PCR set up ~1,000 – 7,000 s; B-lowest, E-highest. Next was the lysis template preparation ~5,000 - 10,000 s; B-lowest and C-highest, followed by the most on the instrument ~5,000 – 18,000 s; B-lowest and D-highest. Overall, B was about 80% less time consuming than A, C and E; while A, B, C, and E were about half as time consuming as D for manual manipulation and instrumentation.

Significance: To our knowledge, such data evaluating the processing times between the commercial PCR systems has not been published. These data can serve as a guideline for time-sensitive food industries examining PCR methods. USDA is an EEO Employer

P1-99 AOAC PTM Certification of the BAC*Gene E. coli* STEC Solution in Two Modular Workflows

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Introduction: Shiga toxin-producing *E. coli* (STEC) are a potentially pathogenic group of *Escherichia coli*, which can cause severe disease at very low infective doses. Seven O-groups are responsible for the majority of the infections in the US and have been classified as adulterants in meat. Their detection is an important topic in food safety.

Purpose: Within the scope of the AOAC PTM certification, the performance of the BAC*Gene E. coli* STEC Solution kits was compared to the USDA MLG5C.00 and FDA BAM Chapter 4a. The study's aim was to validate the two modular workflows for the detection of the Top7 STEC. The use of the PR*Eraser* BAC*Gene* free DNA-removal and the new FastFinder evaluation software with optimized handling were included in the validation.

Methods: Matrix studies were performed for fresh raw ground beef (375 g), fresh raw beef trim (375 g), beef carcass sampling cloth, romaine lettuce (375 g), all-purpose flour, stainless steel environmental sponges and ceramic tile environmental swabs according to AOAC guidelines. All samples were analyzed after a 10-h and 24-h enrichment following the STEC Top7 and *E. coli* O157:H7 workflows and according to the respective protocols of each of the assays. Tests were performed with and without pre-treatment. Results were evaluated with an Excel-based evaluation sheet and the new software.

Results: No significant differences were found between alternative confirmed and reference confirmed results or between candidate and reference method results for all matrices regardless of PCR platform, evaluation software, pre-treatment, or enrichment time.

Significance: The tested workflows provide a reliable, cost and time efficient detection of the Top7 STEC. Furthermore, the combined use of the pre-treatment for free DNA-removal and an automated machine learning based evaluation software with these kits increases the confidence in the results by reducing presumptive positive and questionable PCR results.

P1-100 Validation Studies for the BAC*Gene* Kits, Including Pr*eraser* Free DNA Removal Treatment and Fastfinder Evaluation, As Alternative Methods

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Introduction: Bacterial food pathogens such as *Salmonella*, *Listeria* and Shiga toxin-producing *E. coli* are among the most relevant causes of foodborne outbreaks. Due to their importance for food safety, their reliable detection in food and food production is vital. However, DNA-based alternative detection methods like PCR are susceptible to producing questionable results caused by free DNA or misinterpretation due to PCR curve artefacts.

Purpose: Within the scope of the AOAC certifications the performance of the BAC*Gene* kits was compared to the respective ISO guidelines. The aim of the studies was to validate the use of a pre-treatment that removes free DNA, which could lead to false positive results. Additionally, a new trained machine learning-based evaluation software was introduced and results were analyzed with the previously certified BAC*Gene* Evaluation Sheet as well as the new FastFinder evaluation.

Methods: Thirty replicates were tested for three representative matrices in each of the studies. All samples were analyzed according to the respective protocols, both with and without pre-treatment, and culturally confirmed. The results were analyzed with both the Evaluation Sheet and the FastFinder software.

Results: The results obtained were comparable to the reference methods and that the free DNA removal pre-treatment did not lead to adverse effects on living cells and their detection. The evaluation software provided reliable and comparable results to the reference methods as well as the evaluation sheet.

Significance: The combined use of an evaluation software for improved curve recognition and free DNA removal pre-treatment leads to increased confidence in the results and less cultural confirmations due to presumptive positive or questionable PCR results.

P1-101 Combined Nonthermal Processing and Antimicrobial Packaging for Juice Pasteurization

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Introduction: Nonthermal processing has been extensively studied due to their minimal impact on food quality and nutritional values. Pulsed electric fields (PEF), pulsed UV light (PL), antimicrobial packaging (AP) have been used to treat liquid foods, but limited information is available for their combinations applied in juices.

Purpose: The objective of this study was to investigate the effectiveness of combined PEF, PL, and AP in microbial reduction and impact on quality and nutritional values of juices.

Methods: Bench scale PEF and PL processing systems were used. Apple or orange juices inoculated with *E. coli* K12 or natural microflora were treated by PEF and/or PL and stored in glass jars with antimicrobial caps containing 10 µL of carvacrol (AP). Microbial populations were determined after treatments or during storage at 10°C. Physical and chemical properties of juice samples were also determined. Treatment conditions: PL (5 to 60 s; 1.04 J/cm^2/s), PEF (19, 23, 30 kV/cm), PEF followed PL (PEF+PL), PL followed PEF (PL+PEF), PEF+PL+AP.

Results: Populations of *E. coli* in control, PL, PEF, PL+PEF, PEF+PL samples were 7.8, 7.0, 4.8, 3.9, 3.6 log/ml, respectively, after treatments. There were no significant ($P > 0.05$) differences between PL+PEF and PEF+PL; both treatments reduced *E. coli* to non-detectable level (> 5-log reduction) after 7 days. Both PEF+PL and PEF+PL+AP achieved 5.6-log reduction of Mold & Yeast, however, PEF+PL+AP had lower M & Y counts (2.9 log) than PEF+PL (3.9 log) after 7 days. There were no significant differences in pH, acidity, total soluble solid contents among all samples after treatments and during storage. Increased PL treatment times reduced color a*, b* values, total phenolics and carotenoid contents. Storage studies showed that both treated apple and orange juices had microbial and quality stabilities for 6 weeks.

Significance: This study provides valuable information to juice processors for consideration and design of nonthermal pasteurization of juice products.

P1-102 Evaluation of Improved Automated Rapid Microbiological Assay System

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Introduction: An improved instrument has been developed to automatically detect and record the presence of microorganisms in food and nutraceutical products. The new instrument combined the two legacy instruments, Soleris and Biolumix (referred as LI1 and LI2) in one with accompanying new software that monitors pH, carbon dioxide, or fluorescence indicators in real-time, within a novel broth/agar combination vial.

Purpose: To determine equivalency in performance of the improved instrument against legacy instruments a probability of detection (POD) analysis was conducted and assay detection times were compared between the instrument types.

Methods: The Direct *Pseudomonas* vials (PD-109, colorimetric test) and *E. coli* vials (S2-EC, fluorescence test) were inoculated with low-level inoculum (1 to 5 CFU/vial) to obtain fractional positives of *Pseudomonas aeruginosa* ATCC 9027 and *E. coli* CDC 984. Thirty-two replicates were tested per instrument (32-wells), with two instruments evaluated, totaling 192 samples for PD-109 and 128 samples for S2-EC. The number of detection times (DTs) signaling positive samples and non-detects (NDs) were automatically recorded and the difference in POD (dPOD) analysis was performed on the results.

Results: Average DT of 25.9 hours for PD-109 with improved instrument was faster compared to - legacy instruments that ranged from 27.7 to 28.5 hours, indicating equivalent or better performance. The dPOD between improved instrument and LI1 was -0.02 with a 95% confidence interval of (-0.07, 0.04), to LI2 was 0.00 with a 95% confidence interval of (-0.04, 0.04), indicating no significant difference in detection of *Pseudomonas*. DTs for S2-EC were less than 18 hours with both improved instrument and LI2. The dPOD was 0.00 with a 95% confidence interval of (-0.04, 0.04), showing comparable results for detection of *E. coli*.

Significance: The improved rapid microbiology assay results support a faster time-to-result, reliable instrument performance, easy to interpret accurate results.

P1-103 Performance Evaluation of Real-time PCR for *Salmonella* Detection in Nutraceutical and Dietary Supplements

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Introduction: Rapid methods such as PCR have been making in-roads into the routine nutraceutical and dietary supplements testing. Despite robustness, the widely diverse and ever-growing list of matrices present new challenges to such methods and thereby should be reviewed for compatibility with the adoption of new technology into the laboratory.

Purpose: A study was conducted to evaluate GENE-UP, a real-time PCR (SLM), for the detection of *Salmonella spp.* from four diverse categories [vitamin and components (16), dyes and flavorings (15), herbals (9), protein additives (4)] encompassing 44 matrices in total to determine the compatibility with GU

for the detection of *Salmonella*. The major matrices included were: multivitamin tablets, capsules and premix, CoQ-10, calmagD blend; colorants/flavorings—bilberry and red orange extract, blueberry, pomegranate, citrus flavors; herbal—turmeric extract, green tea, beet powder, chlorella; protein additives—rice protein, whole dried egg.

Methods: All matrices were tested at a test portion size of 10 g where per matrix ≥4 replicates were spiked with *S.* Typhimurium at 10-20 CFU/test portion. *N*= 1-2 replicates were tested as a negative control with similar test portion size, but were not spiked with *Salmonella*. All test samples (*N*=220) were enriched for 20- 22 h in BPW at 42°C. All the presumptive results were confirmed with culture methods i.e., FDA-BAM and/or bioMerieux.

Results: Among 220 tested samples, 100% of the spiked samples were reported as positive by SLM, and all the un-inoculated samples were reported negative. All culture methods were concordant with SLM data.

Significance: The challenge studies conducted here demonstrate the efficacy (fit-for-purpose) of SLM for *Salmonella* detection from nutraceutical and dietary supplements. To our knowledge, a large feasibility dataset like this has not been published and would greatly benefit the nutraceutical industry.

P1-104 Effect of DNA Extraction Method on qPCR Efficiency for Pathogens in Ground Beef

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❖ ***Developing Scientist Entrant***

Introduction: Validating food safety processes can be challenging for food manufacturers due to costs and time associated with traditional "plate and count" methods. Quantitative Polymerase Chain Reaction (qPCR) may increase speeds and reduce costs associated with quantifying pathogens in challenge studies, but requires optimization.

Purpose: The aim of this study was to assess four commercial DNA extraction kits for yield of high levels of inhibitor-free DNA targets from pathogens in ground beef.

Methods: Ground beef was inoculated at 7.71 ± 0.08 log CFU/g *Salmonella* Typhimurium or 8.46 ± 0.08 log CFU/g *Listeria monocytogenes*. The extracted DNA was initially quantified using Nanodrop and Qubit. Kits yielding detectable DNA levels were used in four trials assessing DNA yield, DNA purity, and average qPCR C_t value at 10^0 and 10^{-4} dilutions. The kit with the lowest average C_t value was tested for the presence of qPCR inhibitors using reaction efficiency and R^2 value. The ANOVA and Tukey pairwise test were used to compare C_t values ($P \leq 0.05$, C.I. = 95%).

Results: Three of the four kits yielded detectable levels of DNA. For *Listeria*, kits 2 and 3 resulted in the lowest C_t values at 10^0 (means = 17.7, 17.6); kit 2 had lowest C_t values at 10^{-4} dilutions (mean = 31.2). For *Salmonella,* kit 2 exhibited the lowest C_t values at both dilutions 10^0 and 10^{-4} (means = 17.5, 31.2). Over a five-point standard curve, kit 2 demonstrated an average reaction efficiency of 95.5% and R^2 of 0.998 for *Listeria* and an average reaction efficiency of 96.8% and R^2 of 0.998 for *Salmonella*. Nanodrop and Qubit values did not correlate with C_t values, likely due to beef DNA interference.

Significance: Kit 2 allowed for greatest extraction of pathogen DNA and will be used to optimize qPCR methodology. Further studies will compare "plate and count" methods to qPCR for quantifying pathogens in challenge studies.

P1-105 Evaluation of a Free DNA Removal Protocol with Real-time PCR Assay for the Detection of *Cronobacter* in Dairy Matrices

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Poster

Introduction: The higher sensitivity of real-time pathogen PCR has been widely advantageous, however it has also led to an issue of detecting residual pathogen DNA that seems to be inherent to the production facilities due to the sanitizers, or from the incoming raw material. Dairy production facilities are particularly affected by such trends due to the kill step involved during the production. Such detections divert attention from true problem of detecting the live pathogens.

Purpose: The objective of this study was to evaluate the compatibility of a free DNA removal protocol with a GENE-UP-based Cronobacter (CRO) assay for the detection of *Cronobacter* sp. from dairy powders.

Methods: A pilot set of 20 infant formula (IF) were incubated in BPW at 37°C for 18-24 h. Post-incubation, enrichments were combined with *Cronobacter* DNA ranging from 0-10^6 copies/mL. CRO data was collected before and after the free DNA removal protocol. A follow up survey was conducted with a combination of 52 dairy powders [cereals (6), milk powder (3), IF (43)] were spiked and tested (<5 CFU/test portion) with 11 stressed *Cronobacter* sp. (one strain per matrix). Fifty-eight samples were tested at 25 g size after enrichment in BPW/BPW+10 mg/L novobiocin (for probiotics) at 37°C for 18-24 h. Ninety-two samples were tested at 375 g size after enrichment in BPW+10 mg/L novobiocin at 37°C for 20-28 h. All results were confirmed by culture methods.

Results: Pilot samples treated with DNA removal protocol were negative for DNA spike level of 10^6 copies/mL. For survey, fractional results were obtained. For 25-g samples, 42 were positive and 15 were negative; while for 375-g samples: 64 were positives and 27 were negatives. One sample at each size was confirmed positive on CRO only due to the detection of residual DNA.

Significance: These data indicate the compatibility of a free DNA removal protocol with dairy powders for *Cronobacter* spp. detection using CRO.

P1-106 Development and Validation of a qPCR-based Colony Identification Assay for *Yersinia enterocolitica*

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Introduction: The sole method for isolating *Yersinia enterocolitica* from foods (MFLP-48) published in the Health Canada Compendium of Analytical Methods is culture-based with a lengthy analysis time due, in part, to the number of biochemical tests prescribed to differentiate the organism from the significant background microbiota recovered from food samples.

Purpose: Develop a molecular-based tool to assist in the identification of *Yersinia enterocolitica* isolates across a number of selective/differential agars, and shorten the method turnaround time.

Methods: A novel multiplex qPCR assay was developed with a species marker (*outL*), virulence marker (*ail*), and internal amplification control (IAC). The optimized assay was validated against MFLP-48 for four different agars: Cefsulodin Irgasan Novobiocin agar, MacConkey agar, Xylose Lysine Deoxycholate agar, and CHROMagar™ *Y. enterocolitica* for inclusivity (*n* = 110), and exclusivity (*n* = 56). A transfer study, consisting of a subset of isolates (*n* = 18 inclusivity, *n* = 12 exclusivity) was also conducted.

Results: The validation panel produced a sensitivity of 98% and specificity of 100% for *Yersinia enterocolitica* species detection across all four agars. The transfer study produced 100% agreement with the data generated in the validation study.

Significance: The assay developed can replace up to 18 biochemical confirmation tests prescribed in MFLP-48, significantly reducing turnaround time for a positive result. The savings in time, labor, and cost offered by the novel qPCR method could make surveillance applications more practical.

P1-107 Development of an Ultrafiltration Method for Virus Concentration in Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Human norovirus and hepatitis A virus (HAV) are frequently associated with foodborne viral outbreaks of minimally processed food. Fresh produce, particularly berries and lettuce are highly incriminated. Since viral culture methods are not adapted to food matrices, a concentration step is necessary to detect virus contamination.

Purpose: The objective of this study is to investigate the potential of an ultrafiltration (UF) method for virus concentration and to compare the method developed with the reference method ISO.

Methods: Fresh and frozen produce samples (25 g) were spiked with HAV (10^4 and 10^5 genome copies) in triplicate. Viruses were eluted and concentrated with a commercial ultrafiltration system. Hollow fiber polysulfone tips with ultrafiltration (80–100 kDa cutoff) and 0.05 µm pore size were used with fresh and frozen produces, respectively. Viral RNA was extracted using the Boom method and detected with RT-qPCR. UF concentration step was then compared to the ISO reference method.

Results: Our results show that different elution times (5, 10, 15 or 20 minutes) do not impact significantly ($P < 0.05$, ANOVA) the HAV viral recovery rate (RR) on fresh strawberries, optimal viral RR being 30 ± 13% at 5 min when spiked with 10^4 genome copies. The viral RR on fresh strawberries spiked with 10^5 genome copies was 37 ± 11% using UF compared to 20 ± 6% for the ISO method. UF also showed great viral RR on fresh raspberries (21 ± 9%) and lettuce (65 ± 17%) when spiked with 10^4 genome copies. Lastly, viral RR on frozen strawberries leads to lower RR. All results satisfied the extraction efficiency required by the ISO method targeting ≥ 1% RR.

Significance: Ultrafiltration is a rapid and simple viral concentration method that could be used to detect and prevent foodborne viral outbreaks.

P1-108 Plant Taxon Screening for the Detection of Soya, Maize, and Rapeseed in Food and Feed Samples in Parallel to GMO Screening

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Introduction: Screening for transgenic regulatory elements in food and feed samples is the standard when testing for the presence or absence of genetically modified (GM) plants. Knowing which plant taxon is present drastically speeds up the subsequent identification/quantification of the specific GMO event in the sample. Moreover, it removes any risk of confusion associated with botanical impurities

Purpose: To develop multiplex real-time PCR plant taxon screening and identification assay, that covers the most common GMO-containing plants (soya, maize and rapeseed), to reduce time, effort and cost of GMO analysis. To design an automated solution at a low cost to replace the time-consuming column-based approach

Methods: As an automation solution, the **food**proof® Magnetic Preparation Kit III was used to extract DNA. The **food**proof® Plant Taxon Screening LyoKit, able to detect soya, maize and rapeseed in one single PCR reaction, was tested for inclusivity, exclusivity and limit of detection. Additionally, new multiplex GMO Soya and Maize identification assays complement the GMO screening and identification workflow. The assays comply with ISO 21570 and JRC methods. An internal amplification control is included

Results: Specificity was verified against different modified and non-modified plants. 27 different matrices were tested successfully, including vegetable burgers, flour products and cream cheese. The LOD for the assays is 0.2 target copies/µl. Even in competing matrices, the target could be detected, e.g. 0.1 % soya flour in 99.9 % corn flour

Significance: The flexible GMO LyoKit screening and identification assays offer an easy and cost-effective approach for analyzing genetically modified foods

P1-109 Detection of Listeria spp. from Environmental Surfaces Using CANARY® Technology

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Introduction: Detection of *Listeria* species and *L. monocytogenes* in food manufacturing and controlled environmental agriculture environments is necessary for reducing the risk of food. In addition, it is crucial to provide testing results quickly to facilitate the timely implementation of corrective actions.

Purpose: To evaluate the performance of a novel biosensor technology method to detect *Listeria* species in swab samples taken from stainless steel, plastic, and rubber surfaces.

Methods: A panel of 64 target and 34 non-target microorganisms representing the genus of *Listeria* and other commonly occurring microorganisms found in food manufacturing environments were tested during inclusivity/exclusivity studies. For environmental surfaces, 30 test portions (1 x 1 inch surface areas) were prepared: 20 at a level expected to produce fractional recovery (~1 – 100 CFU/surface depending upon the matrix), 5 at a high inoculation level to produce all positives (~2 – 150 CFU/surface), and 5 uninoculated controls. After swabbing, samples were incubated for 24 hours and tested. Unpaired samples were taken and tested with the U.S. FDA *Bacteriological Analytical Manual* method for comparison. Data were analyzed using a probability of detection model.

Results: The tested method provided 100% inclusivity for the 64 target strains and 100% exclusivity for the non-target strains. There were no significant differences in performance between the test method and FDA-BAM methods for any of the three surfaces tested, as determined by probability of detection analysis.

Significance: These testing results demonstrate the ability of the CANARY® technology to detect *Listeria* species, including *L. monocytogenes*, and is effective for detection of *Listeria* spp. from a variety of environmental surfaces. This technology will provide food manufacturers and growers with an accurate and reliable way to monitor for possible contamination.

P1-110 Assessment of *Salmonella* Precis Method According to the ISO 16140-Part 2 (2016) Standard

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Introduction: Traditional methods for *Salmonella* spp. detection are long established and typically require 3 to 5 days to generate the final result. *Salmonella* Precis method is based on a two-step workflow with an overnight enrichment in the selective One-Broth *Salmonella* followed by streaking onto the chromogenic Brilliance *Salmonella* agar. If characteristic colonies are observed, the end-users can run the Oxoid *Salmonella* Latex Test to quickly confirm the presence of *Salmonella* spp.

Purpose: The method was first validated in 2007. With the publication of the ISO 16140-part 2 in 2016, the available data set has been completed in 2019 by Microsept laboratory to fulfill the current technical rules.

Methods: A total of 266 new samples were tested to complete the sensitivity part in order to get a total of 663 samples distributed in 5 food categories, as well as feeding stuffs and environmental samples. A new matrix/strain pair, a deli-salad inoculated with *S.* Infantis, was added to Relative Limits of Detection (RLOD) set.

Results: All the data from the method comparison study and inter-laboratory study were assessed according to the Acceptability Limits (AL) defined in the ISO 16140-2 (2016). The determined values are all below the AL, confirming that the alternative method produces comparable results to the reference method.

Significance: The data and calculated values fit both with the ISO 16140-2 (2016) requirements. This indicates that the *Salmonella* Precis is a suitable alternative to the ISO 6579 (2017) standard method. With a simple workflow, the *Salmonella* Precis significantly reduces the handling-time and time-to-result. Final results are available within less than 48 hours.

P1-111 Broad Detection of Norovirus GII Using Recombinase Polymerase Amplification and Applications Using Intercalating Dyes

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❖ ***Developing Scientist Entrant***

Introduction: Human norovirus is the leading cause of foodborne illness globally. Detection and quantification of norovirus involves the use of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR); however, it can have limited efficacy in portable and rapid settings. Recombinase polymerase amplification (RPA) is an emerging alternative, which has been reported to outperform PCR in sensitivity, specificity, and simplicity. Previous work has reported an RPA system capable of rapid detection of GII.4 norovirus, but is not able to be used with other GII noroviruses, the genogroup responsible for the majority of cases.

Purpose: The purpose of this study was to develop broadly reactive GII norovirus RPA primers, while also examining the feasibility of intercalating dyes for novel real-time quantification.

Methods: Primers for RPA were designed following TwistDx Inc. guidelines. A total of 41 genotypes sequences from norovirus genogroup GII were downloaded from the NIH GenBank database and aligned in MEGA. Nine primers pairs were screened in gel electrophoresis. After, a SYBR green assay was developed for use in RPA with both DNA/RNA from norovirus GII.4 and MS2. The assay was set at a constant 40℃ in a fluorescence instrument with fluorescence signaling detected every 30 seconds for 40 cycles.

Results: Primers were successful in amplifying norovirus strain using RPA. A winning pair was picked based on band. The SYBR green was successful in detecting the RPA amplicon for a signal in a fluorescence instrument for all targets tested, with detection ranging from 14.61 to 7.30 minutes. Time to signal for norovirus cDNA at a -1 dilution was 7.30 ± 0.75 minutes.

Significance: In sum, this work reports more broadly reactive primers for norovirus detection, as well as successful utilization of intercalating dyes for real-time detection of GII noroviruses in a portable, rapid manner.

P1-112 Detection of Virulence and ESBL Genes in *Salmonella* by Multiplex High Resolution Melt-curve Real-time PCR Assay

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illness caused by consumption of food contaminated with virulent and β-lactam resistant *Salmonella* is one of the major public health problems in the United States. Multiplex real-time PCR assays based on high resolution melt (HRM) curves can be used as an effective technique for rapid and simultaneous detection of multiple drug resistant foodborne pathogens.

Purpose: This study aimed to develop a multiplex HRM real-time PCR for the simultaneous detection of virulence and extended spectrum β-lactamase (ESBL) genes in *Salmonella* in food samples.

Methods: Two sets of multiplex real-time PCR assays were developed for the detection of six virulence and three ESBL genes. The first assay targeted five genes, *hil*A, *fim*H, *sip*A, bla_{TEM} and bla_{SHV}, and the second set targeted *inv*A, *fim*A, *stn* and bla_{CMY}. The assays were validated using genomic DNA from *Salmonella* and non-*Salmonella* strains. A cocktail consisting of *S*. Typhimurium, *S*. Newport and *S*. Enteritidis was prepared in the ratio of 1:1:1 and spiked in food samples at different concentrations. The detection limit and time of the assays were determined by testing the enriched spiked food samples at different time intervals.

Results: All 47 non-*Salmonella* samples processed were found to be negative for the virulence genes but one or more ESBL genes were detected in 11 of the strains. Seventy-one of 77 *Salmonella* serotypes were found to be positive for all six virulence genes. Further, 24 *Salmonella* serotypes had one or more ESBL genes. All target genes, with the exception of *stn*, were detected within 10 h of enrichment at a level of 10 CFU/g *Salmonella* cocktail for all food samples tested.

Significance: The multiplex HRM real-time PCR assays can be used as rapid, specific and cost-effective ways for simultaneous detection of virulence and ESBL genes in *Salmonella* that are present in low concentrations in food samples.

P1-113 Detection of Norovirus Capsid Protein Using an Outer Membrane Protein G

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❖ ***Developing Scientist Entrant***

Introduction: Noroviruses are the leading cause of foodborne illness globally and impose a public health and economic burden. They have a low infectious dose and are highly transmissible, making rapid detection and subtyping crucial for outbreak control. Outer membrane protein G (OmpG) based sensing has shown promise for this purpose for clinical biomarkers in complex matrices, but has not been utilized for foodborne pathogens.

Purpose: The purpose of the study was to develop a rapid, in-field OmpG detection and subtyping method for noroviruses.

Methods: Loop 6 of OmpG was engineered to contain 12 amino acid peptide sequence that binds norovirus. The ability of this OmpG construct to detect GII.4 norovirus was evaluated using single channel recording electro-physiological study. To observe the pore gating behavior 50mV was applied.

Results: The peptide-labeled OmpG exhibited a new, partially open state, with a 5pA decreased signal at 300 mM KCl, 50 mM Tris-HCl (pH 7.5) and a 10pA decreased signal at 1 M KCl, 50 mM Tris-HCl (pH 7.5). Once the target is added, the frequency of the partially opened state increased. The lowest concentration of norovirus capsid protein detected with the generated OmpG construct was 100 nM. Dose-dependent signal is observed in the range of 0 nM to 200 nM of target protein, suggesting the sensor is quantitative. It has a capability of binding and detecting norovirus capsid protein within 10 min.

Significance: This study is the first to apply a promising clinical detection and subtyping technique to norovirus, a foodborne pathogen. This work also provides the foundation for further study on the degree of subtyping resolution for norovirus for which the sensor is capable and the ability to detect virus in complex matrices.

P1-114 Recovery of Human Norovirus Surrogate from Aqueous Solution Using Magnetic Ionic Liquids

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Introduction: Isolation of pathogens from foods is a critical part of controlling foodborne illness outbreaks. However, foodborne viruses are often difficult to isolate due to low concentration and lack of culture methods. Magnetic ionic liquids (MILs) are a diverse class of solvents that can be easily isolated from complex solutions and have proven effective for extraction of bacterial pathogens.

Purpose: In this study, we tested the efficiency of three magnetic ionic liquids for capture and purification of a human norovirus surrogate, bacteriophage MS2.

Methods: MS2 was diluted into sterile 0.1% peptone water to 10^5 PFU/mL and extracted using a cobalt-, manganese-, or nickel-based MIL. The MIL was added to the MS2 suspension, gently mixed for 30 seconds to capture the bacteriophage, and collected using a magnet. The supernatant was then removed and MS2 was eluted into modified Luria broth by gentle mixing for 120 seconds and collected. MS2 RNA was then purified from each fraction using phenol-chloroform extraction and quantified via RT-qPCR.

Results: Average capture efficiencies calculated by loss to supernatant as high as 22% were observed, but were inconsistent—likely due to variation in supernatant collection between peptone and liquid MILs. However, the MILs recovered 3.14 ± 0.39%, 0.89 ± 0.26%, and 1.21 ± 0.80% of the added MS2 for Co, Mn, and Ni, respectively, which is comparable to similar magnetic bead-based methods. Cobalt-based MILs appeared to significantly ($P < 0.05$) outperform the others in terms of recovery efficiency.

Significance: Our results indicate that MILs have potential as a promising capture reagent for concentration and detection of noroviruses, which informs future work on pre-analytical sample processing, one of the major remaining hurdles to efficient detection of viruses in foods.

P1-115 Application of Polydopamine Molecular Imprinted Polymer on a Localized Surface Plasmon Resonance Sensor for Detection of Multi-antibiotics in Chicken Meat

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❖ ***Developing Scientist Entrant***

Introduction: Due to concerns of antibiotic-resistant bacterial strains from animal-derived food products, a significant portion of U.S. poultry industry has voluntarily removed antibiotics from feed and therapeutics. Different countries or organizations have established maximum residue limits (MRLs) as acceptable levels, and therefore, sensitive screening methods for identification and quantification of antibiotic residuals are critical for the stewardship of antibiotics.

Purpose: The objective of this project is to develop a localized surface plasmon resonance (LSPR) biosensing system for rapid, sensitive and selective detection of multi-antibiotics in chicken meat, using polydopamine molecular imprinted polymer (PDA-MIP) as the recognition element.

Methods: Enrofloxacin (ENRO), tetracycline (TETR) and phthalic acid (PHTH) were used as templates, and the PDA-MIP film was fabricated by self-polymerization of dopamine (DA) and purified powder of target antibiotics in Tris buffered saline (pH 8.0) on a LSPR sensor chip. After the removal of templates with sodium dodecyl sulphate (SDS), the developed LSPR/PDA-MIP sensor was used for selective detection of prepared samples. To amplify the detection signals of small antibiotic molecules, competitors conjugated with bovine serum albumin (BSA) were synthesized and reacted with the residual binding sites on the PDA-MIP film. The corresponding sensor coated with non-imprinted polymer (NIP) was modified similarly but without the addition of the antibiotic templates.

Results: The proposed method allowed a detection range of ENRO, TETR and PHTH from 10 to 500 ng/mL, with a detection time of 20 min. The PDA-MIP film demonstrated a greater binding capacity to target antibiotics than the PDA-NIP film. Target antibiotics were discriminated from each other with the use of specific BSA-conjugate competitors.

Significance: The developed LSPR/PDA-MIP biosensing method not only extended the lifetime of modified recognition element, but also reduced the detection time compared with most reported biosensing methods and showed high potential for in-field rapid detection of multi-antibiotic residues.

P1-116 Rapid Detection of Mold Contaminated Beverages Using the Bioluminescent Innovate System

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Introduction: The study demonstrates the use of a bioluminescent rapid microbial method along with traditional sample preparation to detect spoilage molds in contaminated packaging to achieve lean quality for beverage manufacturing efficiency.

Purpose: To demonstrate the performance of the Innovate system in detecting mold contaminated beverages through ATP bioluminescence.

Methods: *Aspergillus brasiliensis* was cultured to produce a <1,000 CFU/mL suspension. Half-liter sport drink bottles were artificially contaminated to create <1 CFU/mL contamination levels to be analyzed after 48, 72, and 96 hours of enrichment. Non-inoculated bottles were also analyzed to determine threshold RLU values. Ten milliliters of product were aliquoted and filtered through a 0.45 µm membrane filter. The membrane filter was transferred to a petri dish, five milliliters of Potato Dextrose Broth was added to the membrane, and the petri dish incubated at 30 ± 2°C for up to 96 h. After incubation two grams of 0.5 mm glass beads were added to the petri dish and placed on an orbital shaker at 200 rpm for five minutes. Fifty microliter samples were analyzed on the Innovate System for mold detection in triplicate.

Results: Detection of the target mold was determined by an RLU value greater than the threshold value. Thresholds were set based on the signal produced from non-inoculated bottles and multiplied by three. The threshold was >3,297 RLUs based on non-contaminated samples. Samples contaminated with mold produced RLUs of 779, 14,513, and 328,461 on each of the respective days. Low inoculums at <1 CFU/mL, 37 CFU/500 mL, were detected after 96 hours and as early as 72 hours of enrichment.

Significance: The combination of filtration sample preparation and bioluminescence allows for rapid detection of molds in a variety of different beverages and package sizes that could be difficult to analyze from a volume perspective.

P1-117 Colorimetric Detection of *Listeria monocytogenes* on Food Contact and Non-food Contact Surfaces Using Paper-based Microfluidics

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Introduction: *Listeria monocytogenes* surface contamination of processing environments is of concern to the food safety industry, as this pathogen may persist for extended time periods, often surviving routine cleaning and sanitation. Consequently, post-processing contamination of foods with *L. monocytogenes* may occur and lead to costly recalls or foodborne illness outbreaks. Environmental testing of food contact or non-food contact surfaces using a multitude of sampling devices and endpoint detection technologies offers a wide range of sensitivity, specificity, time-to-detection, and cost. µPADs (paper-based microfluidics) are suitable candidates for rapid detection platforms, comprising benefits such as low-cost, ease-of-use, and field amenability.

Purpose: Our objective was to develop and optimize a colorimetric enzymatic assay for an µPAD-based detection of *L. monocytogenes* on food contact and non-food contact surfaces.

Methods: µPADs were impregnated with an optimized concentration of 5-bromo-4-chloro-3-indolyl-beta-D-glucoside, indicative of beta-glucosidase activity. Stainless steel, HDPE, and rubber coupons (10x10 cm) were sterilized, then inoculated with 100 µL of 10^1, 10^2, and 10^3 CFU/mL of *L. monocytogenes* or 10^3 CFU/mL *Bacillus pumilus* negative control. Inoculated coupons were dried for 1 hour in a biosafety cabinet at room temperature. Coupons were swabbed with rayon-tipped swabs, calcium alginate swabs, and sponge-sticks, then aerobically enriched in Listeria Enrichment Broth at 37°C, with aliquots removed at 0, 8, and 18 hours. Visual confirmation and ImageJ analysis were performed.

Results: ImageJ analysis and visual assertion revealed beta-glucosidase activity of *L. monocytogenes*. After 18 hours selective enrichment, *L. monocytogenes* spiked at 10^2 CFU/mL was effectively detected on HDPE with sponge-sticks, rubber with rayon swabs, and stainless steel with calcium alginate swabs and sponge-sticks, allowing unambiguous discernment ($n = 54$, $P < 0.05$).

Significance: We developed and optimized a rapid integrated sampling and detection method for *L. monocytogenes* on food contact surfaces. The method is sensitive, specific, inexpensive, and user-friendly, providing a useful alternative to current Listeria environmental testing.

P1-118 Development of Detection Method with Monoclonal Antibody and Nitrogen-doped Carbon Nanodots for *Camylobacter jejuni*

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Introduction: Although the foodborne diseases by *Campylobacter jejuni* infections have been increased, the presence of the pathogen in food is underestimated because of insensitive detection methods. *C. jejuni* is difficult to detect because it exists in viable but non-culturable state. Thus, it is very important to develop a method to detect low levels of this pathogen in food, because the infectious dose of *C. jejuni* can be as low as 100 to 500 cells.

Purpose: The objective of this study was to develop a detection method for *C. jejuni* in food, which has improved sensitivity and shortened detection time.

Methods: Nitrogen-doped carbon nanodots (N-CNDs) were synthesized with citric acid, oleyamine, octadecene, nitric acid, toluene, and ethanolamine. A monoclonal antibody specific to *C. jejuni* was developed through hybridoma cells. Duck samples were placed in filter bags, containing buffered peptone water (BPW) and shaken 20 times, followed by enrinchment with Bolton broth at 42°C for 48 h. The enrichment was streaked modified charcoal-cefoperazone-deoxycholate agar, and identified by colony PCR. In addition, the samples were shaken in BPW and rinsates were transferred to Bolton broth with 10 mg/mL N-CNDs. They were then irradiated to blue light emitting diode at 425 nm for 1 h, followed by detecting *C. jejuni* with the developed antibody.

Results: As a result, the detection efficiency of *C. jejuni* in duck samples was 71.4% in using only Bolton broth and PCR identification for 4 days. However, the use of the Bolton broth containing N-CNDs and 425-nm irradiation for 1 h, and the developed monoclonal antibody obviously improved the detection efficiency of *C. jejuni* up to 85.7%.

Significance: This result indicates that the detection method using the monoclonal antibody and N-CNDs developed in this study can save time and improve the detection efficiency, compared to the conventional culture method.

P1-119 Enhancing Immobilization of Phage on Magnetoelastic Sensor via Chemisorption for Improving Detection of *Bacillus cereus*

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❖ ***Developing Scientist Entrant***

Introduction: Phages are gaining attentions as interesting alternatives to antibodies for capturing the target bacteria specifically on the surface of sensor. Many studies have relied on physisorption of the phages on sensors. However, physisorption results in poor surface coverage of phage, subsequent bacterial capture, and weak interaction between phage and sensor.

Purpose: Therefore, the purpose of this study was to improve the coverage of *B. cereus*-specific phage (KFS-BC1) on magnetoelastic (ME) sensor by using chemisorption methods for capturing *B. cereus*.

Methods: KFS-BC1 was immobilized at various concentrations through physisorption method and chemisorption methods (11-MUA, DTSP, sucrose, L-cysteine, and concanavalin A). The resonant frequencies of the sensors were measured using surface-scanning system before and after immobilization of KFS-BC1. In addition, the coverage of KFS-BC1 on the sensor was calculated after observing the sensor surface using SEM. The phage-immobilized sensors were exposed to various concentrations (2, 4, 6, 8 log PFU/mL) of *B. cereus* suspension at 22°C for 1 h for the comparison of bacterial detection capacity by measuring the resonant frequency shifts (RF) and the density of *B. cereus* on the ME sensors.

Results: RF shifts of phage-immobilized sensor via chemisorption with 11-MUA, DTSP, sucrose, L-cysteine and concanavalin A were 882 ± 97, 400 ± 65, 475 ± 149, 412 ± 56 and 430 ± 110 Hz, respectively, which was higher than KFS-BC1-immobilized sensor via physisorption (385 ± 90 Hz) ($P < 0.05$). When KFS-BC1 was immobilized on the sensor via 11-MUA method, it exhibited the greatest phage density of 194 phages/100 μm^2. In addition, the sensitivity, R^2 and detection limit of 11-MUA-treated sensor were 558.2 ± 37.1, 0.97 and 1.5 ± 0.3 log CFU/mL, respectively.

Significance: This study demonstrated that 11-MUA method enhanced the coverage of KFS-BC1 on the surface of sensor and the 11-MUA-treated sensor could successfully detect the *B. cereus*.

P1-120 Utilizing Next-generation Sequencing and Machine Learning for Semi-quantification of *Salmonella*

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Introduction: The United States Department of Agriculture (USDA) is moving toward a risk-based approach of managing *Salmonella* in the poultry industry, which in part prioritizes quantifying the amount of *Salmonella* either on birds coming in to slaughter or on raw finished product. For example, flocks that are known to be highly contaminated could be scheduled to be processed at the abattoir toward the end of a shift to prevent cross-contamination to flocks with lower levels of contamination. This would in turn positively affect public health as the probability of illness increases with exposure to greater numbers of *Salmonella*.

Purpose: The purpose of this study was to develop a method to semi-quantify, along predefined tiers of contamination, the amount of *Salmonella enterica* present on bootswabs prior to enrichment.

Methods: An overnight culture was quantified by plating serial dilutions and used to inoculate 456 *Salmonella*-negative bootswabs that were sourced from commercial poultry production. The boot swabs were enriched following Clear Labs' bootswab protocol and taken through the Clear Safety Platform™. Using the next-generation sequencing (NGS) data from the Clear Safety Platform, and the known starting colony-forming units (CFU), a machine learning (ML) model was trained to classify *Salmonella*-positive bootswabs as either low or high. The model was derived from the ensemble method, random forest and the data was split 80/20 for training and testing, respectively.

Results: The ML model was able to accurately distinguish all 212 highly contaminated bootswabs. The accuracy of *Salmonella* detection and serotyping of these 456 samples was 100%.

Significance: This research will assist the poultry industry in adopting the recommended strategies by the USDA. Additionally, it provides a novel example of how sequencing data can be leveraged to inform the choices that food producers make in a risk-based manner.

P1-121 Evaluation of the GENE-up® *Salmonella* 2 Real-time PCR Assay for the Detection of *Salmonella* Species in a Variety of Environmental Surfaces

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Introduction: The GENE-UP™ *Salmonella* 2 real-time PCR assay is a Fluorescence Resonance Energy Transfer (FRET)-based real-time PCR test used for rapid and specific detection of *Salmonella* species in foods and environmental surfaces

Purpose: The performance of the alternative method was compared to the Canadian culture-based reference method MFHPB-20 and evaluated according to Health Canada, Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods for testing environmental surfaces from food production environments.

Methods: Forty unpaired stainless steel, plastic, rubber, ceramic, and sealed concrete surface areas measuring 100 cm^2 were inoculated with *S.* Enteritidis, *S.* Heidelberg, *S.* Typhimurium, *S*. Cubana and *S*. Javiana, respectively, at a level (L_1) likely to yield fractional positive results (25% to 75%). Five unpaired surface areas (L_2) inoculated at 10-times L_1 and 5 un-inoculated sample areas were also tested. All surface samples were co-inoculated with *Citrobacter koseri* (interference organism) and *Enterococcus faecalis* (background organism). The inoculated surfaces were dried for 18 to 24 h at 22°C and 50% relative humidity. Acclimated surfaces were sampled with a variety of bacterial carriers and wetting agents and held for 24 h at 4°C followed by enrichment in Buffered Peptone Water (BPW) at 41.5 ± 0.5°C. For the reference method samples were enriched in BPW at 35°C. Samples were tested by the alternative method at 18 h of incubation. All analytical outcomes were biochemically confirmed by the reference method.

Results: Collectively, from the analysis of 500 unpaired samples, the alternative method achieved an overall relative sensitivity of 100%, relative specificity of 97.8%, a false positive rate of 2.2%, a false negative rate of 0%, and a test efficacy of 98.8%.

Significance: This Alternative assay is a suitable method for detecting *Salmonella* in a variety of environmental surfaces after only 18 hours of incubation, thereby significantly reducing presumptive reporting times over the reference method.

P1-122 Evaluation of the 3M™ Molecular Detection Assay 2 for the Detection of *Escherichia coli* O157 (including H7) in a Variety of Foods

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Introduction: The 3M™ Molecular Detection Assay 2 utilizes loop-mediated isothermal amplification combined with bioluminescence to detect *E.coli* O157 (including H7).

Purpose: The performance of the alternative method 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: Unpaired samples spiked with *E.coli* O157:H7 were analyzed by the alternative and reference method. Raw meats (including frozen, processed and unprocessed) and fruit- and vegetable-based products (including raw and raw processed fruit and vegetables, juices, and nut and nut meats) were each inoculated at three levels: 20 samples at a level (L_1) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2) at approximately 10 times L_1 and 5 un-inoculated samples. Samples were also co-inoculated with 10 times L_1 with generic *E.coli* as an interference organism. Alternative samples were enriched in BPW-ISO broth incubated at 41.5 ± 1°C, and tested at 10-18 h of incubation. Reference samples were enriched at 42°C in m-TSB supplemented with 20 µg/mL Novobiocin for 18-24 h. All analytical outcomes were culture confirmed by the reference method.

Results: Collectively from the analysis of 540 unpaired samples, a probability of detection (POD) statistical model determined the alternative method met the criteria outlined by the MMC obtaining a relative sensitivity of ≥98%, relative specificity of ≥ 90.4%, false positive rate of <9.6%, false negative rate of <2% and test efficacy of ≥ 94%.

Significance: This Alternative assay is a suitable method for detecting *E. coli* O157 (including H7) in raw meats and fruit and vegetable based products after 10-18 h hours of incubation, thereby significantly reducing presumptive reporting times over the reference method.

P1-123 Molecular Characterization of *Riemerella anatipestifer* Isolated from Outbreaks in Egypt

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Introduction: *Riemerella anatipestifer* is one of the most important threats to duck raising, particularly in developing countries, as many Egyptians depend on duck as a source of protein in their diets. *R. anatipestifer* infection causes high mortality rates may, as high as 100% and 50 to 80% in ducklings and ducks, respectively.

Purpose: The aim of this study was to isolate *R. anatipestifer* from various farms in Egypt using molecular characterization and genetic analysis of the isolates.

Methods: Traditional microbiological isolation of *R. anatipestifer* from 30 birds (ducks and duckling), molecular characterization of the isolates based on full length outer membrane protein (OmpA), sequencing and BLAST analysis, and phylogeny based on full length *ompA* gene were conducted, and the results were confirmed by 3D prediction of protein.

Results: Five *R. anatipestifer* were isolated from the examined birds. Results showed invasion of a number of different genotypes among the Egyptian duck flocks, indicating progressive circulation of *R. anatipestifer* among these flocks. Phylogeny classified the diversity of *R. anatipestifer* worldwide into two main lineages; each lineage diversified into three main clusters. Our study reports the first genotyping of *R. anatipestifer* based on an immunogenic protein (*ompA*) and confirms the infiltration of different *R. anatipestifer* clusters into the Egyptian duck flocks. Our isolates were found to belong to the two lineages.

Significance: These findings are starting points for advanced investigations of the genetic diversity of *R. anatipestifer* at national and regional levels to better understand their genetic relatedness. Although until now, at least 21 *R. anatipestifer* serotypes have been identified, the molecular techniques regarding its diagnosis, pathogenicity, and antigenicity are not well-attained. So, deeper studies should be done to stop this catastrophic loss of such a valuable protein source.

P1-124 Neutralization of Inhibitory Substances in Oregano, Cinnamon, and Cloves for the Recovery of *Salmonella* Typhimurium and *Salmonella* Abony

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Introduction: Although spices can be a common source of *Salmonella*, isolation of this pathogen can be challenging. Essential oils in spices can inhibit the growth of *Salmonella* with a conventional enrichment dilution of 1:10 in non-selective media. Per the FDA BAM, Chapter 5, Section C.10.c, there are no known methods for neutralizing the toxicity of oregano, cinnamon, and cloves. They must be diluted beyond their toxic levels to recover *Salmonella* (1:100 sample/broth ratio for oregano and cinnamon; 1:1000 for cloves).

Purpose: The purpose of this study was to evaluate several neutralizers for oregano, cinnamon, and cloves with the intent of lowering the sample/broth ratio needed to isolate *Salmonella* Typhimurium and *Salmonella* Abony from these spices.

Methods: Two varieties each of oregano and cinnamon, as well as one variety of cloves were the matrices studied. The matrices were wet-spiked with 3-5 CFU of *Salmonella* Typhimurium and *Salmonella* Abony (Biomerieux Bioball® 30 CFU). Neutralizers evaluated included activated charcoal, corn oil, Dey-Engley Broth, a custom broth containing Tween 80 and sodium bisulfite, and Hi-Media BPW (M14941). Enrichment and isolation were otherwise as described in ISO 6579-1.

Results: For the spices in this study, with no neutralizers, one oregano required a 1:100 dilution, one cinnamon a 1:200 dilution, and cloves 1:500. Activated charcoal successfully lowered each – oregano down to 1:10, cinnamon to 1:50, and cloves to 1:100. Sulfite may have acted as an inhibitor more than a neutralizer.

Significance: Lowering enrichment dilutions can result in significant financial savings in *Salmonella* screening protocols.

P1-125 Prevalence of *Salmonella* and Indicators in Australian Manufacturing Beef Trim

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Introduction: The Food Safety and Inspection Service (FSIS) has the intention to apply a performance standard for *Salmonella* in beef trimmings so a survey of the prevalence of *Salmonella* in Australian manufacturing beef was conducted to assess likely compliance to the standard.

Purpose: To estimate the prevalence of *Salmonella* and counts of indicator microorganisms in Australian manufacturing beef using FSIS methods.

Methods: Samples were submitted from volunteer export meat processors around Australia, proportional to estimated carcass throughput. The N-60 sampling procedure was applied to collect chilled surface slices to achieve approximately 500 g of surface slices in a single bag, with 375 g used for the *Salmonella* test (MLG 4C.02 for screening, AS 5013.10 for confirmation) and the remaining sample (~125 g) used for indicator tests (APC (AOAC 990.12), Coliform/*E. coli* (AOAC 991.14)) and for quantifying *Salmonella* (MLG Appendix 2.05) if detected.

Results: One thousand two hundred and fifty-five samples from 31 meat processors were analyzed. The prevalence of *Salmonella* in Australian manufacturing beef was 0.7%. The *Salmonella* concentration was low (< 0.3 MPN/g, LOD) in all 9 positive samples. Serotypes were *Salmonella* Zanzibar, *Salmonella* Reading, *Salmonella* Chailey, *Salmonella* Agona, *Salmonella* Orion, *Salmonella* Anatum (2 isolates), *Salmonella* Jangwani, *and Salmonella* Anatum var 15+. For indicators, the prevalence and mean concentration for samples above the LOD were 94.9%, 2.38 log, 14.4%, 1.34 log and 4.2%, 1.4 log per gram for APC, Coliforms and *E. coli,* respectively. No correlation was found between the concentration of indicator organisms and the presence of *Salmonella*.

Significance: The survey suggests that Australian exporters will likely meet the FSIS proposed *Salmonella* performance standard of 2 detection from 48 samples in a 52 week period. In addition, none of the detected serotypes figure prominently in US foodborne disease surveillance.

P1-126 Microbial Risk Assessment of *Salmonella* by Duck Consumption in Korea

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illness caused by *Salmonella* is continuously reported in Korea. There is high probability of *Salmonella* contamination in the production process of poultry. In recent, of the poultry products, duck consumption gradually increases in Korea.

Purpose: The objective of this study was to estimate the risk of *Salmonella* foodborne illness by duck consumption in Korea.

Methods: Two hundred five raw duck carcasses were collected from conventional and traditional markets to detect *Salmonella*. The prevalence data were then fitted to the probabilistic distribution to estimate an initial concentration. To describe the fates of *Salmonella* in duck carcass, predictive models were developed with the cell counts collected at 4 to 25°C. In addition, the cell counts reduction on the carcasses by cooking (moist-heat and dry-heat) were examined. Distribution temperature, time, and consumption patterns were surveyed. A dose-response model was searched. With these data, a probability of *Salmonella* foodborne illness/person/day was estimated through a simulation in @RISK.

Results: Of 205 samples, *Salmonella* were detected in 16 (7.8%) raw duck carcasses, and the initial contamination level of *Salmonella* was estimated to be -2.5 log CFU/g, using RiskBeta (17, 190). The predictive models showed that the cell counts increased through distribution conditions. The exponential distribution [RiskExpon 71.185, RiskShift (-0.38407))] showed that average consumption amounts were 57.9 g for moist-heat cooking at 49% of frequency, and 81.3 g for dry-heat cooking at 51% of frequency. Beta Poisson model (α=0.89, β= 4.4×10^{5}) was determined as an appropriate dose response model. With these results, the probability of *Salmonella* foodborne illness by duck consumption was 8.8×10^{-11}/person/day in Korea.

Significance: This result indicates that the risk of *Salmonella* foodborne illness by duck consumption can be considered low in Korea.

P1-128 Frequency of *Salmonella* and *Escherichia coli* O157 in Ground Beef from Butcher Shops in Jalisco State, Mexico

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Introduction: Ground beef is a common vehicle of foodborne pathogens including *Salmonella* and *Escherichia coli* O157:H7. In Mexico, ground beef is regularly fabricated at butcher shops. In our earlier study, *Salmonella* was found in 56.7% of ground beef samples collected from 238 butcher shops in Jalisco State.

Purpose: To determine frequency of *Salmonella* and *E. coli* O157, and enumeration of generic *E. coli* in ground beef fabricated at butcher shops.

Methods: A total of 96 samples were collected from 96 local butcher shops located in 3 municipalities in Jalisco State. Each sample (325 g) was enriched in 975 mL of buffered peptone water (BPW)-ISO at 35°C for 18-24 h. The samples were tested for *Salmonella* and *E. coli* O157 using LAMP-bioluminescent assays, and confirmed by culture, biochemical and serological tests. Additionally, 10 g samples were serially diluted in Butterfield's buffer and plated on 3M™ Petrifilm™ Rapid *E. coli*/Coliform Count Plates. *E. coli* colonies were enumerated after incubation at 35°C/18-24 h.

Results: *Salmonella* was confirmed in 66 (68.8%) samples, and 69 (71.9%) samples were presumptive positive for *E. coli* O157. *Salmonella* frequency was higher than the previous study (56.7%), probably due to sample size differences (325 g *versus* 25 g). Mean *E. coli* counts were 3.5 ± 0.77 log CFU/g, ranging from 1.7 to 6.8 log CFU/g; 39% of samples exceeded the established Mexican standard in for *E. coli* in ground beef at retail (3.7 log CFU/g).

Significance: Good manufacturing practices (GMP) in butcher shops is crucial during ground beef fabrication to reduce the consumers' exposure to foodborne pathogens. The results of this survey have been incorporated in food safety training programs for the inspectors of municipal offices that regulate butcher shops and will help to improve food safety of the ground beef from butcher shops.

P1-129 *Salmonella* Survivability in Rendered Fats Challenged with Different Levels of Moisture and Temperature

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❖ ***Developing Scientist Entrant***

Introduction: High moisture levels introduced to fats after rendering process can lead to *Salmonella* presence and growth. Limited research on strategies to eliminate pathogens in these environments are available. Rendered fat characteristics, such as water activity and fatty acids composition, may contribute to *Salmonella* survivability.

Purpose: The purpose of this research was to evaluate the effects of moisture levels, storage temperatures and fat characteristics on the growth and survival of *Salmonella* in beef tallow, white grease, and poultry fat samples.

Methods: The effects of moisture levels (0%, 0.5%, 1%, and 3%) and temperatures (48°C and 76°C) were evaluated in beef tallow, white grease, and poultry fats. Samples were inoculated with a high (~10^{8} CFU/mL) and a low (~10^{5} CFU/mL) *Salmonella* cocktail (*S.* Newport, *S.* Thompson and *S.* Infantis) with a liquid-to-fat ratio of 25 mL per 450 g. Samples were stored for up to 7 days at 48 and 76°C. Remaining population was evaluated daily with and without enrichment step. Each experiment was conducted in triplicate for each temperature and moisture level, and death rates calculated.

Results: A 4-log CFU/mL reduction was observed in beef tallow samples inoculated with *Salmonella* ($P < 0.05$) after 24 hours at 48°C, while a 2-log CFU/mL reduction was reported in white grease ($P < 0.05$) as compared to time 0. When all products were challenged at 76°C, counts were below detectable limits after 24 hours. Slow death rates were observed in chicken fat: 4.8, 17.3 and 18.9 d^{-1} were the values calculated at 0.5%, 1% and 3% moisture level, respectively.

Significance: This research identified the significant effect of moisture and temperature in rendered fat samples contaminated with *Salmonella* and underlined the need to use time-moisture-temperature data to minimize microbial growth during transportation and storage. Furthermore, the chemical composition of rendered fat seems to play a role in *Salmonella* survivability.

P1-130 Augmenting the Efficacy of Pressure-based Pasteurization of *Escherichia coli* O157:H7 Using Thymol and Mild Heat in Meat Homogenate

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❖ ***Developing Scientist Entrant***

Introduction: As a non-thermal procedure that currently is gaining accelerated industrial importance and momentum, pressure-based pasteurization of *Escherichia coli* O157:H7 could be augmented by utilization of mild heat and natural antimicrobials.

Purpose: The current study investigated inactivation of *Escherichia coli* O157:H7 using mild heat, hydrostatic pressure, and thymol in meat homogenate.

Methods: A six-strain mixture of *Escherichia coli* O157:H7 was exposed to 0 to 9 minutes of six treatments: i) hydrostatic pressure at 4°C; ii) hydrostatic pressure and thymol at 4°C; iii) thymol at 4°C; iv) heat at 40°C; v) hydrostatic pressure at 40°C; vi) hydrostatic pressure and thymol at 40°C. Pressure intensity level of 400 MPa (Hub880 Explorer, Pressure BioScience Inc.), and thymol concentration of 0.15% were used for the experiments of inoculated pathogen in sterilized 10% meat homogenate. Temperature was precisely monitored by stainless steel water jacket surrounding pressure chamber mechanically linked to a refrigerated circulating water bath. Analyses of variance were conducted followed by Tukey and Dunnett's-adjusted means separations.

Results: The six treatments after 9 minutes all resulted in reductions ($P < 0.05$) of the pathogen. Counts before treatment were 5.3 ± 0.4 log CFU/mL and were reduced ($P < 0.05$) to 3.2 ± 0.6 and 2.2 ± 0.3 log CFU/mL after three and six minutes of pressure treatments, respectively. Thymol and mild heat further enhanced the decontamination efficacy of the pressure treatments. At 40°C and in presence of thymol, the counts of 3-, 6-, and 9-minute treatments were all reduced ($P < 0.05$) to <1.0 log CFU/mL.

Significance: Results of the current study could be adapted by stakeholders of pressure-treated products for meeting the regulatory requirements such as the Hazard Analysis and Critical Control Point and Preventive Controls for Human Food rule of FSMA for reducing the public health burden of Shiga toxin-producing *Escherichia coli* O157:H7.

P1-131 Reduction of Shiga Toxin-Producing *Escherichia coli* (STEC) and *Salmonella* on Beef Tissues Subjected to Far-UV SterilrayTM Technology

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✦ ***Undergraduate Student Award Entrant***

Introduction: Efficacious beef carcass interventions are imperative for preventing *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) from entering the food supply. Numerous antimicrobial agents have been investigated; however, interest in alternative interventions, such as ultraviolet light, that do not require chemicals, water, or heat is increasing.

Purpose: This study investigates the efficacy of Far-UV Sterilray™ technology (UV; 220 nm) for reducing *Salmonella* and STEC populations on chilled adipose and lean beef tissues.

Methods: Lean and adipose beef tissues were cut into 50 cm^2 pieces, stored at 4°C, and inoculated with low (1.0 x 10^3 CFU/cm^2) or high (1.0 x 10^5 CFU/cm^2) concentrations of *Salmonella* or STEC. Inoculated samples were subjected to 3,000 mJ of UV or sprayed with ambient water, 200 or 400 ppm of peracetic acid, or 4.5% lactic acid. Treated samples were plated onto Xylose Lysine Desoxycholate and MacConkey (MAC) agars, with or without tryptic soy agar (TSA) overlays, to enumerate *Salmonella* and STEC, respectively. Color data were collected to quantify treatment effects on visual quality.

Results: Tissue and treatment x tissue interaction were not significant ($P > 0.05$) for low or high concentrations of *Salmonella* or STEC. When inoculated with high concentrations of STEC, and plated on MAC or MAC+TSA, treatment was significant ($P < 0.0001$) with UV producing the largest reduction compared to the inoculated, untreated control (1.0 log CFU/cm^2). UV did not alter beef color quality in comparison to the untreated control ($P > 0.05$).

Significance: Far-UV Sterilray™ technology significantly reduced high levels of STEC on inoculated chilled beef tissues, warranting further investigations to optimize and expand its applications in meat processing.

P1-132 *Salmonella* Concentrations, Prevalence, Serovars Distribution and Antimicrobial Resistance Associated with Informal Raw Poultry Processing in Accra, Ghana

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Introduction: Poultry is an important consumer exposure pathway for transmission of *Salmonella* but primary data on *Salmonella* in informal poultry processing needed to guide targeted interventions in Ghana is limited.

Purpose: We provide data on prevalence, distribution and antimicrobial resistance (AMR) of *Salmonella* serovars in informal poultry processing environments in Accra.

Methods: Samples (148) comprising 60 broiler carcasses from 12 live-bird markets (LBM), 33 benchtop swabs, 33 fecal and 22 rinse water samples were assessed for *Salmonella* concentration and prevalence, and confirmed with Microbact and PCR. Polyvalent-O antisera and Kauffman-White Scheme we used for serotyping. AMR of 55 isolates against 14 antibiotics was determined using disc diffusion assay and EUCAST breakpoints. Duncan's multiple range test at $P < 0.05$ was used to determine significant differences in mean concentrations, and prevalence and AMR presented as frequencies.

Results: *Salmonella* was present at 61% (1.3 ± 0.8 log CFU/g) on chicken carcasses, 77% (1.3 ± 0.9 log CFU/g) from rinse water, 81% (2.0 ± 0.8 log CFU/g) from fecal matter and 5% (0.2 ± 0.4 log CFU/g) from benchtops. Concentrations differed ($P = 0.000$) between samples. *S*. Typimurium, *S.* Infantis, *S*. Enteriditis and *S*. Newport were the most prevalent; *S*. Paratyphi B and *S*. Mississippi were associated with carcass only while *S*. Adelaide and *S*. Westhampton were associated with fecal matter and rinse water only, respectively. *S*. Agona, *S*. Senftenberg and the 4 most prevalent serotypes were associated with carcass,

rinse water and fecal matter. All the serovars exhibited multidrug-resistance to ≥6 antibiotics with the exception of *S*. Mississippi, which was resistant to 3 antibiotics.

Significance: The high prevalence of multidrug-resistant *Salmonella* in fecal matter, rinse water and chicken carcasses suggest cross-contamination onto skin of carcasses during processing, and raises concern for public health. Implementation of food safety systems alongside repeated training and consumer awareness is needed to reduce consumer exposure.

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P1-133 *Salmonella* and *Campylobacter* in Chicken Necks, Hearts, Gizzards, and Livers

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Introduction: In 2016, FSIS began an exploratory sampling project for chicken necks, hearts, gizzards, and livers, which are currently exempt from routine sampling and performance standard categorization.

Purpose: This exploratory sampling program assesses the *Salmonella* and *Campylobacter* percent positives in chicken necks, hearts, gizzards, and livers to evaluate if these products represent a public health risk.

Methods: During November 2016–April 2019, FSIS collected and analyzed 164 neck rinsate samples from 60 establishments, 60 heart rinsate samples from 30 establishments, 259 gizzard rinsate samples from 83 establishments, and 211 liver rinsate samples from 81 establishments. *Salmonella* and *Campylobacter* percent positives with a 95% confidence interval (CI) in these 4 products were compared between product types. *Salmonella* analysis was performed using the Microbiological Laboratory Guide (MLG) Chapter 4 methodology. *Campylobacter* analysis was performed using MLG Chapter 41, 30 mL protocol with direct plate methodology.

Results: The average percent positive for *Salmonella* in necks (*n* = 164), hearts (*n* = 60), gizzards (*n* = 259), and livers (*n* = 211) were 48% (CI = 38–59%), 52% (CI = 46–59%), 43% (CI = 38–48%), and 65% (CI = 59–70%), respectively. For those same samples, the average percent positive for *Campylobacter* in necks (*n* = 164), hearts (*n* = 60), gizzards (*n* = 259), and livers (*n* = 211) were 13% (CI = 6–20%), 10% (CI = 6–13%), 34% (CI = 29–39%), and 64% (CI = 58–69%), respectively. Statistical differences were noted in *Salmonella* and *Campylobacter* percent positives for livers from all other products ($P = 0.02$ to $P < 0.001$, depending on the product).

Significance: This exploratory sampling program indicates a high percent positive of *Salmonella* and *Campylobacter* in necks, hearts, gizzards, and especially livers and while necks, hearts and gizzards are typically fully cooked before being consumed, livers are not. These analyses demonstrate the importance of controlling pathogens in these poultry parts in order to prevent foodborne illness.

P1-134 Prevalence and Antimicrobial Resistance of *Salmonella* from Poultry Processing Operations

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Introduction: *Salmonella* is one of the most common causes of foodborne illness in the U.S., and poultry and poultry products play a significant role in transmission. Furthermore, the increase in antimicrobial resistance of *Salmonella* specifically from poultry is of concern to public health.

Purpose: The objective of this study was to determine the prevalence and antibiotic resistance of *Salmonella* spp. isolated from broilers and carcasses.

Methods: Broiler carcass rinses (*n* = 450) and ceca (*n* = 900) samples were collected from processing plants in the U.S. during Fall 2017 and Spring 2018. Samples were enriched overnight in BPW and prevalence of *Salmonella* was determined using the BAX® system. *Salmonella*-positive samples were plated onto BGS and XLT4 agar for further confirmation and then further analyzed for enumeration using the miniaturized MPN method. Antimicrobial resistance (AMR) of the *Salmonella* isolates was determined following National Antimicrobial Resistance Monitoring System (NARMS) protocol.

Results: A total of 450 carcass rinses and 900 ceca samples were analyzed. Of those, 284 samples were positive for both BAX® and selective plating (108 carcass rinses and 178 ceca samples). The mean *Salmonella* populations were 3.5 and 4.0 log MPN/mL for carcass rinses and ceca samples, respectively. Resistance to 14 antibiotics was determined for 243 isolates by the NARMS protocol, with 158 isolates (65%) demonstrating resistance to at least one antibiotic. Forty-one isolates (17%) demonstrated multidrug resistance by showing resistance to at least one antibiotic in three or more drug classes. A total of 105 isolates (43%) were resistant to streptomycin, 96 (40%) to sulfisoxazole, and 68 (28%) to tetracycline, while none of the isolates were resistant to ciprofloxacin.

Significance: This study highlights continued incidence of AMR of *Salmonella* in poultry, reiterating the need to develop and implement mitigation strategies to reduce the prevalence of AMR in foodborne pathogens on poultry farms.

P1-135 Comparison in the Recovery of *Campylobacter* from Poultry Establishments Using Direct Plating Versus Enrichment Methodologies: Amplified Population or Expanded Population?

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Introduction: In August 2018, FSIS implemented the enrichment method for all *Campylobacter* HACCP verification projects, discontinuing the direct plating approach.

Purpose: This study assesses whether the change in method was merely an improvement in sensitivity, resulting in the recovery of more of the same *Campylobacter* species and strains, or whether the enrichment method also resulted in the recovery of a greater diversity of *Campylobacter* strains from poultry samples.

Methods: Data from FSIS poultry samples in the 12 months before transition to enrichment and 12 months following the implementation include *n* = 3,849 *Campylobacter* isolates; a total of 384 isolates from FSIS poultry verification samples by direct plating, and a total of 3,465 isolated following enrichment. Analysis of speciation results and sequence types (using minimum spanning trees) measured the impact of the method change beyond sensitivity.

Results: The data reveal an increase in recovery of *Campylobacter coli* isolates from chicken products, an increase of *Campylobacter jejuni* from turkey products, and the recovery of *Campylobacter lari*, which the previous year of direct plate method did not recover. C. *lari* was recovered from 7.7% of the turkey carcasses samples and 2.6% of comminuted turkey samples analyzed with enrichment. Additional analysis of trends over time is needed to consider if these preliminary findings are consistent over a larger number of data points.

Significance: In a landscape of cultural methods that confirm just one isolate out of each sample, method bias is an unavoidable influence as to which strains are more likely to be recovered. Increased sensitivity from enrichment allows for increased confidence in assessing percent positive for regulated establishments and improved prevalence calculations. The recovery of additional strains may provide improved cluster identification and support for outbreak investigations, as well as a better understanding of *Campylobacter* populations present in poultry to potentially inform better intervention strategies.

P1-136 Improving Microbiological Quality and Safety of Chicken Breast Fillets from Salvage Line during Poultry Processing

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Introduction: Poultry processing operations incorporate numerous antimicrobial interventions to improve the microbiological quality and safety of poultry. While whole birds are subjected to numerous antimicrobial interventions, the chicken parts from the salvage line on the slaughter floor are not subjected to the same antimicrobial interventions.

Purpose: The objective of this study was to evaluate the general microbiological quality and *Salmonella* and *Campylobacter* prevalence on chicken breast fillets from the salvage line subjected to peroxy acetic acid (PAA) generated on-site (OxyFusion™).

Methods: Breast fillets (boneless, skinless; n = 53) from the salvage line on the slaughter floor were aseptically obtained prior-to and after immersion in PAA solution dip tank (7 s exposure) on four separate days of operation. The breast fillets were rinsed manually for 1 min in BPW (400 mL) and the rinsate was transferred to a sterile container and placed in a refrigerated cooler. Aerobic plate counts (APC) and Enterobacteriaceae counts (ENT) were enumerated using Petrifilm method. An aliquot of the rinsate was used for detection of *Salmonella* and *Campylobacter* following a USDA-FSIS method (enrichment) using BAX and additionally, by direct plating for *Campylobacter* on Campy-Cefex agar.

Results: The PAA concentration (mean ± SD) used was 818 ± 238 ppm. The APC and ENT populations were 3.35 and 1.86 log CFU/mL prior to PAA application and 0.76 and 0.18 log CFU/mL subsequent to antimicrobial application. A reduction in *Salmonella* and *Campylobacter* prevalence ($P \leq 0.05$) was observed on breast fillets prior-to (92.1 and 96.3%) and subsequent to (11.5 and 19.2%) PAA application, respectively. Lower prevalence ($P \leq 0.05$) of *Campylobacter* was observed using direct plating of rinse samples on Campy-Cefex agar compared to the enrichment method.

Significance: Use of an on-site generated PAA as an antimicrobial for poultry parts from the salvage line in poultry processing operations can improve the microbiological quality and safety of poultry parts.

P1-137 A Reduced Head-Space Enrichment for BAX® System Detection of *Campylobacter* from Poultry Parts Incubated Under Aerobic Conditions

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Introduction: *Campylobacter* are microaerophilic bacteria requiring strict environmental conditions to grow and survive, yet it is one of the leading causes of foodborne illness in the United States. Anaerobic jars and systems are frequently used to generate the specific microaerobic atmosphere (85% N_2, 10% CO_2 and 5% O_2) needed for the successful isolation of *Campylobacter*. However, an alternative enrichment protocol that utilizes a low headspace container and aerobic incubation can also be used to achieve a reduced oxygen environment.

Purpose: The purpose of this study was to evaluate a commercial real-time PCR assay for detecting *Campylobacter* from poultry parts using this alternative enrichment protocol compared to the USDA FSIS reference culture method.

Methods: Raw chicken drumsticks (325 g) were inoculated with *C. jejuni* to create 20 low-level and 5 high-level samples. Samples were then vacuumed sealed and stored at 4°C for 48-72 hours. Afterwards, samples were mixed with 1,625 mL of BPW. Then, 27.5 mL of the poultry homogenate and 27.5 mL of double-strength Bolton broth were combined in a 50 mL tube. Tubes were tightly capped and incubated aerobically at 42°C for 24-48 hours before analysis by real-time PCR and confirmed following the USDA MLG 41.04.

Results: *C. jejuni* was detected by real-time PCR in 11/20 low inoculated samples and 5/5 high inoculated samples at 48 hours. All results matched culture with 100% agreement. Statistical significance using the probability of detection (POD) determined there were no differences between the methods.

Significance: Overall, these results demonstrate the ability of the BAX® System to accurately and reliably detect *Campylobacter* from poultry parts using a more cost-effective enrichment protocol without using an anaerobic jar to create a low oxygen environment.

P1-138 A Simultaneous Enrichment for *E. coli* O157:H7 and *Salmonella* from Microtally™ Swabs Using the BAX® System

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Introduction: Current methods used to collect and analyze raw beef products for Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* can be labor intensive and result in product loss. These methods could soon be replaced by a more convenient manual sampling device (MSD) that has simplified sampling procedures for beef processors.

Purpose: The purpose of this study was to evaluate a single enrichment for the detection of *E. coli* O157:H7 and *Salmonella* from MicroTally™ swabs using the BAX® System.

Methods: Thirty swabs were co-inoculated with *E. coli* O157:H7 and *Salmonella* Typhimurium at a low (<1 CFU) and a high (5 CFU) level after sampling beef trim. Swabs were equilibrated at 4°C for up to 48 hours and enriched in 200 mL of pre-warmed (42°C) MP media. Samples were incubated at 42°C for 8-15 hours, analyzed by PCR and confirmed at 15 hours using the appropriate USDA FSIS reference method.

Results: For the low-inoculum samples, real-time PCR detected 14 positives (POD 0.70) at 9 hours for *E. coli* O157:H7 and 12 positives (POD 0.60) at 8 hours for *Salmonella* with 100% sensitivity and specificity. When compared to the reference method, dPOD analysis indicated no statistical difference.

Significance: All real-time PCR assays tested in this study delivered accurate results for the detection of *E. coli* O157:H7 and *Salmonella* from MicroTally™ manual sampling swabs equivalent to the reference culture method.

P1-139 Co-evolved Wide Host Range Phage Demonstrated Better Lytic Capacity in a *Felixunavirus* Phage-*Salmonella* Infantis Model on Chicken Meat

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella* Infantis (SI) is considered in recent years an emerging *Salmonella* serovar, as it has been associated with several outbreaks and multidrug resistance phenotypes. Phages appear as a possible alternative strategy to control SI. Bacteriophages over a potential biocontrol solution;

however, its lytic capacity must be investigated. Lytic capacity is defined as: i) decrease in bacterial concentration, ii) increase in viral titer iii) less induction of resistance in *S*I.

Purpose: The purpose was to compare the lytic capacity in a chicken meat model of: (1) phage-12h, a phage of the *Felixunavirus* genera with wide host range pre-exposed to *S*I (obtained from 12 h challenge assay) and (2) phage-wt, the same phage but not exposed.

Methods: Both phages were previously sequenced and phage-12h presented mutations in tail genes presumptively associated with phage adhesion to its SI host. To conduct challenge assays on chicken meat, 1g (2 cm^2) pieces were deposited onto petri dishes. The pieces were immediately inoculated with 50 uL of *SI_rifr* (spontaneous mutation to rifampicin) at 1x10^6 CFU/mL and incubated at 37°C for 1 h. Then, 50 uL of phage stock were inoculated at a concentration of 1x10^6 PFU/mL. Chicken plates were incubated at 4°C for 5 days and bacterial concentration, viral titer, and resistance induction in SI were measured daily. Three independent replicates were conducted.

Results: The SI challenge with both phages indicated that phage-12h showed a higher lytic capacity, as evidenced by: i) decreased bacterial concentration (up to a 4-log reduction compared to 2 log for phage-wt); ii) higher viral titer (5x10^9 PFU/mL for phage-12h and 5x10^7 PFU/mL for phage-wt); and iii) lower bacterial resistance (55% of SI susceptible for phage-12h versus 30% for phage-wt).

Significance: The study showed that pre-exposed phages could represent phages with improved characteristics as biocontrol to control SI in chicken meat.

P1-140 Inactivation of *Salmonella* in Ground Chicken Meat by High-pressure, Allyl Isothiocyanate, and Acetic Acid

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Introduction: Ground chicken meat is one of the most prevalent poultry products associated with salmonellosis outbreaks in the United States. High-pressure processing (HPP) may be used to inactivate foodborne microorganisms with the addition of allyl isothiocyanate (AITC) and acetic acid (AA). However, individual or combined effects of AITC, AA, and HPP on the inactivation of pathogens needs to be established for industry applications.

Purpose: This study was to evaluate treatment parameters of HPP (operation pressure and holding time), AITC, and AA for their effectiveness in the decontamination of *Salmonella* in ground chicken meat.

Methods: Fresh irradiated ground chicken meat was aseptically mixed with selected concentration of AITC and/or AA (w/w), inoculated with *Salmonella* cocktail to about 8.0 log CFU/g initial populations, and subjected to 250, 300, and 350 MPa high pressure treatment for 4, 8, and 12 min at 4°C. The populations of *Salmonella* before and after treatments were enumerated to determine the pathogen reductions (log CFU/g).

Results: AITC and AA alone with concentrations of 0.05–0.075% and 0.0-0.1% (w/w), respectively, were not significant ($P > 0.05$) in reducing *Salmonella* populations (<0.1 log CFU/g). The reductions of *Salmonella* resulted from HPP treatments were 0.6 (250 MPa – 4 min), 1.3 (300 MPa – 8 min), and 2.8 log CFU/g (350 MPa – 12 min) and had a significant ($P < 0.05$) dependence on pressure level. Moreover, there was a significant ($P < 0.05$) synergy of the combined AITC-AA-HPP treatment with the reductions of *Salmonella* ranged from 2.5 to 7.5 log CFU/g in ground chicken meat. A polynomial regression model was developed to describe the effect of HPP, ATIC, and AA on the reductions of *Salmonella* in ground chicken meat.

Significance: The application of AITC-AA-HPP was effective in reducing *Salmonella* in ground chicken meat. The identified treatment parameters and developed model may be used to enhance the product's microbial safety.

P1-141 Shelf-life Extension of Raw Chicken Breasts and Drumsticks by Dip Application of a Novel, Vinegar-based Antimicrobial Solution

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Introduction: Consumers and producers have demanded a longer shelf life of raw chicken to reduce price and eliminate food waste. Verdad® HI100 is a novel, vinegar-based antimicrobial solution for use in raw chicken to extend shelf life.

Purpose: Quantify the shelf life extension of raw chicken following dip application of a novel, vinegar-based antimicrobial.

Methods: Fresh, raw chicken breasts (ca. 190 g) or drumsticks (ca. 130 g), $n = 24$ per treatment, were dipped in 2 L of a novel antimicrobial solution (treatment) or water (control) for 30 s and permitted to drip dry for minimum 1 min. Post-drying, samples were packaged in modified atmosphere (0.4% carbon monoxide, 29.6% carbon dioxide, and balanced nitrogen), and stored (4.4°C) until enumeration (day 0, 7, 14, and 21). Upon enumeration, the sample was transferred to a sterile poultry rinse bag, to which 100 mL of neutralizing broth was added. Samples were hand massaged for 30 s, serially diluted in Butterfield's buffer, and plated on aerobic plate count (APC) 3M Petrifilm™ (48 h at 35°C). Three breasts or drumsticks were analyzed per sampling date.

Results: A population of 6 log CFU/g was considered the spoilage threshold. Control breasts and drumsticks spoiled by ca. day 6 and 7, respectively; meanwhile, treated breasts and drumsticks spoiled at ca. day 17. On sampling day 7, control populations were greater ($P \leq 0.05$) than treatment in both breasts (6.35 log CFU/g versus 3.61 log CFU/g, respectively) and drumsticks (6.00 log CFU/g versus 3.30 log CFU/g, respectively). On sampling day 14, control populations were greater ($P \leq 0.05$) than treatment in both breasts (7.95 log CFU/g versus 4.85 log CFU/g, respectively) and drumsticks (7.82 log CFU/g versus 5.24 log CFU/g, respectively). Control samples were not plated on day 21 due to spoilage organism proliferation.

Significance: The use of a novel, vinegar-based antimicrobial in processing dip tanks can extend the shelf life of raw chicken by up to 10 days.

P1-142 Shelf-life Extension of Water-chilled Whole Chickens without Giblets (WOGs) by Vinegar Powder Addition

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Introduction: Extending the shelf life of raw poultry, such as whole chickens without giblets (WOGs), can increase profitability and reduce food waste. Verdad® Powder N6 is a vinegar powder used in meat products to extend shelf life.

Purpose: Assess the performance of vinegar powder to control spoilage microorganisms and extend the shelf life of chicken WOGs.

Methods: Raw, fresh, water-chilled WOGs ($n = 30$ whole birds weighing ca. 2,400 g) were injected (20% of green weight) with a marinade containing dried vinegar powder (added to 0.3% finished product weight) or without treatment (control). Chickens were individually packaged under 90% vacuum and stored (4°C) until enumeration (day 0, 7, 13, 19, and 23). Upon enumeration, individual WOGs were aseptically transferred to a poultry rinse bag containing 400 mL of buffered peptone water. The WOG was thoroughly rinsed for 1 min following the United States Department of Agriculture (USDA) sampling methodology. Sampling broth was poured into a sterile conical tube for microbial analysis, which was performed by making serial dilutions in Butterfield's buffer and plating on aerobic plate count (APC; 48 h at 35°C) and lactic acid bacteria (LAB) 3M Petrifilm™ (48 h at 35°C), and tryptic soy agar (7°C for 10 d; psychrotrophic counts).

Results: A population of 6.0 log CFU/mL was considered a spoilage threshold. Samples treated with vinegar powder demonstrated microbial control throughout the shelf life. By day 13, APC counts were lower ($P \leq 0.05$) in vinegar powder-treated samples (1.97 log CFU/mL) compared to control (4.66 log CFU/mL). By days 19 day 23, control populations exceeded 6.0 log CFU/mL in all media types, while vinegar powder-treated samples were significantly ($P \leq 0.05$) lower (5.30, 5.39, and 5.43 log CFU/mL on day 23 for APC, LAB, and psychrotrophs, respectively).

Significance: Addition of vinegar powder in raw poultry brine injections can extend the shelf life of raw whole chickens without giblets.

P1-143 Thermal Lethality to *Salmonella* and the *Salmonella* Surrogate *Enterococcus faecium* on Black Solider Fly Larvae Meal

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❖ ***Developing Scientist Entrant***

Introduction: Black soldier fly larvae are an innovative poultry animal feed component. However, microbiological safety of animal feed components must be verified to prevent loss of sanitary processing and to comply with federal regulations.

Purpose: This study was completed to provide data describing the lethality to a selection of human pathogenic *Salmonella enterica* (serovars Senftenberg, Heidelberg, and Typhimurium) during high heat processing (160, 170, and 180°F), and to the *Salmonella* surrogate *Enterococcus faecium* NRRL B-2354.

Methods: Non-inoculated larvae were first irradiated at ~15 kGy to reduce naturally occurring salmonellae and enterococci to non-detectable counts. Following the inoculation of irradiated larvae, decimal reduction time (*D*-value) trials were completed by exposure of inoculated larvae in sealed metallic containers to heating temperatures of 160, 170, or 180°F. Samples of heated larvae were collected at 0.0, 1.0, 2.5, 5.0, 10.0, 20.0, and 30.0 min of heating, and surviving *Salmonella* and *E. faecium* were enumerated on xylose lysine tergitol-4 (XLT4) agar and Kenner Fecal Streptococci (KFS) agar, respectively, each overlaid with tryptic soy agar (TSA) in order to facilitate recovery of sub-lethally injured cells.

Results: At 160ºF, *D*-values of *S.* Senftenberg, a cocktail of *Salmonella* Heidelberg, and Typhimurium, and *E. faecium* were 1.94+1.19 min, 2.18+2.79 min, 2.10+1.30 min, respectively. At 170°F, *S.* Senftenberg (2.16+1.58 min), *Salmonella* Cocktail (0.82+0.15 min), *E. faecium (*1.05+0.35 min) did not statistically differ from one another. At 180°F similar to results obtained at 160ºF and 170°F, *D*-values did not differ between pathogen and surrogate inoculated samples: *S.* Senftenberg (1.72+1.24 min), *Salmonella* Cocktail (1.53+0.96 min), *E. faecium* (0.43+0.13 min) ($P = 0.817$).

Significance: These data indicate the usefulness of *E. faecium* as a *Salmonella* surrogate during thermal lethality validation for high heat processing and may be applied for the protection of food safety in livestock and poultry animal feeds.

P1-144 Use of Sous Vide to Cook Chicken Liver Pâté: Thermal Inactivation of *Salmonella* spp.

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Introduction: Outbreaks of salmonellosis have been attributed to undercooked chicken liver and/or pâté that was purposefully cooked to a rare degree of doneness for preferred quality attributes. Thus, additional research is needed to validate cooking processes, times, and temperatures that eliminate pathogens from pâté without affecting quality.

Purpose: Evaluate the effectiveness of sous-vide cooking on inactivation of *Salmonella* in chicken liver pâté.

Methods: Pâté batter was prepared by blending raw chicken livers, hard boiled eggs (2 units) and a mixture of sautéed onions (10%), salt (0.5%), black pepper (0.25%), and butter (11.2%). The batter was inoculated with a nine-strain cocktail (ca. 6.5 log CFU/g) of *Salmonella* and ca. 225 g portions were aseptically transferred into sterile filter bags, and then heat-sealed. The bags were completely submerged within a container filled with water that was heated using a consumer-type sous-vide device. Pâté was cooked at 54.4, 57.2, 60, or 62.8°C with holding times ranging from 24 to 240 min in a water bath set at 1°C above each target cooking temperature.

Results: A 5-log reduction in pathogen numbers was achieved after cooking pâté at temperatures ranging from 54.4 to 62.8°C for 33 to 240 min. When pâté was cooked to a target instantaneous internal temperature of 54.4, 57.2, 60, or 62.8°C for up to 240, 90, 60, or 35 min, respectively, pathogen numbers decreased by ca. 1.5 to 6.3, 1.8 to 6.4, 0.9 to 6.3, and 1.7 to 5.9 log CFU/g, respectively.

Significance: The data herein provide chefs and consumers with time/temperature options to safely prepare chicken liver pâté using sous vide without appreciably affecting product quality. From a food safety perspective, sous-vide cooking will be especially useful for those food preparers who, for quality and sensorial reasons, prefer to cook pâté at temperatures less than the required 73.9°C (165°F).

P1-145 Peracetic Acid and Cetylpyridinium Chloride to Lessen *Campylobacter* Contamination on Chicken Liver

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Introduction: Foodborne campylobacteriosis has been traced to undercooked chicken liver; we have reported that a high percentage of retail chicken liver is contaminated with *Campylobacter*.

Purpose: The objective of the current work was to test application of peracetic acid (PAA) or cetylpyridinium chloride (CPC) for reduction of naturally occurring *Campylobacter* on chicken liver.

Methods: Chicken liver lobes collected at commercial slaughter were treated by a 15 min dip in 0, 500, 1000 or 2000 ppm PAA at 4ºC or by 15 min dip in 0, 2000, 4000 or 8000 ppm CPC at 25ºC. Following treatment, *Campylobacter* on the surface of liver lobes were sampled by rinse; an aliquot of rinse was removed for plating prior to subjecting the treated lobe to 30 s in a paddle blender to expose internal *Campylobacter*. *Campylobacter* was enumerated from rinse and blended livers by plate count. Data is presented as log CFU/mL and compared by Student's *t*-test; significance was assigned at $P \leq 0.05$.

Results: Naturally occurring *Campylobacter* detected by either rinse or blending of water treated control liver lobes ranged from log 2.8 to 3.1 CFU/mL. PAA treatment at 500 or 1000 ppm did not significantly affect *Campylobacter* numbers. Treatment with 2000 ppm PAA resulted in a significant ($P < 0.05$) reduction of surface *Campylobacter* (log 2.3/mL rinse) but did not lessen numbers detected from blended lobes. CPC at 2000 or 4000 ppm did not significantly lower *Campylobacter* numbers detected. CPC at 8000 ppm resulted in a significant reduction in *Campylobacter* numbers from both rinsed (log 1.8 CFU/mL) and blended (log 2.1 CFU/mL) organs.

Significance: PAA or CPC at levels less than 2000 or 8000 ppm respectively were not effective. High concentrations of antimicrobial processing aids are required to significantly lessen naturally occurring *Campylobacter* on chicken livers.

P1-146 Microbial Shifts in Raw Chicken Marinated with Natural Preservatives

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Introduction: The shelf life of raw chicken is improved by introducing preservatives through marinades, injections, or tumbling.

Purpose: This study investigated the microbial persistence and outgrowth in raw chicken marinated with natural preservatives compared to control.

Methods: Raw chicken was marinated in water, salt, rice starch, vinegar and lemon juice (Control) plus 1.25% Natural Preservative A (NP-A) or B (NP-B), vacuum-sealed and refrigerated. Duplicate 22-gram samples per treatment were analyzed on day 4 and every 3-4 days up to 28 days for pH, Aerobic Plate Count (APC), Lactic Acid Bacteria (LAB), and 16S rRNA Illumina microbiome sequencing.

Results: By day 10, the Control chicken dropped 0.26 pH units, and APC and LAB levels increased initially from $6x10^5$ and $1x10^4$ CFU/g, respectively, to $>1x10^7$ CFU/g in both. The NP-A chicken held pH within 0.10 units through 24 days, and APC and LAB levels remained near $1x10^5$ and $1x10^3$ CFU/g, respectively, until day 21, increasing in both to $1x10^6$ CFU/g. The NP-B chicken held pH within 0.04 units, the APC levels ranged from $2x10^4$-$6x10^4$ CFU/g through 28 days and LAB levels remained at or below $1x10^3$ CFU/g until day 21, increasing to $6.5x10^4$ CFU/g. Microbiome analysis identified, *Ralstonia* in all treatments at day 4 comprising 77% of the population in Control, 39% in NP-A, and 56% in NP-B, however, *Lactobacillus* was also present in NP-A (55%) and NP-B (21%) but not in the Control. By day 7, the Control population shifted to *Photobacterium* (85%) and dominated through day 21. Slight genetic change was seen in NP-A and NP-B until day 21 when the community diversified to include *Leuconostoc* and additional *Lactobacillus* species.

Significance: The natural preservative treatments were able to provide a more stable and balanced environment with less variation in pH, lower microbial levels, and higher microbial diversity, compared to the control.

P1-147 Effects of Photosensitizer Curcumin on the Inactivation of Foodborne Pathogens and Physicochemical Properties of Chicken

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Introduction: There is an increasing global demand for natural antimicrobials. Curcumin is a well-known photosensitizer that can be naturally extracted. It has been shown that curcumin has photoinactivation ability against a variety of microorganisms in media, but studies on foods are limited, especially on poultry and meat.

Purpose: The goal of this research was to investigate the effects of a water-soluble photosensitizer curcumin (PSC) in controlling pathogens and maintaining quality of chicken under refrigerated temperature.

Methods: Chicken skin (5 x 5 cm^2; n = 6), inoculated with 5 log CFU/cm^2 of *Listeria monocytogenes* or a *Salmonella* cocktail, were dipped into 300 mL of distilled water or 300 ppm of PSC for 5 min, or 300 ppm of peracetic acid (PAA, a commercialized antimicrobial) for 10 seconds. PSC-treated samples were subjected to 32.1 kJ/m^2 of illumination by LED (430 nm). Microbial population was monitored during storage. Skin color and pH were measured separately. ANOVA and Dunkan's multiple-range test were used for data analysis.

Results: PSC resulted in 2.9- and 1.5-log CFU reduction of *L. monocytogenes* and *Salmonella*, respectively, right after treatment. Over a 10-day storage period PSC showed the best antimicrobial activities against *L. monocytogenes,* which was significantly lower than water and PAA. Besides, PSC showed equivalent or slightly better activity against *Salmonella* as PAA. Except for Day 10, the *Salmonella* population on PSC-treated samples was significantly lower than water. Out to Day 10, PSC resulted in a significant reduction of *Salmonella* compared to the non-treated control, suggesting an effective post-treatment control in the outgrowth of pathogens. PSC treatment caused no significant changes in pH and color as compared to water.

Significance: Results of this research suggests that PSC could effectively keep pathogen growth under control on chicken without negatively influencing quality. Compared to an existing treatment, PSC provides equivalent or better antimicrobial control.

P1-148 Thermal Inactivation of *Campylobacter jejuni* in Moisture Enhanced Non-intact Chicken Patties by Double Pan-broiling As Affected By Pump Rates and Cooking Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: Non-intact chicken meat may be contaminated with *Campylobacter* during moisture enhancing (MH) process. Limited published information is available for the thermal inactivation of *Campylobacter* in chicken meat.

Purpose: This study aimed to modeling the thermal inactivation kinetic parameters of *Campylobacter jejuni* in moisture enhanced reconstructed non-intact chicken patties with various pump rates and cooked by double pan-broiling sets at different temperatures.

Methods: Fresh 1.5-kg coarse-ground chicken breast, inoculated with *C. jejuni* (3-strain mixture), were MH with NaCl (2.0%) + Na-tripolyphosphate (0.5%) solutions to reach 1%, 5% or 11.1% pump rate. Inoculated samples were then manufactured (hamburger patty maker) into patties (2.1 cm thick and 10.4 cm diameter). Patties were aerobic stored (air permeable plastic film, 4°C, 42 h) before double pan-broiling (Farber-ware®griller, type-k thermocouples) for 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 330, and 360s with griller temperatures set at 200, 300, 400 or 425°F. *C. jejuni* counts were analyzed on Brucella agars under microaerophilic condition. Data (three replicates/four samples each) were analyzed using the using the Mixed Model of SAS, USDA-Integrated-Predictive-Modeling-Program, and USDA-Global-Fit software.

Results: Double pan-broiling for 300-375, 195-210, 165, and 150 s to reach the internal temperature of 76°C in chicken patties with cooking set-up temperatures at 200, 300, 400, and 425°F, respectively. Cooking reduced ($P < 0.05$) *C. jejuni* cells from 5.31-5.80 log CFU/g to <0.3 log CFU/g after 330-360 (200°F), 210 (300°F), 180-210 (400°F), and 150-165 s (425°F) across all MH samples. According to the Weibull-model, the D-value of 1% MH samples (118.2 and 114 s) were lower ($P < 0.05$) than 11.1% samples (139.5 and 124.5 s) when cooked at 400 and 425°F. Buchanan-Two-Phase-model calculated "shoulder" time decreased ($P < 0.05$) from 128 to 95.2 s as pump rate increased from 1 to 11.1% at 425°F.

Significance: Results will be useful by USDA-FSIS to develop risk assessments of *Campylobacter* in non-intact chicken products.

P1-149 VNIR Hyperspectral Imagery and Machine Learning Based Processing for Temperature Dependent Meat Characterization

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Introduction: Visible near-infrared hyperspectral imagery and machine learning technology has revolutionized meat quality assessment and food safety through the use of a non-invasive technique and information distillation process, allowing for objective comprehension of product photonic structure.

Purpose: This technology is used to estimate the spectral band subset, which concisely characterizes the beef meat cooking process and can facilitate the construction of future spectral systems dedicated to temperature-based meat monitoring.

Methods: An exploratory case study consisting of the spectral analysis of a one-inch thick, 48-inch square area oval shaped, boneless, beef shoulder steak was performed. A pseudo-sunlight spectrum light source along with a light diffusive camera box ensuring semi-Lambertian conditions illuminated the steak, which underwent a two-hour, 350°F roasting process. Spectral measurements, using a Resonon, Inc. 300-1100 nm spectral bandwidth field camera, and probe-based temperature measurements were performed every 20 minutes.

Results: Estimated nonnegative matrix factorization eigen spectra delineated specific spectral bands having maximal power contribution to the imagery. Eigen spectral band gradients in the red and blue-yellow spectral band regions suggest that these may be robust gauges for estimating temperature dependent beef state. Gaussian mixture modeling of these same regions shows data clustering undergoing strong temperature dependent modulation further supporting the premise of spectral-temperature meat features. Competitive, leaky learning-based clustering and covariance-based distance metrics using the characteristic spectral bands exhibit strong roasting modulation suggesting a robust spectral parameterization of the meat cooking process.

Significance: The findings suggest specific, exploitable spectral features which can assist businesses in attaining a cost-saving, remote characterization of meat product state (e.g. rare, medium rare, well done) minimizing product waste. This in turn, translates into a reduction of undesired consumer public risk to under cooked meat that can induce illness.

P1-150 Validation of Commercial Antimicrobial Intervention Technologies to Control Salmonella on Pre-rigor, Skin-on Market Hog Carcasses and Chilled Pork Wholesale Cuts

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❖ ***Developing Scientist Entrant***

Introduction: Pork is a significant contributor to salmonellosis, and *Salmonella* control using antimicrobial technologies during processing is of increasing interest by processors.

Purpose: This study compared 8 carcass antimicrobial washes or sprays, and 7 chilled subprimal/trim sprays, applied using commercial equipment and parameters, for their abilities to reduce *Salmonella* contamination in raw pork.

Methods: Hogs were harvested to provide skin-on carcasses, and 8 sides (per 3 replications) were inoculated with a *Salmonella* cocktail (ca. 5 log CFU/cm^2). Each side was treated in a commercial Chad cabinet using a spray [low-volume: 3% lactic acid (LA), 400 ppm peracetic acid (PAA), acidified 400 ppm peracetic acid (aPAA)] or wash [high volume: ambient water (AW), 400 ppm PAA, 400 or 600 ppm bromous acid (DBDMH), 71°C water (HW)] treatment within a randomized complete block study design. Post-treatment *Salmonella* reductions were compared. Chilled subprimals and trim from each side were inoculated and treated with antimicrobial sprays [AW, 400 ppm PAA, 400 ppm aPAA, 400 and 600 ppm DBDMH, 2% LA, modified LA (mLA)] in a subprimal spray cabinet or ribbon mixer (trim). Reductions were determined over 14 days of vacuum-packaged subprimal storage and 4 days of trim storage, along with lactic acid bacterial populations, TBARS and color determinations on non-inoculated products.

Results: High-volume 400 ppm PAA and 600 ppm DBDMH carcass wash treatments resulted in higher ($P \leq 0.05$) *Salmonella* reductions (2.9 and 2.6 log CFU/cm^2, respectively) compared to AW (0.9 log CFU/cm^2). HW achieved a 2.0-log reduction, while other antimicrobial treatments provided 1.4 to 1.7-log reductions. Subprimal treatments resulted in 0.4 to 0.6-log reductions, and trim reductions were 0.1 to 0.2 logs ($P > 0.05$).

Significance: All pre-rigor carcass interventions provided beneficial *Salmonella* reductions, allowing processors flexibility in their operations. *Salmonella* control was less evident at the chilled subprimal and trim stages of processing.

P1-151 Evaluating a Salmonella Lethality Prediction Tool for the Surface of Cooked Meat and Poultry Products

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✦ ***Undergraduate Student Award Entrant***

Introduction: USDA FSIS provides guidelines for cooking ready-to-eat meat and poultry products based on core temperature; however, recent research has demonstrated that, under dry cooking conditions, it is possible to get lower Salmonella lethality on the product surface than at the core. Current approaches to addressing surface survivors are limited in number and scientific support.

Purpose: This study aimed to determine the effectiveness of USDA FSIS Appendix A guidelines when estimating the reduction of Salmonella on the surface of beef and poultry during hydrated surface conditions.

Methods: Beef strips, beef patties, and chicken breast filets were inoculated with an 8-serovar Salmonella cocktail. A full-factorial experiment was conducted, cooking meat and poultry samples to core temperatures of 70 and 73.9ºC, respectively, varying oven humidity (0.7, 30, 70% v/v), temperature (218, 232ºC), and fan speed (low, high). Samples were then immediately cooled, dissected to obtain surface samples, serially diluted, plated, and survivors enumerated. The USDA FSIS Appendix A time-temperature tables were then applied to temperature profiles collected for the surface (when temperatures were below the dew point) to predict lethality outcomes.

Results: *Salmonella* reductions were measured in 42 beef strips, 35 beef patties, and 36 chicken breasts. The time-temperature tables correctly classified lethality outcomes (i.e., classifying as greater or less than 6.5 (beef) or 7.0 (poultry) log) for 26/42 beef strips, 22/35 beef patties, and 20/36 chicken breasts. Most failures (36/45) were "fail-safe", predicting insufficient lethality when experimental results actually exceeded the target lethality. However, 9/45 failures were "fail-dangerous", falsely categorizing a sample as achieving sufficient lethality.

Significance: While the USDA FSIS Appendix A is an important tool to help understand the risks associated with surface *Salmonella* survivors, the classification accuracy was 60%, including some fail-dangerous predictions. Improved approaches to quantifying *Salmonella* surface lethality are still needed.

P1-152 Safety of Shell Eggs as Affected by Rate of Heating during Pasteurization to Inactivate *Salmonella* Enteritidis

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❖ ***Developing Scientist Entrant***

Introduction: Thermal shell egg pasteurization is a technology developed to combat *Salmonella* contamination. The process includes three steps: come-up, holding and cooling. We hypothesize that slow heating rate (SHR) during come-up stage, as practiced commercially, induces responses in *Salmonella* which compromises product safety.

Purpose: Understand how different heating rates during come-up stage could affect *Salmonella* heat resistance during holding stage, *Salmonella* recovery during cooling stage, and expression of *Salmonella* virulence genes.

Methods: Shell eggs, 55-58 g each, were inoculated with *Salmonella* Enteritidis in egg yolk, and submerged in a 59℃- or 53°C-water bath to mimic fast heating rate (FHR) or SHR. After come-up stage, eggs were transferred to a 53°C- or 55°C-water bath to mimic holding stage. The egg internal temperature was monitored by a temperature probe. Survivors in yolk were monitored during holding stage to calculate *D*-values. Remaining eggs from 55℃ holding stage were cooled at 30°C. *Salmonella* recovery rate in yolk within 8 h storage was monitored. For plate count at each time point, 3 eggs were pooled. *D*-values and recovery rates of *Salmonella* in yolk were compared by *t*-test. To study the expression of virulence genes, transcriptional changes were determined using RT-qPCR. Data were analyzed using double ΔCT method. All the experiments were triplicated.

Results: The $D_{53°C}$ of *Salmonella* in yolk subjected to FHR was significantly shorter than that to SHR $D_{53°C}$ (8.9 ± 1.6 vs. 14.4 ± 1.3min). However, $D_{55°C}$ values resulting from the two heating rates were not significantly different. During the first 30 min of cooling, *Salmonella* population in eggs subjected to FHR decreased by an additional 1.3 ± 0.5 log; whereas, no reduction was observed with SHR. SHR caused more profound expression of heat resistant genes than FHR. Both treatments caused upregulation of virulence.

Significance: Fast heating rate at come-up stage could result in safer pasteurized shell eggs, compared to the slow heating rate pasteurization.

P1-153 Quantitative Risk Assessment of *Salmonella* Foodborne Illness through Egg Consumption

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❖ ***Developing Scientist Entrant***

Introduction: There were foodborne outbreaks by *Salmonella*-contaminated eggs in Korea, and consumption of eggs is still high. However, the microbial risk of *Salmonella* foodborne outbreaks by egg consumption has not been estimated in Korea yet.

Purpose: The objective of this study was to evaluate the risk of *Salmonella* foodborne illness by egg consumption.

Methods: Two hundred one egg samples were collected from markets to detect *Salmonella* by plating and gene identification. *Salmonella*-inoculated eggs were stored at 7°C - 30°C, and the cell counts recovered from the eggs were used to develop predictive models, which describe the kinetic behaviors of *Salmonella*. Transportation time and temperature were collected, and consumption patterns for eating raw eggs and cooking (dry heat and moist heat) were surveyed. A dose-response model was searched through literature. With these data, the probability of *Salmonella* foodborne illness was estimated by a simulation in @RISK.

Results: Of 201 samples, *Salmonella* were not detected in eggs, and thus, the initial contamination level of *Salmonella* was estimated, using Beta distribution (1,202). The developed predictive model showed that the *Salmonella* cell counts increased as temperature increased. Temperature and time during transportation were fitted with Pert distribution (10,18,25) and Uniform distribution (0.325, 1.643), respectively. Eggs were consumed raw (1.5%) and after cooking by dry heat (57.5%) and moist heat (41%). Beta-Poisson dose response model ($risk=1-(1+(dose/4.4\times10^{5}))^{-0.89}$) was selected as an appropriate model. The simulation with all data showed that the probability of the foodborne illness was 6.8×10^{-10}/person/day.

Significance: The results indicate that the risk of *Salmonella* foodborne illness by egg consumption seems low in Korea.

P1-154 Effects of Temperature on the Efficacy of Peroxyacetic Acid and Citric Lactic Blend Spray for Beef Carcasses

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Introduction: Antimicrobial interventions are widely researched for reduction of enteric pathogens such as *Escherichia coli* O157:H7 on meat. However, the findings on the efficacy of acid sprays, e.g., peroxyacetic acid (PAA), are inconsistent and often times key spray parameters, e.g., coverage, are not described in the reports.

Purpose: To determine the effect of spray temperature on the efficacy of PAA and citric lactic acid (CLA) spray for beef carcasses using defined parameters.

Methods: We customized a spray system consisting of a spray cabinet, a handheld spray gun and a portable air compressor. Groups of three beef short plates (400 cm^2/side) with primarily exterior carcass surface were each inoculated with 2 ml of cattle fecal slurry (1:10) and were not sprayed, or sprayed with water, 400 ppm PAA or 2.5% CLA at 30 PSI for three minutes. After dripping for 10 min, meat was sampled for enumeration of total aerobes, coliforms and *E. coli*. The experiment was carried out at both room temperature and 1°C, to simulate dressing and chiller conditions. Three independent trials for each temperature were carried out (n = 9). Tukey test was used to separate mean bacterial reductions.

Results: The spray system delivered 0.68±0.05 and 0.72±0.01 mL/cm^2 solution at room and chiller temperatures, respectively. Water did not result in any significant reduction (P >0.05) of any bacterial groups at either spray temperature. PAA reduced 1.44 – 1.65 log CFU units of coliforms and *E. coli* at the two temperatures similarly (P > 0.05) and significantly (P < 0.05) different from water. CLA reduced about 1 log CFU unit more (P < 0.05) of coliforms and *E.coli* at ambient temperature than chiller. The reduction of aerobes by PAA or CLA, however, was significant (P < 0.05) at chiller temperature, but not at room temperature.

Significance: Four hundred ppm PAA and 2.5% CLA are effective interventions for beef. PAA was more effective at chiller than room temperature while CLA was opposite. The parameters set out should be useful to the meat industry for effective implementation of these interventions.

Poster

P1-155 Impact of Supplemental Critical Controls on Salmonella Reductions in Ready-to-Eat Beef Products

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❖ ***Developing Scientist Entrant***

Introduction: USDA FSIS Appendix A guidelines provide time-temperature combinations for *Salmonella* reduction in cooked meat products. Recent research has identified scenarios that meet the published conditions but do not achieve the target *Salmonella* lethality; however, there is limited information on what additional process steps might ensure sufficient lethality.

Purpose: This study aimed to quantify the impact of practical, supplemental critical controls on *Salmonella* lethality in impingement cooked meat products.

Methods: Beef strips and ground beef patties were inoculated with an eight-serovar *Salmonella* cocktail. Each product was cooked (in triplicate) in a pilot-scale, moist-air impingement oven at a controlled temperature (232°C) and absolute humidity (0.7, 15, or 70% v/v) to a minimum core temperature of 70°C (±0.6°C). Five critical controls were evaluated, including adhering to minimum USDA FSIS Appendix A guidelines and pre-/post-process controls (increased endpoint temperature, post-oven carryover time, and pre- or post-oven steam treatments). After cooking, samples were immediately cooled in liquid nitrogen, dissected to remove surface and core samples, and plated to enumerate *Salmonella* survivors.

Results: The efficacy of minimal and supplemental critical controls were product- and process-humidity-dependent ($P < 0.05$). Higher *Salmonella* reductions were observed with added process humidity and in beef strips ($P < 0.05$). No single supplemental control resulted in universal assurance of *Salmonella* reductions; most supplemental treatments were not significantly better than minimal critical control treatments ($P > 0.05$). No treatment reliably ensured >6.5 log reductions of *Salmonella* in ground beef.

Significance: The effectiveness of time, temperature, and humidity controls were highly variable and dependent on product batch. While compliance with current USDA FSIS Appendix A guidelines are critical for the meat industry to provide a safe product, important revisions are needed to reflect the importance of process humidity and product variability in order to ensure food safety.

P1-156 Inhibition of *Clostridium perfringens* in Uncured Turkey Products with Clean-label Antimicrobials during Extended Phase 1 Cooling

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✦ ***Undergraduate Student Award Entrant***

Introduction: The 2017 draft USDA-FSIS Appendix B proposed changes to cooling options to limit growth of *Clostridium perfringens* to 1 log in uncured meat and poultry products. The time restrictions (1 hour between 48.9 - 26.7 °C) present challenges for cooling large diameter products without antimicrobials.

Purpose: To validate the inhibition of *C. perfringens* growth in a model uncured turkey product with clean-label antimicrobials, representing cooling deviations under the 2017 USDA-FSIS Appendix B Option 2 Stabilization Guidelines.

Methods: Eight model uncured turkey products (75% moisture, 1.5% salt) were formulated with no antimicrobial (control) or 1% commercially available dry vinegar (DV), dry vinegar-cultured sugar (DV-CS), or dry vinegar-fruit-spice-extract (DV-FSE), each at pH 6.6 and 6.2. Batter was inoculated with *C. perfringens* spores (3-strain mixture), vacuum-packaged (25 g/pouch), cooked to 70°C and cooled according to the following schedule: Phase 1 (48.9 - 26.7°C) in 2, 3, 4, or 5 hours, Phase 2 (26.7 - 12.8°C) in 5 hours and Phase 3 (12.8 - 4.4°C) in 5 hours. Triplicate samples were enumerated after cooking and at the end of each phase on Tryptose-sulfite-cycloserine agar; each experiment was replicated twice.

Results: *C. perfringens* grew rapidly in all control samples (e.g., >1.5 and >6 log for the 2- and 5-hour extended Phase 1 cooling, respectively). In contrast, treatments containing 1% DV or DV-FSE inhibited 1-log growth for 2- and 3-hours Phase 1 cooling at pH 6.6 and 6.2, respectively. Turkey containing 1% DV-CS at pH 6.6 and 6.2 inhibited 1-log increase of *C. perfringens* when Phase 1 was extended 4- and 5-hours, respectively.

Significance: This study confirms cooling rate, pH and antimicrobials are important factors in controlling growth of *C. perfringens* during cooling. All three dry-vinegar based, clean-label antimicrobials tested in this study can be used by manufacturers to extend Phase 1 cooling of uncured meat and poultry products beyond the recommendations in the 2017 USDA-FSIS Appendix B Option 2 Stabilization Guidelines.

P1-157 Identification of Salmonella spp. and Differentiation between Enteritidis and Typhimurium in One Real-Time PCR Test

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Introduction: 2018 food products contaminated with *S.* Enteritidis (SE) and *S.* Typhimurium (STM) are still the largest source of *Salmonella* outbreaks in the EU. A fast and easy diagnostic tool for detecting the *Salmonella* genus, in particular SE and STM, in food as well as in primary production places is essential to minimize the damage to humans and the food or veterinary markets. Being able to react quickly to salmonellosis outbreaks is difficult with ISO 6579, where the identification process takes up to five days.

Purpose: To validate a rapid tetraplex PCR **food**proof® *Salmonella* Genus plus SE & STM Detection LyoKit with regard to specificity, sensitivity and matrix compatibility.

Methods: The **food**proof® *Salmonella* Genus plus SE & STM Detection LyoKit was analyzed for specificity with 380 *Salmonella* spp. strains including *Salmonella bongori* and *enterica* including all five subspecies, 63 Enteritidis strains, 59 mono/biphasic Typhimurium and 30 non-target strains. Sensitivity was determinate with purified DNA and in combination with different **food**proof® DNA isolation kits. 10^3-10^5 cfu/ml SE and STM, blended with an excess of background flora, was analyzed. Matrix compatibility was tested with different food and primary production samples. 100 naturally contaminated samples were examined after 16 h of BPW enrichment in comparison to ISO 6579.

Results: Specificity results showed 100% success for inclusivity and exclusivity. The kit detected 1 GE and 10^2 cfu/ml of *Salmonella* genus, SE and STM. With strain blending, 10^3 cfu/ml could be correctly analyzed. All tested matrices are compatible with the **food**proof® *Salmonella* Genus plus SE & STM Detection LyoKit. Naturally contaminated samples were tested with comparable accuracy to ISO 6579.

Significance: The new **food**proof® *Salmonella* Genus plus SE & STM Detection LyoKit measured up successfully with ISO 6579, achieving comparable specificity, sensitivity and matrix compatibility.

P1-158 A Multiplex Real-time PCR Kit for the Detection of Food-Relevant *Listeria* Species and Identification of *L. monocytogenes* in a Single Reaction

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Introduction: *Listeria monocytogenes* is considered to be one of the most important foodborne pathogens. It can lead to severe illness, including meningoencephalitis, and abortion, with mortality rates up to 33%. Infections have been traced to the consumption of contaminated foods that have relatively short shelf lives, emphasizing the need for rapid detection methods.

Purpose: *L. monocytogenes* is often found in samples that contain other Listeria spp. Therefore, the detection of Listeria species is used as an indicator for the presence of *L. monocytogenes*. The purpose was to develop a method that detects both in one test, *Listeria* sensu stricto species and *Listeria monocytogenes*.

Methods: As such, BIOTECON Diagnostics has developed the foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit - a rapid, accurate and sensitive qPCR method for the simultaneous detection of food-relevant *Listeria* species and the specific identification of pathogenic *L. monocytogenes* in a single reaction.

Results: To shorten the enrichment time, we have internally validated different rapid enrichment broths for *Listeria*. These broths enable the safe detection of *Listeria monocytogenes* and the food-relevant Listeria species in less than 24 h. Depending on the throughput, the method can be used with different foodproof DNA extraction procedures for both manual and fully automated DNA extraction. All kits, including the rapid enrichment broths and the ISO reference broth, half-Fraser, are currently in the process of being validated according to ISO 16140 by NordVal. This validation is being carried out with a broad variety of food categories including dairy, meats, vegetables, fish, frozen as well as ready to eat foods and environmental samples.

Significance: This is the first lyophilized qPCR kit on the market that is able to detect *Listeria monocytogenes* and all the other food-relevant *Listeria* species: *L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. seeligeri*, and *L. marthii* in a single reaction.

P1-159 Identification of Sus Scrofa (porcine), Bos Taurus, Bos Indicus (bovine) and Equidae (horse, donkey, zebra) in Raw Material and Processed Foods Via Real-Time PCR

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Introduction: Animal species testing is crucial for product quality control, whether due to food adulteration or because of religious requirements, like halal or kosher food. The detection, identification and quantification of potential contaminations in processed and ready-to-eat food has become increasingly important.

Purpose: To develop a multiplex qPCR method, which identifies pork (*Sus scrofa*), beef (*Bos taurus, Bos indicus*) and *Equidae* (horse, donkey, zebra). It adopts a rapid extraction workflow whereby the entire process is carried out in a single tube. This minimizes handling steps and contamination risks, and is more convenient and faster than regular column-based methods. Together with a reference material an estimation of the content can be achieved.

Methods: For DNA extraction, the rapid extraction buffer **food**proof® StarPrep Five was compared with silica based DNA extraction methods. The **food**proof® Animal Detection 1 LyoKit was designed to detect porcine, bovine and equine DNA in specific channels and in one multiplex PCR reaction. A differentiation of equine was done by melting curve analysis, to distinguish between horse, donkey and zebra. For sensitivity testing, a reference material (extracted DNA of 1.0%, 0.1%, 0.01% pork, beef and horse in chicken meat) was used.

Results: Inclusivity and exclusivity was tested to be 100 %. The detection limit of amplification and melting curve for spiked matrices is at least 0.001 % for different matrices like raw meat, milk powder, gelatine or pharmaceutical products. The LOD with **food**proof® StarPrep Five Kit is comparable to column-based DNA isolation methods.

Significance: This method is not only convenient and precise, it also cuts monitoring costs because of its multiplex aspect and additionally saves time.

P1-160 Potential of Edible Gelatin Composite Films Enriched with Clove Oil Nanoemulsions as Chicken Meat Packaging Material

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Introduction: Foodborne diseases are an emerging issue for global public health which can lead to hospitalization and even deaths. One of the novel strategies to control foodborne diseases is the development of active food packaging materials with antimicrobial properties.

Purpose: This study aimed at developing a biodegradable antibacterial meat packaging film to enhance the shelf life of broiler meat which is commonly consumed in Pakistan.

Methods: In this study, food grade clove oil nanoemulsions (NEs) were prepared with two-step homogenization. Stable NEs were incorporated into the film forming solution (FFS) at various concentrations (5, 10 and 20% v/w of gelatin plus carrageenan) to produce packaging films by casting method. The chemical fingerprinting of films was evaluated by FTIR. The antimicrobial, antioxidant activities and water-vapor-permeability were determined through disk diffusion assay, DPPH % inhibition and ASTM E96 methods, respectively. Shelf life was examined by measuring CFU/g of packaged meat.

Results: There were significant differences ($P < 0.05$) statistically in the values of different parameters observed for 5% and 20% NE concentrations. Water vapor permeability of films loaded with NEs was relatively high due to increased mobility of adjacent chains of gelatin molecules and ranged from 14.22 ± 0.29^{c} to 18.78 ± 0.16^{a} for gelatin composite films loaded with 5 and 20% NEs, respectively. Clove oil NEs loaded gelatin films exhibited antioxidant potential which increased significantly (from 39.65 ± 2.22^{c}% to 66.9 ± 1.99^{a}%) with increasing content of NEs from 5 to 20%. Gelatin films loaded with 20% v/w clove oil NEs exhibited highest antibacterial activity against *Salmonella enterica* serovar Typhimurium (7.04 ± 0.03 mm) and *Escherichia coli* (8.30 ± 0.15 mm). Films loaded with 20% clove oil NEs showed excellent results by increasing the shelf life of broiler meat up to 19 days in comparison to control.

Significance: Millions of dollars are spent each year to treat foodborne diseases due to meat products. This packaging material can not only serve as an alternative to conventional plastic-based packaging but also has potential for commercialization.

P1-163 Comparing the Reductions of *Salmonella* and *Listeria monocytogenes* in Different Diameter Salami during Fermentation and Drying

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Introduction: According to the U.S. Department of Agriculture, Food Safety and Inspection Service, dry, fermented sausages, including salami, must achieve a 5-log reduction of *Salmonella* and a 3-log reduction of *Listeria monocytogenes* during processing. Few processes have been validated to achieve these reductions and companies are facing increased pressure from regulators to demonstrate the safety of these products. Numerous factors (recipe formulation, fermentation and drying time, temperature, relative humidity, and diameter) will affect the reduction and survival of *Salmonella* and *Listeria monocytogenes* in salami, and these factors are up to individual processors; therefore, individual processes need to be validated.

Purpose: Quantify and compare the reduction of *Salmonella* and *Listeria monocytogenes* during fermentation and drying of salami with different diameters (50 mm and 80 mm).

Methods: Seasoned pork meat (1.36 kg) was inoculated with *Salmonella* and *Listeria monocytogenes* cocktails (~7 log CFU/g) and stuffed into 50 mm and 80 mm diameter casings. Fermentation (32°C, 95% relative humidity, 24 hours), drying (15°C, 72% relative humidity, 32 days), and storage (21°C, 50% relative humidity, until day 61) occurred in a Stagionello 41262. Salami were sampled throughout the process (n = 3) and survivors were spread-plated on Tryptic Soy Agar overlaid with Hektoen Enteric Agar or Modified Oxford Listeria Agar and incubated at 37°C, 24 to 48 hours prior to enumeration.

Results: After 61 days, a 5.52-log reduction for *Salmonella* and a 4.94-log reduction for *Listeria monocytogenes* was achieved in the 50 mm diameter salami. At the same timepoint (61 days), reductions of *Salmonella* (3.55 log) and *L. monocytogenes* (3.93 log) were significantly lower in the 80 mm diameter salami.

Significance: Casing diameter was shown to have a significant effect on pathogen reduction during fermentation, drying, and storage of salami.

P1-164 New GeneDisc® Method for *Campylobacter* Quantification in Poultry Plants

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Introduction: *Campylobacter* is recognized as the leading cause of bacterial foodborne diarrheal disease worldwide and contaminated poultry meat is considered the most important source of infection in humans. Contamination of poultry meat occurs during the slaughterhouse processing and *Campylobacter* persists throughout the food chain. In order to reduce *Campylobacter* occurrence, EFSA set up a microbiological criterion with a limit of 1,000 CFU/g of sample. In that context, Pall GeneDisc Technologies proposes a new real-time PCR-based method enabling a quantitative result available in less than 2 h.

Purpose: This new method was validated against the ISO 10272-2 reference method on a large range of poultry samples, including environmental samples.

Methods: Thirty samples issued from poultry industry were analyzed by both the GeneDisc method and the reference method. The samples were diluted in Buffered Peptone Water, then, a DNA extraction was carried out from 100 µL of diluted sample before PCR analysis with the GeneDisc plate *Campylobacter*. All samples were confirmed by plating on chromogenic media (mCCDA, CampyFood Agar).

Results: Results highlighted that this new GeneDisc method gave equivalent results to the ISO reference method whatever the sample type (carcass rinse, neck skin, environmental samples) when considering the acceptance limit at 1,000 CFU/g.

Significance: The GeneDisc method for *Campylobacter* quantification enables to drastically reduce the time-to-result, with a result available within 2 hours against 2 days for the culture method according to the ISO 10272-2. Thus, a poultry plant decision maker can quickly set up appropriate actions in case of results greater than the criteria limit of 1,000 CFU/g. Moreover, the poultry sample diluted in BPW for *Campylobacter* enumeration enables to test *Salmonella* spp. detection.

P1-165 New Genedisc® Method for the Combo Detection of *Campylobacter* and *Salmonella* in Poultry Plants

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Introduction: *Campylobacter* and *Salmonella* are the two leading sources of foodborne illness with millions of cases worldwide each year. A strong association has been demonstrated between the consumption of poultry products and sporadic outbreaks of bacterial gastrointestinal disease caused by *Campylobacter* spp. and *Salmonella* spp. in humans. Hazard analysis critical control point (HACCP) programs for food processing have been initiated in poultry processing plants to reduce the risk of foodborne illness. In that context, Pall GeneDisc Technologies proposes a new real-time PCR-based method enabling to simultaneously monitor both pathogens, with presumptive results available in less than 24 h.

Purpose: This new method was validated against the reference methods (ISO 6579 & ISO 10272-1) on a large range of poultry samples, including environmental samples.

Methods: A total of 75 samples issued from the poultry industry were analyzed by both the GeneDisc method and the reference methods. The samples were enriched for 20-24 h, then, a one-step DNA extraction was carried out before PCR analysis with the GeneDisc plate DUO *Salmonella* spp. & *Campylobacter*. All samples were confirmed by plating on chromogenic media (XLD, Brilliance *Salmonella*, mCCDA, CampyFood Agar).

Results: Results highlighted that this new GeneDisc method gave equivalent results to the ISO reference methods whatever the sample type (fresh meat, frozen meat, ready-to heat and ready-to-eat products, environmental samples), the enrichment conditions (micraoerophilic or aerobic), and the contamination level. Moreover, those equivalent results were observed at both time points (20 and 24 h).

Significance: The GeneDisc DUO *Salmonella* spp. & *Campylobacter* offers greater efficiency and flexibility when compared to the reference methods, with a 20-24 h single-step enrichment combined with common sample preparation and common PCR analysis, for monitoring both key pathogens in poultry plants

P1-166 Evaluation of the TEMPO® CAM (Campylobacter) Assay for the Detection of *Campylobacter* from Poultry Samples

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Introduction: Infection with *Campylobacter* bacteria, or Campylobacteriosis, is the most common bacterial cause of diarrheal illness in the United States. *Campylobacter* are present in most warm-blooded animals, however human infection is most commonly associated with consumption of contaminated poultry.

Purpose: The objective of this study was to evaluate the performance of a fully automated enumeration system which utilizes fluorescent indicators and a miniaturized MPN to enumerate *Campylobacter* spp. from several poultry matrices including: Whole bird chicken carcass rinses, Turkey carcass swabs, Chicken Livers, and Raw Ground Chicken, according to the current AOAC validation guidelines.

Methods: All four matrices evaluated were artificially inoculated at 4 contamination levels (uninoculated, low, medium, and high mean log CFU/g), each contamination level being tested in pentuplicate. Whole bird chicken carcass rinses were inoculated with *Campylobacter coli* Duke H91 (0, 2.24, 3.22, 3.60) and rinsed with 400 mL of neutralizing buffered peptone water (nBPW). Turkey carcasses were inoculated with *Campylobacter jejuni* ATCC 33291 (0, 1.77, 2.26, 2.53) and 100 cm^2 of each was swabbed with a sponge rehydrated in nBPW. Chicken liver samples (325 g) were inoculated with *Campylobacter lari* ATCC 35221 (0, 1.11, 2.00, 2.30), and 325-g raw ground chicken samples were inoculated with *Campylobacter coli* ATCC 43478 (0, 2.88, 3.90, 4.82), both were diluted in 1,625 mL of buffered peptone water (BPW). All replicates were run on both the candidate method and the reference method, USDA/FSIS MLG Ch. 41.04 direct plating analysis protocol. Colonies from the USDA reference method were confirmed via latex agglutination.

Results: In the method comparison study, the automated enumeration system demonstrated no statistically significant differences from the reference method for any of the matrices tested. The mean difference was calculated between the candidate and reference method log results with a 95% confidence interval for each contamination level.

Significance: These data support the reliability and use of TEMPO® CAM for the enumeration of *Campylobacter* spp. from poultry. Enumeration can provide additional information useful for the control of *Campylobacter* presence in the poultry processing plant.

P1-167 Salmonella Lethality in Fully-cooked Bacon and Evaluation of a Non-pathogenic Surrogate Enterococcus faecium for Validation Purposes

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✦ ***Undergraduate Student Award Entrant***

Introduction: Ready-to-eat (RTE) bacon, like other RTE meats, require sufficient *Salmonella* lethality to comply with USDA FSIS regulations. Unlike other RTE meats, bacon cooking is primarily controlled based on final product yield. However, there is very limited information supporting pathogen lethality in commercial fully-cooked bacon.

Purpose: This study was designed to evaluate whether current commercial cooking practices provide sufficient *Salmonella* lethality for fully-cooked bacon.

Methods: Commercially produced traditional and thick-cut bacon slices were inoculated with *Enterococcus faecium* NRRL B-2354 or an 8-serovar cocktail of *Salmonella*. Inoculated samples were cooked in triplicate on a wire mesh tray in a pilot-scale moist-air impingement oven (humidity 70% v/v, 20% fan speed) at temperatures of 232°C (up to 6 min) or 177°C (up to 10 min) to a commercially-relevant yield (40%). Samples were immediately cooled, weighed for yield calculations, serially diluted, and plated on differential media to enumerate survivors (37°C, 48 h). Additional triplicate trials were run at 177°C for 30 and 60 s cook times, yielding under-cooked bacon, to evaluate *E. faecium* as a potential *Salmonella* surrogate.

Results: All treatments achieving the target product yield achieved >6.5-log reductions of *Salmonella.* Additionally, all products that were cooked below the target yield (average 75%) also achieved >6.5-log reductions of *Salmonella* and *E. faecium*. Fully and partially-cooked bacon achieved average maximum surface temperatures of 121 ± 7.8 and 92 ± 1.0°C, respectively. No statistical relationship between *Salmonella* and *Enterococcus* could be estimated due to the large reductions observed.

Significance: Experimental results indicate that typical commercial cooking practices for fully-cooked bacon ensure that the target 6.5-log reduction of *Salmonella* lethality is ensured. This scientific evidence is important in meeting a need for processors to comply with USDA FSIS regulations.

P1-168 Prevalence and Antibiotic Resistance of Salmonella and Campylobacter on Raw Retail Chicken Breasts

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Introduction: Raw poultry products frequently contain *Salmonella* and *Campylobacter*, which are among the top five pathogens that contribute to foodborne illnesses, hospitalizations, and deaths in the United States. The severity of infection and the public health burden from *Salmonella* and *Campylobacter* can be much greater when these bacteria are antibiotic resistant.

Purpose: The purpose of this study was to determine the percentage of raw, packaged, retail chicken breasts that test positive for *Salmonella*, *Campylobacter*, and generic *E. coli* and compare the results to regulatory data at the plant level. This study also investigated the antibiotic susceptibility profiles of the *Salmonella* and *Campylobacter* isolates.

Methods: Raw, packaged, chicken breast samples were retrieved in January/February 2018 from 27 metro areas across the US. USDA/FSIS MLG methodology was used to isolate and identify *Salmonella* (*n* = 672), *Campylobacter* (*n* = 499), and generic *E. coli* (*n* = 597) from 400-g samples. Methods outlined in the latest edition of the NARMS Manual of Laboratory Methods were followed for antimicrobial susceptibility testing of a subset of *Salmonella* (*n* = 52) and *Campylobacter* (*n* = 16) isolates.

Results: *Salmonella* was found in 8.6% of chicken breasts, *Campylobacter* in 4.2%, and generic *E. coli* in 60.4%. Having a "3" rating in USDA's *Salmonella* Categorization of Individual Establishments for chicken parts was predictive of having a high level of *Salmonella* in our data set ($P \leq 0.05$). A total of 73.1% of *Salmonella* isolates and 62.5% of *Campylobacter* isolates tested were resistant to 1 or more classes of antibiotics, with 48.1% of the *Salmonella* isolates being resistant to 3 or more classes of antibiotics.

Significance: Chicken is the most popular meat in the United States, and consumers may be exposed to multi-drug resistant *Salmonella* and *Campylobacter* through consumption of retail chicken breasts. Efforts are needed to reduce *Salmonella* and *Campylobacter* contamination in chicken and promote more judicious use of antibiotics in food animals.

P1-169 Beef Microbiomes and Biofilm Formation by Bacteria Transferred to Food Contact Surfaces

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Introduction: Food contact surfaces can be sources of spoilage and pathogenic organisms for meats, especially those able to persist over time.

Purpose: The objectives are to survey the microbiota of several beef cuts, determine the microbiota that can transfer from beef to two common food contact surfaces, stainless steel (SS) and high-density polyethylene (HDPE), identify isolates that bind to these surfaces, and evaluate selected isolates for biofilm formation in single strain- and dual species-biofilms with *E. coli* O157:H7.

Methods: Top round, flank, chuck, and ground beef were purchased from 3 different retail stores. In triplicate, SS or HDPE coupons (2 cm x 5 cm) were placed on approximately 15-g beef portions for 3 hours at 10°C. Coupons were then transferred to conical tubes to submerge coupons halfway in PBS for 24 hours in 10°C. The coupon surfaces and additional triplicate portions of beef cuts were plated on TSA and incubated at 25°C for 5 days. Additionally, the beef and coupon samples were collected for 16S rRNA gene amplicon sequencing and isolates were picked for single strain- dual species biofilm formation using a crystal violet binding assay (72 h, 10°C) in 96-well plates.

Results: A total of 136 bacterial operational taxonomic units (OTUs) were identified among beef cuts while 196 bacterial OTUs were identified among coupon samples. While there were no core genera among beef cuts from 3 stores, the core genera detected among all coupons were *Caulobacter* and *Pseudomonas*. Average aerobic plate counts from beef ranged from 3.27 ± 0.44 log CFU/g to 7.70 ± 0.20 log CFU/g while average aerobic plate counts from coupons ranged from none detected to 4.85 ± 0.38 log CFU/cm^2. Sixty-one of 962 beef isolates, 31 of 211 SS isolates, and 29 of 199 HDPE isolates were strong biofilm-formers (OD_{590}>1.000).

Significance: The microbiota identified on the coupons may help determine the communities able to attach and potentially form biofilms on common materials used as food contact surfaces.

P1-170 Control of *Bacillus weihenstephanensis* in Pasteurized Liquid Whole Eggs Formulated with Nisin

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Introduction: *B. weihenstephanensis* is capable of causing food poisoning through production of emetic toxin at temperatures as low as 8°C and has the potential for producing diarrheal enterotoxins. It is able to grow at < 7°C and has been isolated from eggs and liquid egg products. Additionally, spores can survive pasteurization and can germinate into vegetative bacteria with the ability to multiply under favorable conditions.

Purpose: The purpose of this study was to evaluate the control of *B. weihenstephanensis* in pasteurized liquid whole eggs with nisin during storage at refrigerated and abuse refrigerated temperatures.

Methods: Whole liquid eggs mixed with nisin was pasteurized and filled into 950-g retail cartons. Three production lots of egg cartons were syringe-inoculated with *B. weihenstephanensis* (*Bacillus* Genetic Stock # B6A21, B6A22, B6A23) spores to achieve an initial level of 2-3 log CFU/g. The product was held at 4°C for 30 days and then transferred to both 7 and 10°C for 60 days to simulate the potential abuse temperature storage through the 90-day shelf life. Three individual inoculated cartons were plated for *B. weihenstephanensis* on MYP agar at each time point. Additionally, two un-inoculated cartons were plated for *B. weihenstephanensis*, aerobic plate counts and yeast and mold at each time point. The acceptance criteria were less than 3-log increase of *B. weihenstephanensis* over initial inoculum levels during storage.

Results: *B. weihenstephanensis* counts decreased ($P < 0.05$) to undetectable levels by week 8 in lot 1 and in lot 2 and 3 by week 6 at all storage temperatures. No significant trend for the growth in background microflora was observed in un-inoculated controls.

Significance: The whole liquid eggs formulated with nisin evaluated in the study controlled the outgrowth of *B. weihenstephanensis* when stored at 4°C for 30 days, and 7 and 10°C for the remaining 60 days.

P1-171 Microbial Survey during the Shelf Life of Retail Non-meat Based Foods

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Introduction: The microbial communities of plant-based meat alternatives differ both in composition as well as overall abundance.

Purpose: This study investigated the microbial flora associated with plant-based meat alternatives purchased at retail.

Methods: Multiple packages from 5 different plant-based meat alterative products were purchased at local retailers and stored at refrigerated temperatures (4°C). The packages had similar lot codes. At several time points over the stated shelf life, including one time point past, 22-gram samples were analyzed for Aerobic Plate Count (APC), Lactic Acid Bacteria (LAB) and Yeast/Mold (Y/M). When possible, isolates were collected, gDNA isolated and subjected to either 16S or ITS sequencing for microbial identification.

Results: The pH for all products were similar and ranged from 6.2 to 6.8. Despite the neutral pH, yeast and mold counts were relatively low for all products tested, with the highest level reaching 3.5x10^4 CFU/g for one sample tested after its expiration. Interestingly, three products had higher levels of LAB and APC (>1x10^6 CFU/g) several days before their stated expiration dates and all three were either ground products or ground and formed patties. The two products with the lowest LAB and APC counts (<1x10^3 CFU/g), even past their expirations, were a formed breakfast patty and sausage product. This was a bit unexpected as both contained spices, unlike the ground products, which are known to be a source of microbial contamination. The 16S and ITS sequencing identified organisms often associated with other refrigerated foods including *Leuconostoc, Carnobacterium, Pseudomonas, Lactobacillus* and *Candida* spp.

Significance: The rapid growth of plant-based meat alternatives has spurred tremendous innovation in organoleptic and process technology. Understanding the microbial communities in these products is key to helping them maintain their shelf life and quality as demand and distribution of these products continues.

P1-172 Isolation and Identification of Molds on Secondary Quality Pickling Cucumbers

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Introduction: Production of value-added products from a variety of surplus vegetables by fermentation can help reduce food waste. However, little is known about the identity and fate of molds and their potential toxins in fermenting vegetables, including cucumbers.

Purpose: To isolate naturally occurring fungi from secondary quality cucumbers and determine if potential toxin-producing strains were present.

Methods: Fresh pickling cucumbers free of soil or obvious injury were placed inside sealed plastic bags and incubated at room temperature for up to 7 days. For samples exhibiting mold growth, mycelium sections (2-5 sq. mm) were excised and placed onto MEA or DRBC agar plates. Isolates were purified by at least three serial passages on agar for 5 days at room temperature. Fresh cucumbers were then stab inoculated with the isolates to reproduce spoilage. Cell morphology, sexual and asexual spores were examined using brightfield microscopy. DNA extraction and amplification of the ITS region by PCR was followed by BLAST analysis of high quality (Q > 25) sequences using the NCBI fungal sequence database.

Results: Morphologically distinct mold isolates (n = 17) from two lots of cucumbers (n = 8) were obtained. All isolates were of the phylum *Ascomycota*, and 13 were *Fusarium* spp., including seven sequence types (STs). The remaining isolates included *Galactomyces, Alternaria,* and *Acremonium* spp. For these samples, approximately two isolates per cucumber, the probability of obtaining *Fusarium* was $P = 0.94$. Microscopic examination of vegetative cell structures and spore types revealed morphological features consistent with the ITS barcoding assignments. Stab inoculation confirmed the isolates reproduced the original cucumber spoilage.

Significance: Because *Fusarium* strains were present among the isolates there is the potential for fumonisin toxin synthesis, which could persist upon further processing. Additional work will include determining the prominence of various mold species on spoiling cucumbers and how fermentation affects and biologically active toxins.

P1-173 Application of Functional Ice to Improve Microbiological Quality of Tilapia during Storage and Transportation

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❖ ***Developing Scientist Entrant***

Introduction: Seafood is a highly perishable commodity and is prone to spoilage during storage and transportation. Spoilage of seafood can lead to food waste and food loss and ultimately impact food security especially in low-income countries. There is a need to develop novel, yet practical methods to reduce spoilage during storage and transportation. Functional Ice (FICE) is an innovation over regular ice used in seafood storage that actively suppresses microorganisms during seafood storage.

Purpose: A study was conducted to validate the antimicrobial efficacy of FICE made using peracetic acid (PAA) on reducing bacteria on fish surface.

Methods: Whole, eviscerated tilapia was treated with FICE made with different concentrations of PAA (100, 200 and 300 ppm). The controls were potable water for ice preparation and rinsing of tilapia with a chlorine solution of 20 ppm. The product was stored in a 2:1 ratio (ice to tilapia) in coolers placed at room temperature. Microbiological sampling was conducted at 0, 8 16 and 24 hours (n = 96) to determine the aerobic mesophilic bacteria counts, total coliforms, *Escherichia coli* and lactic acid bacteria. A Hedonic test (scale 0 – 9) with no training panelist was developed to assess organoleptic attributes. The differences between treatments were analyzed using ANOVA with LSMeans ($P \leq 0.05$).

Results: After 24 h of storage, FICE at 200 and 300 ppm PAA concentration obtained significantly low counts ($P < 0.05$) of aerobic mesophilic bacteria (1.37, 1.27 CFU/g), total coliforms (0.20, 0.00 log CFU/g), *Escherichia coli* (0.00, 0.00 log CFU /g) and lactic acid bacteria (0.40, 0.34 log CFU/g), respectively, compared to other treatments. Sensory analysis indicated that FICE treated tilapia was "acceptable" to the panelists.

Significance: Functional ice made with peracetic acid can be used as a practical method to actively suppress spoilage microorganisms as well as reduce spoilage.

P1-174 Bacterial Communities Associated with Shrimp Decomposition during Storage at 0°C and 36°C

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Introduction: Bacterial communities associated with seafood products change under various storage conditions. Understanding how microflora changes during storage is critical in discovering biomarkers of decomposition for method development.

Purpose: The objective of this study was to quantify and identify bacteria associated with shrimp stored at 0 and 36°C. Understanding changes in bacterial communities during storage will aid in linking sensory, chemical, and microbiological data to utilize the most efficient method(s) for detection of decomposition in seafood products.

Methods: Freshly dead, beheaded shrimp were incubated at 0 and 36°C for 20 days and 12 hours, respectively. At each sampling point, every 4 days at 0°C and every 4 hours for 36°C, 10 g were blended 1:10 with sterile saline. Serial dilutions were plated onto TSA and incubated at 4°C for 2 weeks or 36°C for 48 hours, for the 0 and 36°C stored shrimp, respectively. Total bacterial counts were determined, and 48 colonies were purified for identification by 16S rDNA gene sequencing.

Results: At 0°C, total bacterial counts increased from 4.9 to 8.8 log CFU/g after 20 days. At 36°C, total bacterial counts increased from 5.4 to 8.7 log CFU/g after 12 hours. At 0°C, the initial bacterial community consisted primarily of *Psychrobacter*, *Acrobacter*, and *Planococcus* spp. By day 20, it consisted predominantly of *Shewanella* and *Pseudoalteromonas* spp. At 36°C, the initial bacterial community was highly diverse, including *Shewanella, Vibrio, Photobacterium, Psychrobacter,* and *Planococcus* spp. By hour 12, the dominate bacteria were *Vibrio* and *Photobacterium* spp.

Significance: These data suggest that bacterial communities responsible for decomposition are temperature influenced and that no single bacteria/bacterial community can be used as a decomposition indicator. Further understanding bacterial communities responsible for decomposition and their relationship to chemical indicators will help in advancing rapid and reliable methods for detection of decomposition in shrimp.

P1-175 Microscopic Studies on *Aspergillus flavus* Infection in Bambara Groundnut (*Vigna subterranea* (L.) Verdc)

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Introduction: Bambara groundnut (*Vigna subterranea* (L.) Verdc) is susceptible to fungal infection and aflatoxin contamination. *Aspergillus flavus* grows in the legume and produces aflatoxins as secondary metabolites. However, there is sparse information on the infection pattern of *A. flavus* in Bambara groundnut.

Purpose: This study aimed at understanding the infection pattern and changes in the seed structure of Bambara groundnut due to *A. flavus* infection.

Methods: Sixty healthy and previously irradiated Bambara groundnut seeds were artificially inoculated with spore suspension of aflatoxigenic *A. flavus* (2 x 10^6 spores/mL) and stored at 25 ± 2°C and 75 ± 2% relative humidity for 14 days. Samples were withdrawn at 24 h intervals for 4 days, then at 7 days and 14 days. Using scanning and transmission electron microscopy, the mode of entry of *A. flavus*, changes to the seed coat, storage cells and tissue structure due to fungal infection were studied.

Results: The seed coat of Bambara groundnut was rapidly colonized by *A. flavus* spores within 24 h of inoculation. The infection of internal tissues of the cotyledon was through the ruptured seed coat, resulting in a disruption of the cellular architecture. Cell wall collapse, the presence of cavities in parenchymatous cells and ruptured storage cells resulted from *A. flavus* infection of the seed over the storage period. All (100%) the treated seeds under study showed visible signs of *A. flavus* infection.

Significance: Information from this study will help in the development of effective post-harvest management practices to mitigate *A. flavus* infection in Bambara groundnut.

P1-176 The Mystery of Exploding Wine Bottles

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Introduction: Wine production is a growing component of the Oklahoma beverage industry. As the number of new wineries is increasing, challenges associated with wine quality are also increasing.

Purpose: This study was designed to identify the cause of undesirable gas production in Cynthiana wine from an Oklahoma winery.

Methods: A total of 14 different wine samples (9 Cynthiana and 5 Vignole) were received from the winery and stored at 4°C until analyzed. The winery indicated that two of the Cynthiana variety wines had gas production and explosion issues reported by customers. The winery had also conducted telephonic interviews with complaining customers and collected information on how wine bottles were stored and used before explosions. To identify the quality issues, wine samples were analyzed for reducing sugar, free and total SO_2, alcohol %, pH, and yeast and mold at the Oklahoma State University Analytical Lab (ISO 17025) using appropriate AOAC official methods. All experiments were repeated at least three times. Mean and standard deviations were calculated using JMP Pro 13 statistical software.

Results: It was observed that all wine samples tested contained reducing sugars levels of 33.75 to 48.75 g/L. Free SO2, total SO_2, alcohol, and pH ranged from 24 to 185 ppm, 124 to 327 ppm, 9.89 to 15.52 and 4.30 to 5.14, respectively Yeast was present at 23 CFU/ml in only one of the two wine samples implicated in the gas production and explosions. The customer interview by winery also revealed that only the partially consumed bottles stored at the ambient temperature were involved in the gas production and explosion. Based on the available information, it was concluded that the high pH and low free SO_2 made wines susceptible to cross-contamination by consumers.

Significance: Wineries producing high pH and low SO_2 containing sweet wines should consider microbial cross-contamination risk associated with consumer handling of wines.

P1-177 Visible Light Illumination by 405 NM LEDs Can Prevent Mold Spoilage of Strawberries and Tomatoes

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Introduction: Molds like *Botrytis cinerea* and *Rhizopus stolonifer* cause spoilage of strawberries and tomatoes during transportation and storage, leading to fruit wastage and compromising food security. One of the main causes of this problem is that refrigeration, despite its benefits, cannot kill molds.

Purpose: The objective of this research was to design and develop a visible light-based method that can be used to complement refrigeration to prevent mold spoilage of fruits, namely strawberries and tomatoes, and extend their shelf life.

Methods: The antifungal effect of 405 nm LEDs was first established *in vitro* by illuminating spores of *B. cinerea* and *R. stolonifer* on DRBC agar for 6 h. Subsequently, studies on fruits were conducted by spot-inoculating strawberries and tomatoes with the mold spores and illuminating them at 7°C and 60% relative humidity over a period of 72 h. The antifungal effect was then expressed as the percentage survival fraction and plotted as a function of the time.

Results: The *in vitro* trials demonstrated complete inactivation of both the species on DRBC agar, with 6 h of illumination producing an antifungal effect of 100%. On strawberries, treatment with 405 nm LEDs reduced *B. cinerea* and *R. stolonifer* by 67% and 19%, respectively, after 72 h. The corresponding antifungal effect on tomatoes was 79% and 70%. There was no appreciable difference discernible in the appearance of the illuminated and control strawberries or tomatoes. All experiments were conducted at least three times with duplicate plating.

Significance: These results demonstrate that visible light illumination with 405 nm could prevent mold spoilage of strawberries and tomatoes in a safe and chemical-free manner. The visible light technology thus shows promise to be applied in the cold chain to complement refrigeration and offset its limitations.

P1-178 An Intervention Applied to Meat Trimmings before Grinding Addressed a Recurring Spoilage and Shelf-life Problem in a Commercial Setting

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Introduction: "Meatloaf Mix" and "Chef's Blend" are popular case-ready products composed of blended ground beef, veal, and pork. When addressing a common spoilage problem in these products of package bloating it is essential to also consider effects on pathogen proliferation. Therefore, functional ingredients that inhibit growth and increase susceptibility to heat of pathogens are good candidates for shelf life extension.

Purpose: Determine the efficacy of applying Defenstat™ with and without vinegar to trim as an intervention to control spoilage.

Methods: Ground beef/pork blend packaged in chubs and ground beef/veal/pork blend packaged in modified atmosphere packaging (MAP) trays were produced from meat trimmings treated with either Defenstat™, Defenstat™ plus dry vinegar, or no treatment (Control). On days 1, 7, 15 and 25 following production the levels of aerobic and anaerobic bacteria, psychrophiles, and lactic acid bacteria (LAB), as well as *Carnobacteria*, *Leuconostoc*, *Brochothrix*, *Pseudomonas*, *Hafnia* and *Serratia* were measured, while organoleptic qualities (odor, color, gas production) were monitored. The Day 1 products were evaluated by a consumer taste panel.

Results: At Days 1, 7, and 15 both treatments reduced chub meat blend aerobic and anaerobic plate counts by 1.6 log CFU/g, and MAP meat blend counts by 1.0 log CFU/g. At Day 25 aerobic and anaerobic plate counts were 2.2 and 1.2 log CFU/g lower than controls for chub and MAP products, respectively. Specific spoilage organisms showed varying responses to the treatments by time, with *Carnobacteria*, *Hafnia,* and *Serratia* the most sensitive and *Leuconostoc* the least sensitive. Control products showed bloating and off odors on Day 7, while treatments remained unchanged through Day 25. Consumer taste panel rated treated products equal to, or preferred to controls

Significance: The treatments reduced and limited the growth of spoilage organisms and addressed problems of shortened shelf life without adversely impacting consumer taste preference.

[USDA is an EEO Employer]

P1-179 Combined Effect of Natural Polysaccharide and Citrus Oil Marinade to Extend the Shelf-life of Ready-to-Eat Deli Chicken

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Introduction: Due to the increased demand for natural strategies to control pathogenic bacteria and food spoilage, effective compounds for use as antimicrobials have been sought by food manufacturers. Ready-to-eat meats are particularly a concern since they may be consumed without further cooking or processing and are known to be highly susceptible to the growth of both spoilage and pathogenic microorganisms. Oils derived from citrus fruits alone or combined with other natural antimicrobials have proven to inhibit growth or kill foodborne human enteric pathogens.

Purpose: The purpose of this study was to evaluate the efficacy of orange oil combined with a patent pending chitosan blend infused marinade to inhibit the growth of spoilage organisms in RTE deli chicken.

Methods: Chicken samples were subjected to the following marinade treatments: 1% orange oil, 3% orange oil, 1% orange oil with chitosan blend, 3% orange oil with chitosan blend, chitosan blend alone, and control (no oil or chitosan). Samples were marinated for a total of two minutes before being placed in a Whirl-pak bag and stored at 4°C. Microbial evaluations were performed on Days 0, 3, 5 and 7.

Results: Steady growth of initial background microflora was observed in the control by Day 5 which was significantly different ($P < 0.05$) compared to all of the other treatments which remained constant. After Day 5 the treatments containing orange oil alone appeared to significantly ($P < 0.05$) stimulate growth, counts approximately 2 log CFU/g higher than the control counts by Day 7.

Significance: Results of this research provide a natural alternative that can be incorporated into a marinade to assist shelf life extension by decreasing spoilage microorganisms.

P1-181 Pine Needle (*Pinus densiflora*) Extract-mediated Synthesis of Zinc Oxide Nanoparticles and Comparative Antimicrobial and Antioxidant Activity of Agar/ZnONP-KOH with Agar/ZnONP-PN

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Introduction: Zinc oxide nanoparticles(ZnONPs) have antimicrobial activity. However, ZnONPs have to be synthesized by bio-based methods due to non-toxic, eco-friendliness, economical and safe food packaging.

Purpose: The objective of this study was to synthesize ZnO nanoparticles mediated pine needle extract and compare biodegradable agar-based ZnONP-KOH (chemical-synthesis) films with ZnONP-PN (green-synthesis).

Methods: Pine needles were extracted by hot water at 80℃ for 3 d. For the synthesis of ZnONP-KOH and ZnONP-PN, 0.221 g of Zn nitride was mixed with distilled water and diluted pine needle extract, respectively, and heated at 100℃ for 1 h. Then, 1 M KOH was mixed in the solution until neutral pH. ZnONPs were analyzed by FE-SEM, XRD and FT-IR. For the preparation of agar films, agar/ZnONPs films were made by the solution-casting method. A total of 100 g of agar/ZnONPs was added in 20 mL of broth containing active *S. aureus* and *E. coli* O157: H7 at 36℃ and the colonies were counted at the same interval times. The antioxidant activity of films was tested by DPPH and ABTS. FE-SEM, FT-IR, XRD and UV-barrier properties were analyzed.

Results: The success of the synthesis of ZnONPs and preparation of agar/ZnONPs was observed by FT-IR, FE-SEM and XRD. Although the antioxidant activity of agar/ZnONP-KOH was not shown, those of agar/ZnONP-PN exhibited 50% of DPPH and 88% of ABTS. The agar/ZnONP-KOH exhibited stronger antimicrobial activity than agar/ZnONP-PN. Although the population of *S. aureus* was not changed in agar/ZnONP-KOH, the growth as 1 log CFU/mL of *S. aureus* was observed in agar/ZnONP-PN. In *E. coli*, agar/ZnONP-KOH was shown the reduction of 1 log CFU/mL but agar/ZnONP-PN did not change the population.

Significance: Green-synthesized ZnONPs had the weaker antimicrobial activity than the chemical method, however, bio-based ZnONPs had strong antioxidant activity, not shown in chemical-synthesized ZnONPs. Therefore, ZnONP-PN presented the potential of competitive material to chemical-synthesized ZnONPs.

P1-182 Synthesis of Silver Nanoparticles Mediated Pine Needle (*Pinus densiflora*) Extract and Its Application for the Preparation of Carrageenan-based Antioxidant and Antimicrobial Films

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Introduction: Silver nanoparticles (AgNPs) have strong antimicrobial activity. However, to make non-toxic, economical, eco-friendly and safe food packaging, AgNPs should be synthesized by bio-based methods. Also, recently, biodegradable films have attracted attention because the problems of plastics have been serious.

Purpose: The objective of this study was to synthesize silver nanoparticles mediated pine needle extract and develop biodegradable functional food packaging film such as carrageenan-based nanocomposite films.

Methods: Pine needles were extracted by hot water at 80℃ for 3 d. For the synthesis of AgNP-PN, diluted pine needle extract was mixed with 0.085 g (1%), 0.17 g (2%) and 0.255 g (3%) of silver nitride, respectively, and heated at 100℃ for 90 min. AgNP-PN was analyzed by FE-SEM, XRD and FT-IR. For the preparation of Carr-AgNP-PN, 4 g of carrageenan, 1.2 g of glycerol and 30 mL of AgNP-PN were mixed in 120 mL of distilled water and heated at 80℃ for 30 min and Carr/AgNP-PN films were made by the solution-casting method. 100 g of nanocomposite films were added in 20 mL of broth containing active *S. aureus* and *E. coli* O157: H7 at 36℃ and the colonies were counted at the same interval times. The antioxidant activity of films was tested by DPPH and ABTS. The mechanical properties such as FE-SEM, FT-IR, XRD and UV-barrier were analyzed.

Results: The success of the synthesis of AgNP-PN and preparation of Carr/AgNP-PN was observed by FT-IR, FE-SEM and XRD. The composite films exhibited strong antimicrobial activity against *S. aureus* and *E. coli*. Carr/AgNP-PN$^{3\%}$ was shown the 3-log CFU/mL and 2-log CFU/mL reduction of *S. aureus* and *E. coli*, respectively. DPPH and ABTS were significantly different, and those values of Carr/AgNP-PN$^{3\%}$ were 82% and 99%, respectively. Film transparency at 280 nm significantly decreased from 74.8 ± 1.3 to 0.5 ± 0.1.

Significance: Carr/AgNP-PN was shown the stronger antimicrobial activity against gram-positive than gram-negative, the high antioxidant activity and good UV-barrier.

Poster

P1-183 Potential of Curcumin Nanoemulsion Based Coatings for Post-harvest Preservation of Fresh Grapes

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Introduction: Green grapes are highly perishable and vulnerable to contamination with foodborne pathogens. Thus, edible coatings can be applied to the fruits to protect and maintain texture, control volatile compounds, assure flavor retention, decrease respiration, as well as to control the release of antioxidant substances and nutraceuticals that can increase the shelf of fresh fruits.

Purpose: This study aimed at developing an edible coating based on food grade nanoemulsions (NEs) with potent antimicrobial activities to enhance the shelf life of green grapes, which are prone to microbial contamination and spoilage during post-harvest stage.

Methods: In this study, food grade sunflower oil NEs loaded with curcumin (5-10 mg) were prepared with two step homogenization (initially with rotor stator homogenizer at 495 g for 5 min then with high pressure homogenizer for 3 cycles at homogenizer pressure 60-120 MPa). Antimicrobial potential against *E.coli* ATCC 8739 and *Staphylococcus aureus* ATCC 25923 was evaluated by disk diffusion assay. The chemical finger printing was evaluated through Fourier transform infrared spectroscopy (FT-IR). In preservation studies, weight loss and browning index of the grape samples were evaluated for 14 days at 25°C. The results were analyzed by using ANOVA.

Results: Food grade NE with curcumin (10 mg) was most stable with a mean particle size of 88.45±4.3 nm and a zeta potential of -26.3±0.12 mV. The antibacterial activity of curcumin loaded NE was due to methylene (C-H) bend evaluated by FT-IR. There were statistically significant differences ($P < 0.05$) in the weight loss and browning index of grapes preserved without coating (39.25±6.22^{a} % and 476±17^{a}, respectively) versus with coating (27.33±4.1^{b} and 390±7.55^{b}) with higher values found for samples without coating.

Significance: Food grade NE based coatings possess unique features that favors their application to not only reduce post-harvest losses but as an alternative to non-biodegradable food packaging.

P1-184 Incorporation of Antimicrobial Bio-based Carriers onto Plastic Surface for Enhanced Antimicrobial Activity

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Introduction: Microbial cross-contamination from food contact surfaces is a potential route for the transmission of spoilage and pathogenic microorganisms to food products. There is an unmet need for improving sanitation of food contact surfaces to reduce risk of cross-contamination from farm-to-fork continuum, such as processing, preparation, and storage of food. Thus, development of an antimicrobial coating that provides a localized concentration of the antimicrobial agents on diversity of exiting food contact materials is highly desirable.

Purpose: This work demonstrates a scalable strategy for development of rechargeable antimicrobial coatings on food contact surfaces via covalent immobilization of bio-based carriers on poly(vinyl alcohol-*co*-ethylene) (PVA-co-PE) films.

Methods: To enhance effective levels of bound chlorine to the surface, yeast cell microcarriers were used as carriers for the encapsulation of food grade *N*-halamine polymer ε-Poly-L-lysine (EPL).Yeast cell wall particles based antimicrobial microcarriers were immobilized onto a PVA-*co*-PE surface by covalent bonding.

Results: The rechargeability of this coating was demonstrated by repeated recharging and quenching test. This coating can inactivate 5 logs of model pathogenic bacteria (*E. coli* O157:H7 and *L. innocua*) in wash water without and with high organic load (chemical oxygen demand = 500 mg/L) in 2 min and 20 min, respectively. This antimicrobial coating has been demonstrated to effectively reduce the cross-contamination between fresh produce and plastic films ($P < 0.01$). Furthermore, the ability of this coating to reduce the formation of *L. innocua* biofilms was also demonstrated ($P < 0.01$). The coatings developed in this study reduced the numbers of *L. innocua* cells adhered to the surface by 2.7-3.6 log CFU/cm^2 compared to native PVA-*co*-PE films.

Significance: These properties of antimicrobial *N*-halamine materials will significantly enhance the efficacy of surface sanitation, prevent the cross-contamination of fresh produce, and reduce biofilm formation on the food contact and nonfood contact surfaces.

P1-185 Evaluation of InvisiShield Antimicrobial Packaging to Reduce *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and Human Norovirus Using the Antimicrobial Chlorine Dioxide

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Introduction: Chlorine dioxide (ClO_2) has been used for many years as an antimicrobial in a variety of settings. Its use on foods has been limited as ClO_2 often produces negative organoleptic effects.

Purpose: This study had two objectives: 1) To characterize a novel ClO_2 packaging system for its efficacy against *E. coli*, *Salmonella* and *L. monocytogenes* on tomatoes and human norovirus (HNV) on stainless steel (SS); and 2) To determine changes in organoleptic properties of sliced tomatoes packaged with ClO_2 treatment.

Methods: Tomatoes were purchased commercially and aseptically sliced. Six slices per replicate were inoculated with 10 µL of a bacterial cocktail (2-3 log CFU/mL) containing five strains of either *E. coli*, *L. monocytogenes* or *Salmonella*. SS coupons were inoculated with 20 µL of a 20% stool suspension positive for HNV (GII.4 Sydney). Tomatoes or SS coupons were packaged per manufacturer's instructions using treatment or control packaging. Tomato packages were held at 7ºC for 2, 4, 7, 10 and 14 days ($n = 10$). Packages containing SS coupons were held at room temperature for 1 and 2 days ($n = 3$). For sensory analysis, 42 panelists participated in a triangle test of difference using control and treated tomatoes stored at 4ºC for 3 days.

Results: Treated tomatoes were rated as not significantly different in appearance, flavor or texture attributes compared to the control ($P < 0.05$) in the sensory analysis. Bacterial strains were not detected on the sliced tomatoes after 2 days, while controls saw an average of 0.89 log increase after 14 days. HNV showed 5.93 ± 0.05 and 5.89 ± 0.05 log reduction relative to control after 1 and 2 days, respectively.

Significance: Antimicrobial packaging systems can be used to increase the shelf life and improve the safety of food products by adding another hurdle that microorganisms must overcome. InvisiShield reduced the numbers of bacteria on tomatoes without compromising their organoleptic properties and showed initial efficacy against HNV. Future work focuses on investigating treatment efficacy against HNV on food products.

P1-187 Refining a *Listeria monocytogenes* Predictive Risk Tool for Retail Deli Departments

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a major concern for the retail food industry, specifically retail delis. Recent studies have highlighted delis departments as being a risk for environmental *L. monocytogenes* contamination. However, in-store testing for *L. monocytogenes* is not typically performed due to lack of regulatory requirement and considerable cost and liability associated with positive results.

Purpose: This on-going study was designed to validate the performance of a recently developed predictive tool based on mathematic modeling for assessing *Listeria monocytogenes* contamination risk at retail.

Methods: Forty retail deli departments located throughout the United States are enrolled to validate a previously developed logistic regression predictive risk tool. Ten food contact and non-food contact surfaces previously identified as high risk for *L. monocytogenes* contamination are swabbed in each store twice over 12 months. Whole genome sequencing is used for confirmation of *L. monocytogenes* and *Listeria* spp. Stores were screened using the *L. monocytogenes* predictive model and data will be utilized for validation. A Firth's bias-corrected logistic regression model was developed to predict the probability of a retail deli having high *L. monocytogenes* prevalence (>10%); the same regression model will be used for validation in this study.

Results: To date, 42.1% (8/19) of stores sampled resulted in at least one *L. monocytogenes* positive site, however 89.5% (17/19) of sampled stores were positive for *Listeria* spp. One store was 30% (3/10 sites) positive for *L. monocytogenes* and 50% (5/10) positive for total *Listeria* spp. Of the ten sites sampled, squeegees had the most *L. monocytogenes* positives (5/19 stores), while drains had the highest *Listeria* spp. contamination rate. The predictive model will be validated upon sampling completion.

Significance: Given the limitations of in-store sampling, a novel approach to assessing *L. monocytogenes* risk through a combination of predictive modeling and minimal sampling could be of major value to the retail food industry.

P1-188 A Survey of the Prevalence of Foodborne Pathogens on Selected Local Food Products Procured from Farmers' Markets in Central Virginia

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Introduction: The popularity of local foods is showing no sign of slowing down, as evidenced by the increasing number of farmers' markets offering a wide variety of fresh food products to customers.

Purpose: This study aimed to survey the prevalence and characterization of foodborne pathogens on selected local food products procured from farmers' markets in Central Virginia.

Methods: A total of 194 samples sold by 43 vendors at 12 registered farmers' markets in Central Virginia were obtained between March 2017 and November 2017. Bacterial isolates were tested for their susceptibility to 12 antimicrobials representing nine different categories. Pulsed-field gel electrophoresis (PFGE) profiles of the isolated *E. coli* were further explored for genomic diversity and environmental association to identify potential pathways of bacterial transmission concerning the routes of contamination.

Results: The highest aerobic mesophilic and psychrotrophic counts were observed from street corn (9.20 log CFU/g) and ground beef (9.69 CFU/g), respectively. The highest level of coliforms were found in pork sausage (2.62 log MPN/g). Detection of 24.7% *E. coli*, 17.5% *Listeria* spp., 1.5% *Campylobacter*, and 1.0% *Salmonella* were discovered in tested samples. Ampicillin, amoxicillin-clavulanic acid, and streptomycin showed the highest frequency of resistance among *Campylobacter* (100%) and *Salmonella* (100%) isolates while tetracycline showed the highest frequency of resistance in *E. coli* isolates (35%). Approximately 16.1% *E. coli*, 61.8% *Listeria*, 100% *Campylobacter,* and 100% *Salmonella* isolates exhibited resistance to three or more categories of antimicrobials, meeting criteria for multidrug resistance. Matching *E. coli* PFGE profiles obtained from different food products (pork chop and pork kidney) processed at the same facility manifested the potential of inter-transmission of the bacteria.

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.

P1-190 Cross-Contamination is a Continuous Challenge to *Listeria monocytogenes* Control in Retail Grocery Produce Environments

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❖ ***Developing Scientist Entrant***

Introduction: Our recent study found *Listeria monocytogenes* was prevalent in some but not all retail grocery produce environments. Risk factors including infrastructure designs, hygiene behaviors, and sanitation practices significantly impacted *L. monocytogenes* prevalence.

Purpose: This study evaluated the impact of a deep clean intervention on facility infrastructure, behavior management, sanitation practices, and *L. monocytogenes* prevalence by surface type in retail produce environments.

Methods: A 110-question survey on infrastructure, behavior management, and sanitation practices was distributed to 30 retail produce managers among seven U.S. states three times over 16 months. Concurrent monthly *L. monocytogenes* environmental sampling was conducted. Wilcoxon sum rank test with Tukey post-hoc test was used to identify significant factors that impacted *L. monocytogenes* prevalence ($\alpha = 0.05$). A generalized linear mixed model was used to assess longitudinal association among survey factors, deep clean, and *L. monocytogenes* prevalence by surface type.

Results: Post-deep clean, *L. monocytogenes* prevalence was significantly correlated with cross-contamination events. Higher *L. monocytogenes* prevalence on non-food contact surfaces (NFCS) was correlated with interdepartmental traffic ($P_{adj} < 0.05$) and in-house guacamole production ($P_{adj} < 0.05$), the latter of which was also significant for overall prevalence ($P_{adj} < 0.05$). Scrub brush usage during floor cleaning was found correlated strongly with higher *L. monocytogenes* prevalence on NFCS ($P_{adj} < 0.05$). More frequent cleaning of produce retail case down to the coil associated with lower *L. monocytogenes* prevalence both on NFCS and overall ($P_{adj} < 0.05$). However, higher *L. monocytogenes* prevalence correlated strongly with "issuing full wage for sick leave" compared to no sick leave wage ($P_{adj} < 0.05$, NFCS and overall), and "giving specific examples and detailed explanations during new employee orientation" compared to absence of this training method ($P_{adj} < 0.05$, NFCS).

Significance: This study highlights cross-contamination as the most significant risk factor in produce environments with high *L. monocytogenes* prevalence; interventions should focus on viable, practical strategies to reduce cross-contamination.

P1-191 Introduction of Hygiena InSITE *Salmonella* as a Rapid Method for Surface Surveillance of Stressed *Salmonella*

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Introduction: The contamination of surfaces with *Salmonella* species is one of the major routes for cross-contamination. InSITE *Salmonella* is a simple-to-use, rapid device for screening surfaces for *Salmonella* species.

Purpose: The InSITE *Salmonella* device is designed to swab a large area for possible *Salmonellae* contamination, pre-enrich and then selectively enrich in the same device giving a presumptive positive in 24 hours from sample collection. This allows better and more rapid surveillance of high-risk surfaces.

Methods: *Salmonella* Typhimurium ATCC 14028 and *Salmonella* Arizoniae ATCC 13314 were grown and diluted into diluent. Sterile stainless steel squares were inoculated with 100 µL from each dilution -1 through to -9 and air dried overnight under asepsis. Each square was swabbed with one InSITE *Salmonella* device and incubated at 6 hours in the pre-enrichment phase, a further 18 hours in the selective phase. Devices are positive if a vivid yellow color appears in the viewing window. All devices used in the study were analyzed for confirmation of positivity using the BAM *Salmonella* confirmation steps with a final identification by biochemistry. Directly inoculated devices without a drying step were performed as controls.

Results: The drying of the *Salmonella* on the stainless steel surface rendered the bacteria stressed. Dilutions -1,-2,-3 and -4 were all positive for both *S.* Typhimurium and for *S*. Arizoniae. The -5 dilution dried was also detected using the InSITE *Salmonella* device. All dilutions from the directly inoculated devices were positive. All devices were then confirmed for negativity and positivity using a confirmation protocol from BAM, the confirmation was 100% for both positives for *Salmonella* but also negative for *Salmonella* from those swabs that indicated a presumptive negative at 18 hours.

Significance: An easy to use enclosed device that will reliably indicate presumptive positive and negative *Salmonella* from surfaces has advantages to many food producers and manufacturers.

P1-192 Ability of No Rinse Food Contact Sanitizers to Impart Undesirable Flavors to Food Via Cutting Boards

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Introduction: No Rinse Food Contact Sanitizers (NRFCS) are widely used in retail food establishments, but it is not known if residual sanitizer left on cutting boards can negatively impact the flavor profile of food. Sanitizers that negatively impact food flavor could have issues in adoption, which increases food safety risk.

Purpose: The purpose of this study was to test the ability of three NRFCS to impact the flavor of prepared foods when using freshly sanitized cutting boards.

Methods: Brand new NSF® certified polypropylene cutting boards were rinsed in deionized water and then thoroughly sprayed with one of three NRFCS: chlorine (200 ppm), benzalkonium chloride (BAC; 200 ppm), or ethanol (29.4%). Water was used as a control. Wet cutting boards sat for 60 seconds and were then allowed to air dry in a fume hood. Once dry, raw whole carrots (vegetable) and cooked egg whites (protein) were prepared into uniform pieces on the cutting board. Taste testing was then performed according to ASTM E1885-18 (Standard Test Method for Sensory Analysis – Triangle Test). Method states ten correct judgements out of 18 subjects are required to obtain a 95% significance level to determine if the NRFCS impact the flavor of foods in this study.

Results: Panelists were unable to detect a difference between the carrots and egg whites prepared on treated cutting boards (i.e., ethanol, chlorine, and BAC) compared to water alone. This suggests that NRFCS in this study did not significantly impact the flavor profile of carrots or egg whites prepared on the cutting boards ($P > 0.05$).

Significance: When used according to label instructions, ethanol-, BAC-, and chlorine-based No Rinse Food Contact Sanitizers do not impart detectable flavors to foods, highlighting the importance of following label use instructions for these products.

Poster

P1-193 Characterizing Microbial Cross-Contamination on Full-sized Surfaces Using a Traditional 'Cloth and Bucket' Disinfection Method

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Introduction: Cleaning using reusable cloths submerged in buckets containing soiled water or disinfectants may contribute to spreading microorganisms and is regularly practiced in food-service, schools, and other settings.

Purpose: To characterize the degree of cross-contamination of microorganisms on full-sized surfaces using 'cloth and bucket' method with PBS or a quaternary ammonium compound-based disinfectant (QAC), with and without soil.

Methods: A laminate Surface (S1; 6 ft^2) was inoculated with 10^9 to 10^{10} of a *Listeria innocua* and *Escherichia coli* cocktail, MS2 bacteriophage, or *Bacillus cereus* spore suspension. Once the inoculum dried, S1, followed by three additional, clean Surfaces (S2 through S4) were wiped consecutively with a cloth submersed periodically in PBS, 400 ppm QAC, or 400 ppm QAC with 5% soil – three replicates each per treatment. A 1 ft^2 area of each surface was swabbed (8 samples), and bucket liquid was sampled (3 mL). Organisms were enumerated by plating on selective agar (bacteria) or by plaque assay (bacteriophage).

Results: For all Surfaces, there was cross-contamination with *L. innocua* and *E. coli* and a high concentration of bacteria was detected in the bucket with PBS. *L. innocua* and *E. coli* were not detected when using QAC, with or without soil. With PBS and QAC with soil, MS2 cross-contamination was observed through S3 and S4, respectively, with a high bacteriophage concentration detected in the bucket. MS2 was not detected when using QAC. Cross-contamination with *B. cereus* spores occurred efficiently to all Surfaces even in the presence of QAC without soil, and a high concentration of spores was detected in the bucket.

Significance: Our study suggests that the use of reusable cloths in 'cloth and bucket' systems could potentially promote cross-contamination and re-contamination of surfaces, particularly for spores. Additional work characterizing cross-contamination of viruses, and optimizing methods for direct comparisons between organisms is warranted.

P1-194 Evaluating the Performance of an Ethanol-based Sanitizing Surface Wipe Using a Newly Developed Quantitative Carrier Test Method

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Introduction: Sanitizing wipes for hard surfaces (e.g., tables) are a rapidly growing category, especially in settings involving food. However, efficacy testing of sanitizing wipes is often performed in a manner without consideration for bacterial decontamination via wiping, nor the potential for cross-contamination.

Purpose: The purpose of this study was to investigate the antimicrobial performance of an ethanol-based sanitizing wipe using a newly developed quantitative carrier test ("QCT-3").

Methods: Nine sterile stainless-steel disks (1 cm diameter) were inoculated with 10 µl (~4 log CFU) of *Staphylococcus aureus* (ATCC 6538) suspension in a soil load (bovine mucin, yeast extract and bovine serum albumin) and allowed to air dry. Disks were loaded onto a platform (30.0 x 60.5 cm) constructed to allow for immediate neutralization of disks at the desired contact time. An additional platform containing nine uninoculated disks was placed beside the test platform and was designed to quantify bacterial transfer via wiping. Wiping (PURELL Surface Alcohol Wipes, GOJO) was performed twice with constant pressure (3-4 pounds) and began from the contaminated platform to the transfer platform. After 60 seconds, disks were immediately placed in 10 mL of neutralizing solution (PBS+0.1% Tween-80 +0.1% sodium thiosulfate). Neutralized samples were serially diluted and plated onto brain heart infusion agar plates and allowed to incubate at 36 ± 1°C for 48 ± 4 hours. Three independent experiments were performed.

Results: Wiping achieved greater than 4-log CFU (>99.99%) reduction in *S. aureus* CFU, suggesting a combination of killing and removal. No transfer of *S. aureus* to clean disks was observed, suggesting most bacteria removed were inactivated or sequestered in the wipe applicator itself.

Significance: Antimicrobial wipe testing using the QCT-3 method more accurately reflects real world behaviors and efficacy than other test protocols. Using this method, the ethanol-based sanitizer wipes tested were shown to be effective against *S. aureus*.

P1-195 Survey of Microbial Contamination of Touch Screens Used by the Public in Retail Food Establishments

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Introduction: Electronic touch screen devices are widely used in establishments where food items are prepared and sold. Little is known about the occurrence of microorganisms on these surfaces.

Purpose: To determine levels of total aerobic bacteria, fecal indicators, and pathogens on electronic touch screen devices located in retail food establishments in the United States.

Methods: Sponge-Sticks (3M Corporation) were used to collect 201 surfaces samples (touch screens, restroom door handles, ATMs, credit card readers, and employee phones) from grocery and convenience stores, quick serve restaurants, and casual dining restaurants located in Atlanta (GA), Cleveland (OH), Denver (CO), and Orange County (CA). Twenty of the samples were field blanks, run as a process quality check. The entire surface area for each screen was swabbed and all surfaces sampled were measured for surface area. Total aerobic bacteria, total coliforms, and *Escherichia coli* were enumerated using culture methods. Adenoviruses, and Genogroups I and II of human noroviruses, were also quantified using real-time PCR.

Results: Self ordering kiosk touch screens averaged 3.85 log CFU total bacteria across all locations, which was not significantly different than restroom door handles (3.72 log CFU*; P* > 0.05). Coliforms were detected on 35.4% (64/181) of samples; the majority of coliform positive samples were from convenience and grocery stores (89.1%, 57/64), which were significantly more likely to be positive for coliforms (*P* < 0.05) than other locations. *E. coli* was not detected in any of the samples. Of the coliform positive samples, 22% (14/64) were positive for adenovirus, while none were positive for GI or GII norovirus.

Significance: Electronic touch screen devices become contaminated with potentially pathogenic microorganisms during use, which may increase the risk of infection for users. These results address knowledge gaps pertaining to the types and quantities of microbes present on these devices, which informs future studies and risk models.

P1-196 Microbiological Survey of Sushi Sold in Ontario

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Introduction: Consumption of sushi represents a foodborne illness risk as it is served raw and undergoes significant handling during preparation. The risks have not been evaluated for sushi sold in Ontario where its consumption is growing.

Purpose: This study assessed hygienic-sanitary quality of sushi through testing of selected pathogenic and indicator microorganisms and overall microbial composition via a molecular method.

Methods: Sushi containing raw fish ingredients (salmon, tuna, halibut, eel, trout, mackerel, red snapper, butterfish, tilapia, pollock, fish-eggs), cooked shrimp, artificial crab and/or rice, were purchased from restaurants and grocery stores across Ontario utilizing convenience sampling. Samples were tested for *Listeria monocytogenes*, *Staphylococcus aureus, Bacillus cereus, Vibrio* sp., *Escherichia coli,* coliforms, and aerobic mesophilic counts, following ISO/IEC17025 laboratory standards. A subset of samples were also analyzed for overall microbial composition based on PCR and sequencing of the 16S rRNA gene using MiSeq.

Results: Of 250 samples tested, 4.4% contained *L. monocytogenes* with contamination levels below 2 log CFU/g and 0.8% were positive for *V. cholera*e. Coagulase-positive Staphylococci were found in 0.8% samples (1 to >2 log CFU/g). *B. cereus* was found in 4.9% of samples (1 to >4 log CFU/g). *E. coli* was found in 2.4% of samples (1 to 3 log CFU/g) and total coliform counts ranged from 1 to >5 log CFU/g. Aerobic mesophilic counts ranged from 1 to >7 log CFU/g with 12.4% of the samples being >6 log CFU/g. Unpaired MiSeq analysis of 30 samples revealed the presence of presumptive *Salmonella* and *L. monocytogenes* in two separate samples, and two additional samples with multiple presumptive pathogens (*Campylobacter*, *Salmonella* and *Listeria*), which were undetected through the standard testing approach.

Significance: This survey fills an important data gap on the microbiological quality of sushi sold in Ontario, supporting efforts for mitigating risks associated with this complex food type.

P1-197 Assessing Brazilian Food Establishments' Hygienic Handling of Leafy Vegetables and Their Microbiological Quality

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Introduction: All food establishments including vegetables in their preparations should adopt good practice procedures to ensure the proper hygiene of leaves and, consequently, to offer safer food to consumers.

Purpose: This study aimed to gather information about Brazilian food establishments' hygienic handling of leafy vegetables and to evaluate whether their microbiological quality meets the current legislation.

Methods: A total of ten food establishments located in the city of Piracicaba, SP - Brazil, were visited and a 14-question survey was applied, addressing the origin of the vegetables, washing steps, temperature conditions during storage and exposure. In addition, 30 samples of leafy vegetables were collected and

analyzed for total coliforms and coliforms at 45°C using the standard MPN method. Samples were also tested for *Salmonella* spp. using the ISO 6579:2002 method.

Results: Of all ten food establishments, five obtained leafy vegetables from farms and the other five from suppliers. Pre-washing was performed by eight establishments. Disinfection was carried out in all them through immersion of vegetables in chlorinated water (mean of 40 mg/L), of which eight performed rinsing. Washed vegetables were stored under refrigeration in seven establishments. Among those establishments in which vegetables were available as self-service meals (n = 8), four included a refrigerated counter to display them. The mean counts of total coliforms and coliforms at 45°C were 2.5 ± 0.7 and 0.9 log MPN/g, respectively. None of the samples was positive for *Salmonella*, in accordance with what is established by the Brazilian Surveillance Agency for fresh vegetables.

Significance: These data can contribute to a better understanding of the surveyed Brazilian food establishments' hygienic handling of leafy vegetables. This is important to support the implementation of proper control measures to ensure the safety of these foods.

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P1-198 Understanding Supply-chain Food Safety Vulnerability of Foods in Model Meal Kit Delivery Boxes

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Introduction: Recently, the demand for online meal kit delivery has significantly increased. These kits consist of ingredients including meats that are pre-portioned for the customer's convenience and are kept cold using ice packs and some insulation inside the box. The kits are then transported from the packing location to the consumer mostly on non-refrigerated trucks and may take up to 48 h to be delivered leading to food safety risks.

Purpose: To investigate temperature profiles of various foods inside a model meal kit delivery box exposed to simulated isothermal conditions.

Methods: Model meal kit boxes were reconstructed to represent a typical meal kit delivery box. Each kit consisted of a cardboard box lined with a shredded-paper insulating material, four ice-packs raw ground chicken (454 g), whole breast fillet (360 g), a cardboard partition, two ingredient packs and temperature probes. The boxes were placed in an incubator set at 26°C or 37°C for 48 h. Temperature data (3 trials) was analyzed using polynomial regression.

Results: Chicken breast crossed 4°C after 6.25 hours in the 26°C incubator, and 3.3 hours in the 37°C incubator. The temperature profile of chicken breast can be described by the second order equations y = 2E-06x2 - 0.0025x + 3.8103 and y = 4E-06x2 + 0.0006x + 2.7908, respectively. Ground chicken surpassed 4°C after 27 hours at 26°C and 8.5 hours at 37°C. The third order equation y = -3E-09x3 + 2E-05x2 - 0.0121x + 4.0205 explains the ground chicken temperature profile at 26°C conditions, and the second order equation y = 3E-06x2 -0.005x + 4.161 describes the temperature curve at 37°C.

Significance: Temperature abuse during supply-chain of the meal kit delivery systems can increase food safety risks. Companies can design experimental models to predict temperature changes and improve food safety of the meal kit delivery system.

P1-199 Survey of Locally Small Produce Growers' Perception of Antibiotic-resistance Issues at Farmers Markets

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Introduction: Farmers markets (FM) meet consumers' desire for locally-grown fresh produce. Antibiotic resistance (AR) has been identified in bacteria isolated from fresh produce from local FMs.

Purpose: This study was to determine local small produce growers' awareness and attitude toward AR risks from fresh produce sold at FMs.

Methods: Surveys were conducted (face-to-face questionnaire interview) at 2 FMs in WV, 1 FM in Detroit, and 1 FM in western PA from August to November in 2019. Questions included basic information of age, gender, and education level, awareness of AR risks, concerns, the same field rotated for produce and livestock, type of fertilizers, source of irrigation water, and interest to take AR training. Data were analyzed using R-software to compare the difference between locations. Chi-square tests of independence were employed to examine bivariate relationships between categorical variables (P = 0.05).

Results: Survey response rate was 20% (40/200, 42.5% males and 57.5% females) and no difference (P > 0.05) in answers to each question was found among various locations. A total of 87% of participants heard AR-risk and 60% believed that the issue was caused by use of antibiotics in human health vs 40% thought it was caused by AR-bacteria on produce; 60% used plant compost vs 25% used chemicals as farm/garden fertilizers (P < 0.05); 45% used rain water vs only 27.5% used municipal water for irrigation (P < 0.05). Fifty-five percent believed government vs 12.5% for vendors is responsible for AR-risk (P < 0.05). Additionally, 82.5% never converted to growing produce in the same fields in which was previously raising livestock while 7.5% did this (P < 0.05). Only 12.5% participants were interested in attending an AR prevention training in addition to GAP/FSMA.

Significance: The survey data will help identify risk factors for AR when combined with research findings and allows local state government agencies to make better-informed decisions regarding food safety policies related to AR risks.

P1-200 Comparison of Sanitary Management Status in Community Child Centers with or without Sanitary Guidance Visits by Dietitians

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Introduction: Community Child Centers (CCCs) are welfare service operations established by the Ministry of Health and Welfare to extend cares and provide meals to children of poor families to grow in a healthy and happy environment.

Purpose: It is a growing trend in local governments that dietitians of CCFSM (Center for Children's Foodservice Management) visit Community Child Centers to perform sanitary guidance visits. Therefore, this study was performed to identify the effect of sanitary guidance visits in CCCs by comparing sanitary management status by such visits.

Methods: By conducting a survey on the status of sanitary management, by e-mail and post mail, from August – December 2017, with managers at 253 CCCs in Gyeongnam province, 159 (62.8%) returned their responses. Survey questionnaire were composed identically of the check list (6 areas with 30 items) used by CCFSM dietitians in their sanitary guidance visit, and the answers were to respond by Likert 5-point scale. Chi-squared test and t-test have been applied by SPSS 23.0.

Results: CCCs with sanitary guidance visits by CCFSM dietitians (Supported) were 44 centers (27.7%) while Non-supported were 115 centers (72.3%). Total average points for the 30 questionnaire items showed that Supported (4.69±0.25) marked substantially higher scores than Non-supported (4.38±0.29) and performed better sanitary management (P < 0.001). From all of the 6 areas, average scores at Supported marked substantially higher scores: P < 0.001 at all

areas of 'Environment & facility', 'Personal hygiene', 'Raw material', 'Process management', 'Storage management', and 'Other matters'. Even in the 'Vegetables not heated are washed and disinfected thoroughly" which was the lowest marked item at the Supported (4.05±0.91) has shown a substantial difference (P < 0.001) from the score of the Non-supported (3.25±0.85).

Significance: To enhance sanitary management level at CCCs, all CCCs are recommended to register at CCFSM.

P1-201 Effects of Disinfection on Raw Vegetables and Fruits Not Heated in Children's Foodservices

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Introduction: Since fruit and vegetables going through cultivation and distribution can increase the level of microbial contamination, microbial reduction measures are required in non-heating cooking at foodservices for children with weak immunity.

Purpose: The purpose of this study is to experiment on the effectiveness of various methods of cleaning and sterilizing raw fruit and vegetables among the CCPs developed for children's foodservices in Korea to confirm the most effective method.

Methods: After taking 10 grams of each raw vegetable of six kinds (lettuce, cucumber, cherry tomato, paprika, apple, pepper) in the state of non-washed, washed with water, washed with chlorine (100 ppm, after five minutes soaking and rinsed), washed with baking soda (1 percent solution, after 5 minutes soaking and rinsed) and washed with vinegar (2 percent solution, after 5 minutes soaking and rinsed), measured APC and coliform counts. Diluted the homogenized sample solutions (APC: 100~10-5, coliform:100~10-3), performed pour plating method three times in repetition by distributing 1 mL of the solution on Petri dishes in duplicate and cultivated them. The results were statistically analyzed by Excel Xlstat.

Results: APC results in lettuce for 'washed in chlorine (<30 CFU/g)' was substantially lower (P < 0.01) comparing with non-washed (6.61±0.48 log CFU/g), washed with water (6.61±0.48 log CFU/g), washed with baking soda (4.96±0.21 log CFU/g), washed with vinegar (5.30 ±0.61 log CFU/g). Other vegetables and fruit have not shown effective sterilization difference as their microbiological quality were good from the state of non-washed. As 'washed with chlorine' turned out to be the most effective sterilization method, it was suggested for HACCP plans in sterilizing raw vegetables.

Significance: Still some of the Korean children's foodservices use baking soda of vinegars to sterilize raw vegetables, it has been verified through this experiment that sterilization by chlorine is the most effective method. Therefore, regardless of whether applying HACCP or not, sterilization by chlorine shall be recommended to be used widely.

P1-202 Good Manufacturing Practices and Microbiological Quality in Cafeterias of the School Meal Program in Santiago, Chile

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Introduction: The Chilean school meal program provides food to students in low-income schools. The Ministry of Education oversees the program, but private companies run the program in school cafeterias. Good Manufacturing Practices (GMP) are mandated in Chile through the Chilean Food Hygiene Regulation (RSA), and it regulates the minimum food safety conditions for all food facilities. Compliance level of school cafeterias to GMP, microbiological quality of foods, or cleanliness of kitchen surfaces are unknown.

Purpose: To determine GMP compliance of school cafeterias in the National School meal program and the microbiological quality of their food and surfaces.

Methods: In Santiago, Chile, 25 school cafeterias were visited from September-December 2019. A checklist of 104 questions was applied, including sections (9) such as "Environmental control" (premises), "personnel practices," "waste management," among others. Compliance levels were: suitable (>81% compliance), fair (60-80%), and critical (<59%). One food sample and 6 food contact surface samples were taken for microbiological testing from each cafeteria. Samples were analyzed following BAM methodologies, and RSA criteria determined microbiological compliance with Chilean regulations.

Results: Most cafeterias did not meet basic GMP levels; 5 schools were classified as "fair" and 20 were "critical." "Ingredients and warehousing" (99%) had the best compliance and the lowest was for "waste management" (23.2%). Only 3% (5/150) surfaces met limits for microbiological parameters of cleanliness (APC, *Enterobacteriaceae*, *E. coli*). From food samples (salads, n = 13; main dish, n = 6; and desert, n = 6), only salads exceeded acceptable microbiological parameters: 46% (6/13) exceeded the limits for APC and/or *Enterobacteriaceae*. Fortunately, food samples were free of foodborne pathogens.

Significance: School cafeterias need to improve GMP compliance and cleanliness in their kitchens to reduce the risk of food poisoning. A nationwide study would provide more information to help to address the problem.

P1-203 Safety and Regulatory Implications of Clean Label: Stats, Trends, Challenges and Lessons Learned

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Introduction: The Clean Label movement is fundamentally changing how some foods are produced, handled, and consumed and as a result questioning the relevance and validity of some regulations and food safety guidelines.

Purpose: Identify what matters most for safety and shelf life in the method of production and formulation for clean label food products.

Methods: This presentation will review published and unpublished data along with case studies on the shelf life and safety of clean label products to identify relationships with emerging types of food ingredients and novel food processing technologies against the backdrop of prevailing food safety regulations and guidelines.

Results: Quantitative and qualitative data from the food manufacturing sector will illustrate the importance of defining what constitutes a Clean Label product or process in terms relevant to shelf life and food safety. This presentation will provide a practical, science-based pathway for categorizing Clean Label ingredients and food processing technologies for a systematic safety monitoring and eventually, regulatory oversight.

Significance: Understanding the fundamentals of Clean Label formulation, reformulation, and processing in terms relevant to categorization, shelf life and food safety will not only be reassuring to consumers and the industry but also help regulatory agencies with a framework for oversight and possibly, certification to help level the playing field.

P1-204 A Study of the Freshness of Scallops

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Introduction: Scallops are widely consumed globally and thus, it is important to preserve their quality. The K value, determined by the amount of inosinic acid (IMP), is a common seafood quality index, but it may not be a reliable indicator of scallop quality.

Purpose: The purpose of this study was to investigate the effects of various factors on scallop quality and IMP-degrading enzyme activity and clarify the cause of the rapid increase in K value in scallops.

Methods: Scallops were homogenized in twice the volume of ultrapure water. Homogenates were dialyzed against cold water at 4°C for 2 days and the resulting dialysate solution was diluted with a crude enzyme solution containing 0.5 mL of 25 mM IMP, 2.4 mL of buffer (pH 6.0-7.6; 0.2 M maleic acid/0.2 M Tris/0.2 M NaOH), 0.1 mL of 50 mM $MgCl_2$, 50 mM $MgSO_4$, 20 μM inosine (HxR), 20 μM hypoxanthine (Hx), and 0.1 mL of water. One milliliter of the enzyme solution was added to the reaction mixture and incubated at 30℃ for 24 hours, after which 1 mL of 10% perchloric acid was added and the precipitate was removed by centrifugation (12,000 rpm, 7 min). Free phosphoric acid levels, which reflect enzyme activity, were determined using the molybdenum blue method.

Results: The change in pH and increased HxR and Hx production, caused by changes in scallop quality, did not affect IMPase activity. The IMPase activity of control scallops was 25.0 ng/g/min at pH 6.4, whereas the addition of Mg^{2+}, which is produced with changes in scallop quality, increased IMPase activity to 106 ng/g/min with $MgCl_2$ and 294 ng/g/min with $MgSO_4$ (both, $P < 0.05$ versus control).

Significance: These results suggest that Mg^{2+}, produced by changes in scallop quality, may activate scallop IMPase and cause a rapid increase in K value.

P1-205 Effect of Soy Sauce Pickling on Taste Components of Tuna

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Introduction: Tuna consumption is popular globally. The taste component of seafood is due to inosinic acid (IMP), which is degraded by IMPase. Therefore, it is necessary to suppress IMPase activity, to maintain umami. Tuna is typically stored in salt and soy sauce, in addition to general cold storage. Salt and low temperatures are known to inhibit IMPase activity, but there is no detailed report on the effects of soy sauce pickling.

Purpose: The purpose of this study was to investigate the effect of soy sauce on IMPase activity and the preservation of bluefin tuna.

Methods: Bluefin tuna flesh was homogenized with ultrapure water and dialyzed for 2 days. The dialysate (1,000 μL) was added to a solution containing 500 μL of soy sauce, 500 μL of 25 mM IMP, and 2.0 mL of reaction buffer and incubated at 30℃ for 24 hours. The reaction was stopped by adding 1.0 mL of 10% perchloric acid and the precipitate was removed by centrifugation. Free phosphoric acid levels, which reflect enzyme activity, were determined using the molybdenum blue method.

Results: Soy sauce inhibited IMPase activity in bluefin tuna to a greater extent than saline, when used at the same concentration ($P < 0.05$). The melanoidin substance formed by the Maillard reaction between reducing sugars and the amino compounds in soy sauce inhibited IMPase activity ($P < 0.05$). Moreover, a soy sauce seasoning mixture consisting of soy sauce, cooking liquor, and sweet sake, which is used for pickling, significantly inhibited IMPase activity in bluefin tuna ($P < 0.05$).

Significance: Soy sauce and a commonly used soy sauce seasoning solution were shown to be effective at preserving bluefin tuna, due to the effects of salt and melanoidin present in soy sauce.

P1-206 Effect of Slurry Ice and Flake Ice Preservation Techniques on the Microbial and Physicochemical Properties of Black Drum (*Pogonias cromis*)

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Introduction: Soon after seafood is harvested, bio-deterioration starts if left unpreserved. The loss of physical/chemical characteristics creates huge economic loss for fish harvesters. The use of slurry ice as an emerging technique in seafood preservation presents promising results compared to conventional seafood chilling techniques like flake ice.

Purpose: Evaluate the benefits of using slurry ice as a chilling method in comparison with flake ice for seafood in the Gulf of Mexico.

Methods: Thirty-six (36) freshly caught black drums of equal size were collected from boats within 12 hours of harvesting. Fish was separated into two groups and stored in insulated containers for 18 days. One group was stored in flake ice (FI) in an ice-fish ratio of 2:1. The second group was stored in slurry ice (SI) in an ice-fish ratio of 3:1. The fish temperature was monitored continuously. Fish fillets were analyzed for microbial stability and physical/chemical quality at 2-day intervals. Mean separation were analyzed using ANOVA and Tukey's studentized range test at $\alpha = 0.05$.

Results: The average temperature for FI and SI treated samples ranged from -1.57°C to -3.30°C, and -2.89°C to -5.03°C, respectively. Aerobic plate count for FI samples reached 6.4±0.17 log CFU/g at day 12, while SI never exceeded 5.1±0.88 log CFU/g. Changes in color, pH, and texture showed no significant difference ($P > 0.05$) between treatments. SI allows for better storage of the fish, keeping bacterial load lower than FI. This feature was, however, not enough to guarantee physical/chemical quality for a longer period.

Significance: Findings present fish harvesters with a cost-effective alternative for fish preservation to minimize post-harvest loss of product, amidst information on the properties of the preserved product.

P1-207 Reduction of Fecal Coliforms and Male-specific Coliphage after Chlorine and Ultraviolet Disinfection during Wastewater Treatment

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Introduction: Shellfish growing areas may be affected by sanitary conditions at wastewater treatment plant (WWTP) point source discharge, prompting either an open or closed status. Fecal coliforms (FC) are the regulatory standard used to determine the classification of a growing area. Recently, male-specific coliphage (MSC) has been proposed as an alternative indicator for sanitary quality.

Purpose: To evaluate the efficiencies for reduction of indicator organisms WWTPs using different methods of disinfection; and determine the impact of these discharges by monitoring sentinel oysters.

Methods: FC and MSC were enumerated from influent and final effluent of two secondary activated sludge WWTPs: one plant used chlorine while the other used UV light disinfection. Sentinel oysters were deployed, at thirteen different occasions over the course of a year, for two weeks near the outfall of each WWTP. FCs were enumerated in oysters using APHA MPN and in sewage using mTEC membrane filtration. A double agar overlay was used to enumerate MSC in sewage and oysters.

Results: While each treatment plant was effective in reducing FC levels (>99%), UV disinfection had a consistently higher reduction rate than chlorine disinfection. MSC levels in sewage ($n = 13$) were reduced to a greater extent by the UV treatment (100%) than by chlorine treatment (77-99%). FC levels in sentinel oysters ($n = 12$) near the chlorine plant (<20 to 5,400 MPN/g) were greater than those near the UV plant (<20 to 230 MPN/g). Similarly, MSC levels in oysters ($n = 13$) near the chlorine plant (12 to 3,353 PFU/100 g) were greater than those near the UV plant (<10 to 12 PFU/100 g).

Significance: Irrespective of size and location of the two types of treatment plants, the UV method of wastewater treatment resulted in a greater reduction of both indicator organisms, reflected by lower levels of both FC and MSC in the sentinel shellfish.

P1-210 Quantitative Microbial Risk Assessment for Highly Pathogenic *Vibrio* spp. in Sea Squirt in Korea

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Introduction: As sea temperature rises, the number of foodborne illness caused by raw seafood intake increases. Although highly pathogenic *Vibrio* spp. (*Vibrio cholerae* and *Vibrio vulnificus*) cause the foodborne illness through raw seafood intake, there is no microbial risk assessment for sea squirt in Korea.

Purpose: This study evaluated the risk of foodborne illness by highly pathogenic *Vibrio* spp. through sea squirt consumption.

Methods: For exposure assessment, the prevalence of *V. cholerae* and *V. vulnificus* on sea squirt was evaluated, and predictive models were developed. Distribution temperature and time data were collected by a survey. Consumption amount and frequency for the sea squirt were obtained by a survey. The prevalence data for *V. cholerae* and *V. vulnificus*, distribution temperature, time, consumption amount, and frequency data were fitted with @RISK fitting program to obtain appropriate probabilistic distributions. Dose-response models for *V. cholerae* and *V. vulnificus* were searched for in the literature. Eventually, simulation models were prepared using collected data in @RISK to estimate the risk of pathogenic *Vibrio* spp. through sea squirt consumption.

Results: Initial contamination levels of *V. vulnificus* and *V. cholerae* in sea squirt were -3.7 log CFU/g and -3.2 log CFU/g, respectively. The developed predictive models with the Baranyi model (primary model) and polynomial model (secondary model) were appropriate to describe the fates of the *Vibrio* spp. The consumption frequency of sea squirt was 0.26%, and the daily consumption amount was 68.84 g per person. Beta-Poisson model [$P=1-(1+Dose/\beta)^{-\alpha}$] was selected for dose-response. The simulation with these data series showed that the risk of *V. cholerae* and *V. vulnificus* foodborne illness per person per day from raw sea squirt consumption were 1.02×10^{-12} and 2.66×10^{-15}, respectively.

Significance: This result indicates that highly pathogenic *Vibrio* spp. in sea squirt could be considered low in Korea.

P1-211 Risk Assessment of Highly Pathogenic *Vibrio* spp. (*Vibrio vulnificus* and *Vibrio cholerae)* in Gizzard Shad

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Introduction: Highly pathogenic *Vibrio* spp. (*Vibrio vulnificus* and *Vibrio cholerae*) can be contaminated in aquatic products. Gizzard shad is usually consumed raw in Korea, and thus, there is a possibility for their contamination in gizzard shad from the aquatic environment, but the microbial risk assessment has not been conducted.

Purpose: This study evaluated the risk of highly pathogenic *Vibrio* spp. by raw gizzard shad consumption.

Methods: Two hundred twelve gizzard shad samples were collected from fishery markets and restaurants to detect *V. vulnificus* or *V. cholerae* by most probable number (MPN)-PCR assay. A predictive model was developed to describe the kinetic behavior of the *Vibrio* spp. at 7-25°C. Distribution time and temperature for gizzard shad were collected. Consumption amounts and frequency for gizzard shad were investigated, and dose-response models for *V. vulnificus* and *V. cholerae* were searched. Simulation models were developed with collected data to estimate probabilities of *V. vulnificus* and *V. cholerae* foodborne illness in @RISK.

Results: The initial contamination level of *V. vulnificus* was estimated as -3.5 log CFU/g by Beta distribution (2,212). Also, the initial contamination level of *V. cholerae* was estimated as -3.3 log CFU/g by Beta distribution (3,211). The developed predictive model showed *Vibrio* spp. growth. Pert distribution (0,4,72) and ExtValueMin distribution (15.8904,2.8188) showed that the mean time and temperature were 14.7 h and14.3°C, respectively. Pert distribution showed that average consumption amount was 70.32 g at 0.1% of frequency. The simulation showed that the risks of foodborne illness caused by *V. vulnificus* and *V. cholerae* by raw gizzard shad consumption were 8.40×10^{-11} and 2.43×10^{-10}, respectively. Therefore, the risk of pathogenic *Vibrio* was low in gizzard shad.

Significance: These results should be useful in evaluating the risks for highly pathogenic *Vibrio* foodborne illness by raw gizzard shad consumption.

P1-212 Quantitative Microbial Risk Assessment for Highly Pathogenic *Vibrio* spp. (*Vibrio cholerae* and *Vibrio vulnificus*) in *Ganjang-gejang*, Soy Sauce-marinated Raw Blue Crab

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Introduction: *Ganjang-gejang* is a Korean traditional dish, made with raw fresh blue crab by marination with soy sauce and spices. Because the food is consumed after the marination without additional heating, there is a possibility for highly pathogenic *Vibrio* spp. infection to consumers.

Purpose: This study estimated the risk of foodborne illness by highly pathogenic *Vibrio* spp. (*V. vulnificus* and *V. cholerae*) through the *Ganjang-gejang* consumption in Korea.

Methods: One hundred eighty-two blue crabs and *Ganjgang-gejang* were collected from the west coast, east coast, south coast, and Seoul in Korea to detect *V. vulnificus* and *V. cholerae*. Predicted models were developed to describe fates of *Vibrio* spp. in *Ganjang-gejang* with the cell counts data at 4 to 20°C. Temperature and time data were collected from fish market, supermarket, and restaurants. Consumption amounts and frequency were surveyed. To evaluate dose response, models were searched. With these data, simulation models were prepared in @RISK to estimate the probabilities of the foodborne illness.

Results: The initial contamination levels were estimated to be -3.9 log CFU/g by Beta distribution (1,183) for *V. vulnificus* and -3.3 log CFU/g by Beta distribution (3,181) for *V. cholerae*. The predictive models showed the *Vibrio* cell counts in *Ganjang-gejang* decreased at average storage temperature (5°C), determined by Log-Logistic distribution (1.7156,2.8187,3.6117,RiskTruncate(2,14)). The average consumption amount was 79.81 g, shown by Log-Logistic distribution (0.11813,13.616,1.1774) at 0.91% of frequency. Beta-Poisson model[$P=1+Dose/\beta)^{-\alpha}$] was used for dose-response models (*V. vulnificus*: $\alpha=9.3\times10^{-6}$, $\beta=1.1\times10^{5}$; *V. cholerae*: $\alpha=1.31\times10^{-1}$, $\beta=1.49\times10^{7}$). The simulation with the collected data showed the probabilities of the foodborne illness were 5.90×10^{-15} for *V. cholerae* and 1.57×10^{-17} for *V. vulnificus*.

Significance: The result indicates that the risk of highly pathogenic *Vibrio* spp. by *Ganjang-gejang* consumption is low in Korea.

P1-214 Study of the Survival of *Vibrio parahaemolyticus* in the Stomach Compartment in a Simulator of the Human Intestinal Microbial Ecosystem (SHIME), in Presence of Food

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Introduction: *Vibrio parahaemolyticus* (*Vp*) is the leading cause of bacterial gastroenteritis linked to the consumption of raw or undercooked seafood worldwide. Despite being poorly tolerant of acidic environments, *Vp* can overcome the stomach barrier and reach the intestinal mucosa, causing diarrheal disease.

Purpose: To evaluate the survival of four clinical isolates of *Vp* after 2 h in the stomach compartment of a dynamic *in vitro* simulation platform (SHIME - Simulator of the Human Intestinal Microbial Ecosystem), in the presence of oysters.

Methods: The SHIME reactors contents were: prepared oyster juice (OJ, pH ~ 6.2), mixed with a simulated gastric fluid (pH 2.0) in a final ratio of 1:1. The four *Vp* strains were individually inoculated in each bioreactor from 5.9 to 7.0 log CFU/mL and initial and final pH values were set as 5.5 and 4.0, respectively. SHIME samples were collected at different time points (0, 60 and 120 min) and plated on LB agar with 3% sodium chloride. Experiments were repeated twice, using two different batches of OJ, totaling 4 SHIME runs for each *Vibrio* strain.

Results: Differences were observed in the growth of the *Vp* strains, ranging from -0.5 to + 0.5 log CFU/mL, after 60 min. The final pH (4.0) was reached after 90 min of run time, and at the end of the experiment (120 min), a reduction in numbers of *Vp* ranging from 1.0 to 1.8 log CFU/mL was observed.

Significance: Although it is known that food can aid in the protection of pathogens during their passage through the stomach, the recovery of greater than 4.5 log CFU/mL of *Vp,* after prolonged exposure to acid stress, may be indicative of a positive correlation between acid resistance and virulence, since all the *Vibrio* strains studied were isolated from clinical cases.

P1-215 Evaluation of the BAX® System Real-time PCR Assay for *Vibrio* for the Detection of *Vibrio cholera*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus* in Raw Seafood Products

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Introduction: *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus* are human pathogens commonly found in raw seafood and the number of infections due to *Vibrio* spp. has shown an upward trend internationally due to the culture of consuming raw seafood. The rapid detection of these pathogens is important both for the food industry and regulatory authorities.

Purpose: The performance of the BAX® System Real-Time PCR Assay for the detection of *Vibrio* species was compared to the Canadian culture-based reference method MFLP-37 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: Paired samples consisting of raw fish and crustaceans (crab meat, kingfish, shrimp, tuna) and mollusks (oysters, clams, mussels and scallops) were each inoculated with *V. cholerae*, *V. vulnificus*, or *V. parahaemolyticus* at three levels: 20 samples at a level (L_1) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2) at approximately 10 times L_1, and 5 un-inoculated samples. Samples (50g) were enriched in 450 mL of APW broth and incubated at 35°C for 16-20 h and tested by the alternative and reference methods. Regardless of the analytical outcome, all enrichments were streaked onto TCBS and CHROMagar *Vibrio* (incubated 18-24 h, 35°C), and mCPC agar (incubated 18-24 h, 40°C) with further confirmation of suspect colonies by biochemical tests. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was also used as a rapid method for the rapid identification of suspect colonies.

Results: Collectively from the analysis of 270 paired samples, a probability of detection (POD) statistical model determined the alternative method exceeded the criteria outlined by the MMC obtaining 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: This Alternative assay is a suitable method for detecting *V. cholerae, V. vulnificus, or V. parahaemolyticus* in raw fish, crustaceans and mollusks after 16-20 hours of incubation, thereby significantly reducing presumptive reporting times over the reference method.

P1-216 Detection of Human Norovirus and Other Human Enteric Viruses in Sanaga Clams, Cameroon.

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Introduction: Since clams are filter feeding organisms, they can accumulate microorganisms that may be pathogenic for consumers, such as human enteric viruses that are responsible for 20% of foodborne outbreaks. Despite the high endemicity of diarrheal and hepatic diseases in Central Africa, no study has investigated the presence of human enteric viruses in wild clams harvested in this part of the world, that represent an important food source for local populations.

Purpose: The purpose of this work was to evaluate the prevalence of human noroviruses and others human enteric viruses in Cameroonian clams.

Methods: Twenty-five samples of 10 to 15 clams were collected from the Sanaga River in Cameroon at different periods of the year. After digestion of the digestive tissues with proteinase K and viral lysis with guanidium thiocyanate, nucleic acids (RNA) were extracted using a magnetic ramp. Norovirus GI and GII (NoV GI, NoV GII), Hepatitis A and E viruses (HAV, HEV), Rotavirus (RV), Sapovirus (SV) and Astrovirus (AV) were quantified using rRT-PCR with standard curves. The Mengo virus was used as an extraction control and synthetic RNA as rRT-PCR external control. NoV GI, GII and HAV genotyping was done by RT-PCR, cloning and sequencing of partial capsid gene regions.

Results: A total of 68% of samples were contaminated with NoV GI, GII, HAV, AV and/or EV, while RV, HEV and SV were absent. HAV showed the highest prevalence (52%), followed by NoV GI (32%) and NoV GII (24%), with respective concentrations ranges between 1 – 2.5; 0.2 – 3.9 and 0.3 – 2.4 log RNA copies/g of digestive tissues. Genotyping identified NoV GI.2; GII.3, GII.4_Sydney 2012, GII.6 for NoV; and G.IA, G.IIIB for HAV.

Significance: These results suggest that Cameroonian clams can accumulate some human enteric viruses as observed for other shellfish species. It also highlights that they can cause viral food poisoning in consumers if they are not properly treated.

P1-217 Microbiological Quality and *Salmonella* Prevalence in Catfish from Small Louisiana Wild-caught Catfish Processors

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❖ ***Developing Scientist Entrant***

Introduction: Since 2017, the US Department of Agriculture (USDA) started regulating fish products from the order Siluriformes. USDA is conducting research to stablish a baseline of *Salmonella* spp. in catfish.

Purpose: Determine microbiological quality and *Salmonella* spp. prevalence in catfish harvested from small Louisiana wild-caught catfish processors.

Methods: Catfish samples were collected monthly from nine wild-caught catfish facilities within 24 hours after processing. Raw catfish fillets were evaluated for aerobic mesophilic bacteria (AB), total coliforms, *E. coli*, *Staphylococcus aureus* and *Salmonella* spp. Samples were plated in duplicates. For *Salmonella* spp., USDA methodology was followed. *Salmonella* spp. suspect colonies were confirmed by PCR and sequenced by the full length 16s method.

Results: During 2018 and 2019, 489 samples were collected from nine wild-caught catfish facilities located in Louisiana from the *northeast* (one), *central* (three), *southwest* (three), and *southeast* (two) regions. The counts for APC, *E. coli*, total coliforms, and *Staphylococcus aureus* were 5.01±0.70, 0.58±0.89, 2.16±0.77, and 0.73±1.02 log CFU/g, respectively. For AB, *E. coli,* total coliforms and *Staphylococcus aureus* no significant difference was found during the two years of catfish collection. However, significant differences (P<0.05) were found by region having the highest counts of AB and total coliforms in the *northeast* region; and for *Staphylococcus aureus* in the *southeas*t region. From the 489 samples, 33 (6.75%) samples were suspected positive for *Salmonella* spp., from those samples 26 (5.31%) were confirmed as *Salmonella* spp.

Significance: The findings of the study showed that the microbiological load in catfish fillets from small wild-caught catfish facilities in Louisiana is low, in addition to a low prevalence of *Salmonella* spp. This information will help regulators and industry to better understand the safety associated to this seafood commodity.

P1-218 Influence of Surface Material, Sanitizer Concentration, Shear Stress, Contact Time, and Water Temperature on Surface-adhered Fungal Spoilage and Bacterial Pathogen Control

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❖ ***Developing Scientist Entrant***

Introduction: Cross-contamination within the food production environment is an important source of spoilage microbes and foodborne bacterial pathogens, and is largely mediated through environmental sanitation.

Purpose: The goal is to evaluate the relative impact of sanitation program features on microbial retention of surface-adhered spoilage organism and bacterial pathogen.

Methods: A collection of *L. monocytogenes* (n = 3) and the black yeast *Exophiala* spp. (n = 2) were grown on coupons (2.4 by 3.5 cm^2) stainless steel with a 0.81 mm finish (SM) and a 3.18 mm finish (RM), high-density polyethylene (P), Buna-N rubber (R), cement (C), and epoxy-sealed cement (E). Treatment variables included sanitizer concentration (0, 0.6, 1.2 & 2.4 mL/L), contact time (30 s and 5 min), shear stress (0.034 and 2.42 Pa), and water temperature (15.6, 23.9 and 32.2°C), which were applied in a custom bioreactor.

Results: Surface roughness and hydrophobicity did not significantly impact the level of initial microbial attachment, but did significantly impact ease of removal. *L. monocytogenes* were reduced by 1.5±1.5, 1.0±0.5 and 1.8±0.7 log CFU/coupon on SM, RM, and P under the most intense treatment at 23.9°C, and 4.2±0.4, 5.2±0.5 and 4.7±0.5 log CFU/coupon on coupons R, C, and E. *Exophiala* spp. were reduced below the limit of detection on coupons SM, RM, and P. Meanwhile, no significant reduction was observed on the remaining coupons. Application of CFD simulations provided estimations of wall shear stress in a bench-scale bioreactor system. *L. monocytogenes* and *Exophiala* spp. removal were differently effected by the variables (surface material, sanitizer concentration, water temperature, shear stress, and contact time), which all significantly (P < 0.05) contributed to microbial reductions.

Significance: Determination of outcomes related to sanitation program efficacy will allow processors to balance tradeoffs between quality and safety with cost and waste stream management appropriate for their facility.

P1-219 Quantitative Risk Assessment of *Bacillus cereus* in Salted and Fermented Squid (Squid *Jeotgal*)

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❖ ***Developing Scientist Entrant***

Introduction: Squid *Jeotgal* is a fermented squid that is salted and seasoned with spices. Because spices may have *Bacillus cereus* spores. There is a possibility for *B. cereus* growth in the Squid *Jeotgal* during fermentation and storage.

Purpose: The objective of this study was to estimate the risk of *B. cereus* in squid *Jeotgal*, through consumption in Korea.

Methods: *B. cereus* in 50 squid *Jeotgal* samples, collected from markets in Korea, were enumerated on CHROMagar™ B. cereus. Temperature and time data of distribution and display for squid *Jeotgal* were collected. Primary model was developed with the Weibull model to calculate *Delta* (time required for first decimal reduction) and ρ (shape of curves), using *B. cereus* cell counts collected from 7°C to 35°C. The *Delta* values were then analyzed with a secondary model (exponential equation). Consumption patterns and a dose-response model were also obtained. With all collected data, a simulation model was prepared in @RISK to estimate the probability of *B. cereus* foodborne illness.

Results: Of 50 squid *Jeotgal* samples, 23 samples (46%) were contaminated with *B. cereus*, and the initial contamination level was estimated to be 1.1 log CFU/g by the Uniform distribution. *B. cereus* cell counts gradually decreased during distribution. Lognormal distribution showed that consumption amount and frequency were 16.62 g and 0.96%, respectively. There was a dose-response model for *B. cereus*. Thus, the minimum infection dose (10^5 log CFU/g) was used for hazard characterization by using discrete distribution. Subsequently, a simulation with collected data showed that the probability of foodborne illness by *B. cereus* per person per day through the consumption of squid *Jeotgal* was 0.

Significance: This result indicates that the risk of *B. cereus* in squid *Jeotgal* is very low in Korea.

Poster

P1-220 Histamine and Proteolytic Bacteria Levels in the Fermentation of *Carcinus maenas*

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❖ ***Developing Scientist Entrant***

Introduction: *Carcinus maenas*, commonly known as green crab, is an aggressive invasive species found on the east coast of the United States and Canada. Mitigation efforts have focused on finding marketable uses for green crabs, including development of value-added products such as fermented condiments (crab sauce).

Purpose: The purpose of this study was to understand the effect of salt level and fermentation time on the microbial profile and histamine levels in a fermented green crab sauce prototype.

Methods: *Carcinus maenas* was trapped on the Maine coast and frozen until use. Whole crabs were finely chopped in a Kolsch bowl cutter (3,200 g) and combined with Kosher salt (10, 20 or 30% w/w). All treatments were prepared in triplicate and were fermented at 75°F. The product was sampled after 60, 90, and 120 days of fermentation. Biogenic amine content was determined by HPLC using a Waters AccQ·Tag. The population of proteolytic bacteria was determined by spread plating on skim milk agar. Data were analyzed by MANOVA followed by Tukey's HSD post hoc test in R studio.

Results: No significant differences ($P > 0.05$) in histamine content at different salt concentrations or time points were observed, the average histamine content being 67.1 ± 16.4 µg/mL. The population of proteolytic microbes was significantly lower in high salt treatments, compared to 10% salt samples. The population of proteolytic bacteria followed a nonlinear trend for all treatments, but reached a maximum of 6.6, 4.8 and 3.5 log CFU/mL in the 10, 20 and 30% salt treatments, respectively, on the last sampling date (d 120).

Significance: This data shows histamine levels are above the legal limit (50 ppm) regardless of salt concentration. Higher salt concentrations (20 and 30%) were associated with lower levels of proteolytic bacteria, which is associated with longer fermentation times in the production of crab sauce.

P1-221 Multidetermination of Nitrofurans and Chloramphenicol in Aquaculture Products by Enzyme-linked Immunosorbent Assay

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Introduction: Veterinary drugs such as nitrofurans and chloramphenicol are used in aquaculture products to control disease and improve food safety. However, the occurrence of residues in aquaculture products pose major public health concerns when consumed by humans. Thus it is necessary to have a fast, effective, affordable testing regimen such as ELISA in place.

Purpose: To harmonize the extraction protocols of AgraQuant® Nitrofurans (AHD, SEM, AOZ Plus and AMOZ Plus) and Chloramphenicol plus for ELISA screening purposes for shrimp so that one sample extract can be used for all five kits. To validate ELISA Drug Residue kits ((AHD, SEM, AOZ Plus and AMOZ Plus) and Chloramphenicol plus by comparing to the LCMS/MS reference method for the analysis of shrimps.

Methods: Vannamei shrimp (1 kg) obtained from supermarket was sent for LCMS/MS analysis in Nitrofurans (AHD, SEM, AOZ and AMOZ) and Chloramphenicol and determined to be non-detectable. Twenty non-detectable shrimp samples of 1 g each were fortified with either 0.5 ppb AHD, 0.5 ppb SEM, 0.5 ppb AOZ, 0.5 ppb AMOZ or 0.15 ppb Chloramphenicol according to the half of MRPL (minimum required performance limits). This limit was set by European Union Commission Decision 2003/181/EC. The spiking controls used were analyzed by LCMS/MS to confirm the concentration. Another 20 blank samples were used as control. The samples were extracted using ethyl acetate and analyzed using the ELISA.

Results: The average recovery of the 20 spiked shrimp samples were 98.0% (0.49 ppb)for AMOZ, 98.0% (0.49 ppb) for AOZ, 90.0 % (0.45 ppb) for SEM, 94.0% (0.47 ppb) for AHD and 93.3% (0.14 ppb) for Chloramphenicol. The CV% for the results were 0.7%, 0.4%, 0.6%, 4.8% and 3.9% in AMOZ, AOZ, SEM , AHD and Cholorampheniçol, respectively.

Significance: The AgraQuant® Nitrofurans and Chloramphenicol plus ELISA kits offer a rapid and reliable tool for testing drug residues in shrimps.

P1-224 Source Tracking Metabolically-active Bacterial Communities from Rooftop Harvested Rainwater to Irrigated Soil and Produce

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❖ ***Developing Scientist Entrant***

Introduction: Rooftop harvested rainwater (RHRW) is gaining increasing interest as a potential irrigation water source. Previous studies have indicated the presence of both biotic and abiotic factors affecting RHRW quality. However, very few studies have investigated the potential transfer of microorganisms, particularly metabolically-active bacteria, from rainwater to irrigated soil and produce.

Purpose: To bridge this knowledge gap, we tracked and characterized metabolically-active bacteria from irrigation water (ambient rain, first flush tanks, secondary tanks and municipal water) to irrigated soil and produce (chard) using 5-bromo-2'-deoxyuridine (BrdU) (a DNA label) coupled with next-generation sequencing techniques.

Methods: A total of 364 samples (irrigation water = 64, soil = 160 and produce = 140) were collected from a vegetable raingarden in Maryland, U.S.A from June to August in both 2018 and 2019. Subsamples were treated with BrdU. DNA from all treated and non-treated samples was extracted and PCR-amplified for the V3-V4 hypervariable region of the 16S rRNA gene and sequenced using the Illumina HiSeq 2500. The sequencing data were analyzed using QIIME, multiple R packages and SourceTracker.

Results: Irrespective of sample type, BrdU-treated samples were characterized by statistically significantly lower alpha diversity. Bacterial profiles observed in BrdU-treated samples, irrespective of sample type, were *Pseudomonas veronii*, *Pseudomonas lurida*, *Sphingomonas* spp., *Aeromonas* spp., *Arthrobacter* spp., *Sediminibacterium* spp., *Bacillus* spp., *Janthinobacterium lividum, Curvibacter lanceolatus* and *Geobacillus thermodentrificans.* Using the SourceTracker tool we were able to track and quantify the relative contributions of the irrigation water to the bacterial community of the irrigated produce.

Significance: Our preliminary findings 1) suggest that the presence of metabolically-active bacterial pathogens from irrigation sources is important, as contamination events can occur through the use of RHRW to irrigate food crops; and 2) can be applied to develop appropriate on-farm RHRW treatment technologies prior to irrigation.

P1-225 Coupled DNA-labeling and Sequencing Approach Enables the Detection of Viable But Non-culturable Vibrio spp. in Irrigation Water Sources in the Chesapeake Bay Watershed

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Introduction: Brackish waters are increasingly being explored as potential irrigation water sources to ensure future food security due to immense pressure on existing freshwater resources. However, brackish waters may harbor human pathogens including *Vibrio* species.

Purpose: Thus, there is a need to improve understanding of the prevalence of *Vibrios* in tidal brackish waters intended for use as irrigation water sources. Nevertheless, the presence of viable-but-nonculturable (VBNC) *Vibrio* spp. in brackish water stymies our existing detection methods.

Methods: To overcome this knowledge gap, we used a combination of 5-bromo-2′-deoxyuridine (BrdU) labeling, enrichment techniques, along with 16S rRNA sequencing to identify the metabolically-active fraction of *Vibrio* spp. in irrigation water from four sites (reclamation plant, pond, non-tidal freshwater creek and tidal brackish water creek) from May to September 2018 (n = 180 samples). Additionally, standard culture methods were used to enumerate *Vibrios* in all enriched non-BrdU treated water samples.

Results: Our coupled DNA-labeling and sequencing method revealed the presence of metabolically-active *Vibrio* spp. in all sampling sites, while the culture method only showed the presence of *Vibrios* in three of the four sampling sites. Specifically, we observed the presence of metabolically-active *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* using both methods at all sites. Interestingly, we were also able to detect the presence of metabolically-active *Vibrios* in non-enriched BrdU treated samples.

Significance: This approach not only refines our understanding of the prevalence of VBNC *Vibrios* in multiple water types but also can be applied to develop on-farm water treatment technologies that may be necessary to improve the quality of brackish water sources as climate change continues to impact our freshwater resources.

P1-226 Quantification of *Salmonella enterica* in Maryland Irrigation Ponds

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Introduction: Ponds are an important irrigation water source for produce crops in the Mid-Atlantic. Specific knowledge of pond water quality is important for safe, continued use of these irrigation sources.

Purpose: Quantify *Salmonella enterica* populations in irrigation ponds in Maryland and evaluate relationship with *Escherichia coli* levels.

Methods: Eleven on-farm ponds were sampled on the Eastern Shore, Western Shore and Piedmont Regions of Maryland, for a total of 21 visits between June and October. Sixty water samples were filtered on-site using a modified Moore swab technique in which 10 L, 1 L and 100 mL volumes of water were passed through cheesecloth to be used in a Most Probable Number (MPN) technique. Cartridges were pre-enriched in Lactose Broth, enriched in TT Hajna Broth with iodine and streaked for selective isolation of *Salmonella* onto XLT4. Water was collected for standard membrane filtration through 0.45 µm mixed cellulose ester filters for enumeration of *E. coli* on MI agar, as per EPA Method 1604.

Results: Out of 21 pond visits, 10 ponds from 7 farms were found to be positive for *Salmonella*, ranging from 0.03-2.9 MPN/L of water. Only 3 ponds were positive more than once. Therefore 77% of ponds were *Salmonella*-negative and the overall average was 0.14±0.07 MPN/L. Conversely, *E. coli* was detected in all but one pond sample (1.60±0.13 log CFU/100 mL). There was no correlation between the two species, even when considering only *Salmonella*-positive samples. No statistical differences in *Salmonella* or *E. coli* levels were detected by sampling month, although *E. coli* was highest in July and lowest in September (2.13 and 1.05 log CFU/100 mL, respectively; P = 0.09).

Significance: *Salmonella* presence in ponds was variable and at very low concentrations. *Salmonella* risk in pond waters should be monitored as *E. coli* was not an adequate indicator of *Salmonella* risk.

P1-227 Effect of Source Water Type and Quality on *E. coli* Removal by Zero-valent Iron Sand Filtration: A Conserve Study

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Introduction: Outbreaks of *Escherichia coli* O157:H7 infections have been linked to water used to irrigate leafy greens. Previous experiments have shown that zero-valent iron (ZVI)/sand- filtration can reduce *E. coli* in surface water. However, specific parameters of the ZVI filter design (iron concentration, contact time) and influent water type (surface, treated) may affect *E. coli* reduction.

Purpose: To determine if different percentages of ZVI and water types affect *E. coli* reductions in filtered surface water.

Methods: ZVI sand filters were constructed with either 50% zero-valent iron particles and 50% sand particles (0.400-0.625 mm) or 35% ZVI/65% sand particles within 40 PVC pipes (4 in. diameter, 12 in. length). Two liters of autoclaved pond water (PW) or laboratory deionized water (DI) were inoculated with *E. coli* TVS 353 (10^4 CFU/mL) and pumped through filters, followed by the addition of 8 L of uninoculated PW or DI. *E. coli* populations were determined from each liter of effluent, and water quality parameters were measured. Six filtration trials for each water type were conducted. Previously developed linear models and principal component analysis (PCA) was used to evaluate the role of influent and quality parameters on *E. coli* reductions.

Results: *E. coli* levels in DI were significantly (P < 0.05) lower by 2 log CFU/mL compared to PW after ZVI filtration, indicating that water quality may influence ZVI efficacy. Overall, *E. coli* populations were reduced by 98% over six filtration trials in DI; however, a 63% reduction was achieved in PW. Increased levels of oxidation-reduction potential (ORP), conductivity and turbidity negatively influenced *E. coli* reductions by ZVI filtration. ZVI filters containing 50% ZVI/50% sand showed slightly more reduction than the 35% ZVI/65% sand filters but were not significantly different (P = 0.48).

Significance: *E. coli* reduction by filtration is affected more by water type and quality than by ZVI concentration in filters.

P1-228 Identification of an In-line Agricultural Water Treatment Method Based on Microbiological and Chemical Characterization

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❖ ***Developing Scientist Entrant***

Introduction: New practices for mitigating food safety risks are constantly evolving. Preharvest agricultural water has been associated with contamination of fruits and vegetables and the Produce Safety Rule (PSR) has put forth strategies for growers to manage the risk posed by preharvest agricultural water. Treatment of preharvest agricultural water which are referenced within the PSR lack in studies demonstrating the effectiveness of many treatments.

Purpose: This work has begun to address these knowledge gaps by determining the efficacy of in-field preharvest agricultural water treatment for inactivating *E. coli* and coliforms.

Methods: Five treatments [Ultraviolet Light (UV-C; minimum UV dosage of 30,000 μWs/cm^2), Chlorine (5 ppm free chlorine after 2.46 s contact time), Peracetic Acid (PAA; 5 ppm after 2.46 s contact time), or the combination of PAA or Chlorine for the first and last 5 min of operation along with UV-C (PAA + UV-C or Chlorine + UV-C) were evaluated. Water was treated for 60 min with physicochemical variables measured every minute of the treatment. Water samples were collected at intervals of 0, 5, 15, 30, 45, and 60 min intervals. Colilert/Quanti-Tray 2000 analysis was used for determining the *E.coli* and coliform populations/100 mL with 16 replicates.

Results: *E. coli* and coliform counts were significantly decreased for all treatment times compared to the untreated control, with a mean reduction of coliforms/100 mL ranging from 1,440-1,790 MPN ($P < 0.05$). All treatments effectively reduced the populations of generic *E. coli* to undetectable levels (1 MPN/100 mL). Significant differences were also observed among the different treatments where combination treatments were shown to perform significantly better compared to the individual treatments. Amongst physicochemical properties, turbidity and conductivity was significantly ($P < 0.05$) lower in treated water samples.

Significance: This data will help provide in-field validation of preharvest agricultural water treatments which successfully inactivate target indicators of bacterial foodborne pathogens.

P1-229 Genomic and Phylogenetic Characterization of *Salmonella* Newport from East Coast by Using WGS Data

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Introduction: *Salmonella* Newport is one of the top three illness causing serovars in the US and PFGE pattern JJPX01.0061 isolates have been linked to recurrent tomato-borne outbreaks from the Delmarva peninsula.

Purpose: The genomic diversity and phylogenetic relatedness amongst *S*. Newport environmental isolates from the East Coast was investigated and PFGE pattern JJPX01.0061 isolates persistence and distribution in the Delmarva region was characterized.

Methods: Environmental *S*. Newport isolates ($n = 547$) spanning 12 years from 11 East coast states were subjected to whole genome sequencing and assembled. Publicly available genomes from NCBI Pathogen Detection Browser ($n = 156$) were selected to identify further relatedness. All genomes were analyzed with CFSAN SNP Pipeline using *S*. Newport CFSAN000825 (closed genome) as a reference to generate the SNP matrix. A phylogenetic tree was constructed based on total SNP variation and visualized with location and year on each branch. Antimicrobial resistance genes and *Salmonella* Pathogenicity Islands were identified in each isolate.

Results: Of the 547 isolates, 530 were identified as Lineage III and sequence types: ST118 ($n = 341$), ST5 ($n = 159$), and ST350 ($n = 28$). Genomic diversity clustered the isolates into seven clades including Pattern61 Delmarva Clade (I, $n = 344$), Non-Pattern61 Delmarva Clade (II, $n = 189$), and Multi-states Clades. SNP differences ranged from 0 to 1238 SNPs and were randomly distributed with respect to position in the reference genome. SNP difference ranges within Clades I and II were 0-63 and 0-134 SNPs, respectively. All identified pattern JJPX01.0061 isolates (ST118) grouped into eight distinct clusters separated by <30 SNPs. Eight SPIs were identified (SPIs 1 to 5, SPIs 12 to 14, and C63PI). All 530 *S*. Newport carried *aac(6')-Iaa* gene.

Significance: Analysis revealed a Pattern61 Delmarva Clade indicating long-term endemic persistence of this subtype within this microcosm and revealed diversity of *S*. Newport from this environment. The study offers important data for better source-tracking of tomato-borne outbreaks in the Delmarva region.

P1-230 Foodborne Pathogens in Surface Water from the Maule Region, Chile

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Introduction: Maule is a main agricultural region in Chile and it exports foods worldwide. Many farms in Maule irrigate food crops with surface water from rivers, streams and creeks; however, the microbiological safety of these waters has not been studied. *Salmonella* spp, *Listeria monocytogenes* and STEC are known foodborne pathogens that could be present in water, while *Arcobacter* spp. are emerging foodborne pathogens and an indicator of fecal contamination.

Purpose: To investigate the presence of foodborne pathogens in surface waters from Maule, Chile.

Methods: Water samples ($n = 300$) were obtained from Claro ($n = 150$) and Lontué ($n = 150$) river basins from rivers, creeks, and streams. Samples were obtained monthly (May-August) from 30 sampling sites in each basin. For sampling, 10 L of water were filtered through a Modified Moore Swab in each site. Samples were analyzed for the presence of *Salmonella* spp. and *L. monocytogenes* following BAM methodologies, and detection of *Arcobacter* spp. and STEC were done by previously described methods.

Results: All four pathogens were detected in different percentages: *Salmonella* spp. and *L. monocytogenes* were isolated in 14% and 17.7% of samples, respectively. STEC and *Arcobacter* spp. were detected in 7.5% and 43.3% of samples, respectively. Sites with persistent contamination with a particular foodborne pathogen (presence >2 times) were found for *Salmonella* spp. (5 sites), *L. monocytogenes* (4 sites), and *Arcobacter* spp. (27 sites). Also, 13 (21.6%) sites tested positive to at least one pathogen in all 5 samplings and 5 (8.3%) sites were free of pathogens in all sampling days. The four pathogens were found at the same time in 2 samples (0.7%).

Significance: Irrigation waters of Maule are contaminated with foodborne pathogens increasing the risk of foodborne illnesses. Urgent measures need to be taken to improve the safety of waters used to irrigate food crops in Maule.

P1-231 Fate and Biofilm Formation of Wild-type and Pressure-stressed Foodborne Pathogens of Public Health Concern in Surface Water

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Introduction: Since the historic outbreak of cholera in the Soho area of London that served as the cornerstone of modern epidemiology, spread of infectious diseases in surface and sub-surface water has been a persisting public health challenge.

Purpose: The current study investigated fate and biofilm formation of wild-type and pressure-stressed pathogens of public health concern in low-nutrient environment at 4, 25, and 37°C.

Methods: A four-strain cocktail of *Listeria monocytogenes*, six-strain cocktail of *E. coli* O157:H7 and five-strain cocktail of *Salmonella enterica* serovars were used to inoculate surface water and samples were enumerated for 28 days to determine the fate and proliferation of the pathogens at 4, 25, and 37°C. Additionally, biofilm formation of the pathogens for wild-type and pressure-stressed phenotypes were monitored on surface of stainless steel and rubber coupons for 28 days. Sessile cells were separated from the coupons using sonication for 5 minutes. The project was conducted in three independent repetitions, each considered as a blocking factor of a randomized complete block design. Each block additionally contained two repetitions with each repetition repeated in duplicate as microbiological replicates. The study was analyzed using Tukey-adjusted ANOVA at type I error level of 5%.

Results: At 4°C and after 28 days, all three wild-type pathogens were reduced ($P < 0.05$) but remained detectable in the inoculated samples. Counts of biofilms for both wild-type and pressure-stressed phenotypes, however, were reduced ($P < 0.05$) to the detection limit on rubber and steel coupons. Survival and proliferation of planktonic and sessile cells were more pronounced at higher temperatures for both phenotypes.

Significance: The current study indicates the above-mentioned pathogens of public health concern could proliferate in surface water at various temperatures for prolonged amount of time. Pressure-stressed phenotypes were able to form biofilm and proliferate in low-nutrient environment in a rate comparable to the wild-type pathogens.

P1-232 Antimicrobial Resistant Bacteria in Superficial Water in Central Chile

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Introduction: The food market is currently demanding high standards of safety in plant products intended for human consumption. Irrigation of crops with microbiologically polluted water is one of the important sources of food contamination. Therefore, the evaluation antibiotic resistance in agricultural water is a pivotal point. In this study we evaluated the presence of both environmental and enteropathogenic resistant bacteria at various sampling sites in two rivers that are used to irrigate crops in rural area in central Chile.

Purpose: The main objective of this work was to detect antimicrobial resistant bacteria in superficial water that has direct agricultural use.

Methods: River water (10 L) was processed to detect resistant enterobacteria using a modified Moore swab protocol, enriched with peptone water incubated at 37°C overnight. The enriched samples were seeded in MacConkey agar supplemented with ciprofloxacin (CIP) and ceftazidime (CAZ) and incubated at 37°C. Isolates were identified by MALFI-TOF and antibiotic susceptibility profile was performed. Environmental antibiotic resistant was detected by the filtration of 1 L grab sample and the concentrated bacteria were seeded in R2A agar plates and incubated at 3 temperatures (37, 25 and 15°C). Bacterial-banks were built using the criteria of colony morphotypes and then challenged with antibiotic from different families.

Results: Multi drug resistance organisms (MDRO), both environmental and pathogenic, were found at all temperatures, with beta lactamase and quinolones having a high frequency of detection. Two isolates from *Aeromonas* spp. and *Stenotrophomonas* spp. showed resistance to all evaluated antibiotics.

Significance: Our results will allow a better understanding the role of superficial water in the dispersion and maintenance of the antibiotic resistant bacteria.

P1-233 Antimicrobial Resistance of *Enterococci* in Surface and Recycled Water: A Conserve Study

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Introduction: The *Enterococcus* genus includes opportunistic bacterial pathogenic species known to accumulate antimicrobial resistance (AMR) traits. Determining AMR patterns of this bacterial taxon is important for water quality management in agriculture.

Purpose: To evaluate the distribution and AMR of two *Enterococcus* spp., *E. faecalis* and *E. faecium,* in surface and recycled waters.

Methods: *Enterococcus* isolates ($N = 744$) recovered from 12 surface and recycled water sites over two years were identified as *E. faecium*, *E. faecalis* or other by PCR amplification of *ddlA* and *sodC* genes. Antimicrobial susceptibility testing (AST) was performed on select isolates ($N = 199$; *E. faecium* = 49, *E. faecalis* = 150) using the microbroth dilution method with the NARMS GPN3F plate. Minimum inhibitory concentrations for 14 antibiotics were determined. Nominal logistic regression model analysis was employed to assess seasonal and water type effects on species distribution and antimicrobial resistance.

Results: Out of 744 isolates, 70 *E. faecium* (9.4%) and 309 *E. faecalis* (41.5%) were identified. Representative isolates from each sampling time and site were selected ($N = 199$) for AST. Of these, 188 (94.5%) exhibited resistance to at least one antibiotic. Only 4.1% of *E. faecium* and 6.0% of *E. faecalis* were pan-susceptible. Twenty-seven *E. faecium* (55.1%) and 25 *E. faecalis* (16.7%) isolates exhibited multidrug resistance, whereas 14 *E. faecium* (28.6%) and 78 *E. faecalis* (52.0%) isolates were single-drug resistant. Associations were found between both season and water type and *E. faecalis* resistance. Resistance to tetracycline ($\chi^2 = 4.36$, df = 6, $P < 0.05$), levofloxacin ($\chi^2 = 5.15$, df = 6, $P < 0.05$) and multidrug resistance ($\chi^2 = 7.49$, df = 6, $P < 0.01$) were more likely in winter than summer or spring. Resistance to ciprofloxacin ($\chi^2 = 3.97$, df = 6, $P < 0.05$) and rifampicin ($\chi^2 = 5.11$, df = 6, $P < 0.05$) was more likely in non-tidal than tidal river isolates.

Significance: *E. faecalis* and *E. faecium*, were ubiquitous in surface and recycled waters available for crop irrigation and AMR was prevalent and associated with season.

P1-234 Diversity and Resistance to Extended Spectrum β-Lactams in *E. coli* from Multiple Irrigation Water Sources: A Conserve Study

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❖ ***Developing Scientist Entrant***

Introduction: Pathogenic and antimicrobial-resistant *Escherichia coli* are a major public health concern. Due to the possibility of crop contamination and antimicrobial resistance gene transfer to other pathogens, characterizing *E. coli* in irrigation water is important for agriculture and food safety.

Purpose: To determine the phylo-grouping and resistance to extended spectrum β-lactam (ESBL) antibiotics of *E. coli* recovered from surface and reclaimed water in the Mid-Atlantic region of the USA.

Methods: A total of 751 *E. coli* isolates from 12 different sites (2 irrigation ponds, 1 tidal river, 5 non-tidal rivers/creeks, 3 reclaimed water effluents and 1 post-food processing water) were identified with species-specific PCR amplification of the *uidA* gene. Phylo-typing (groups A, B1, B2 and D) was done by amplification of three virulence genes: heme transport (*chuA*), stress response (*yjaA*) and lipase/esterase (*tspE4C2*) genes. Antimicrobial susceptibilities to cefotaxime, cefoxitin, ceftriaxone, cefuroxime and ceftazidime were tested using the Kirby-Bauer method.

Results: Phylogenetic analysis revealed that most isolates belonged to group B1 (362/751; 48.2%), comprising enterohemorrhagic, enteropathogenic and Shiga toxin-producing *E. coli.* Groups B2 and D, including intestinal and extraintestinal strains were represented by 17.6% (133/751) and 21.9% (165/751), respectively. Group A which includes mostly commensal strains was represented by 12.1% (91/751) of isolates. Only 17 isolates (2.3%) exhibited resistance to β-lactams; most (41.2%) belonged to group A (7/91; 7.7%), with 6/7 exhibiting resistance to a single antibiotic; cefoxitin. Four isolates from group B1 (4/362; 1.1%), 4 from group B2 (4/133; 3.0%) and 2 from group D (2/165, 1.2%) exhibited resistance to multiple antibiotics. Resistant isolates were recovered from non-tidal rivers/creeks (n = 11), reclaimed wastewater (n = 4), tidal rivers (n = 1) and pond water (n = 1).

Significance: Water sources available for food crop irrigation contained a large percentage of potentially pathogenic *E. coli*. Non-tidal river strains were more likely to exhibit resistance to β-lactams.

P1-235 Prevalence of Foodborne Pathogenic Bacteria and Shiga-toxigenic *Escherichia coli* Virulence Genes in Conococheague Creek, Pennsylvania

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Introduction: Investigation of microbial contamination in water sources represent valuable inputs to understand the water microbial quality and it is essential for alerting the community regarding the use of surface water and the potential health hazards.

Purpose: The purpose of the study was to determine the prevalence of *Salmonella* and *E. coli* O157:H7, and Shiga-toxigenic *E. coli* virulence genes (*stx1* and *stx2*) in Conococheague creek, PA.

Methods: During March, 2018, to March, 2019, weekly water samples were collected from 6 sites along the Conococheague creek, PA (n = 228). Water samples (100 mL/sample) were filtered through a 0.45 µm membrane filter using a vacuum manifold and the filters were then directly placed on mTEC agars to enumerate the populations of generic *Escherichia coli*, or enriched to monitor the presence of bacterial pathogens (*Salmonella* and *E. coli* O157:H7) by real-time quantitative PCR and the prevalence of the virulence genes (*stx1* and *stx2*) of Shiga-toxigenic *E. coli* by conventional PCR and gel electrophoresis.

Results: Overall, generic *E. coli* populations in water samples ranged between 2.2 to 3.5 log CFU/100 mL during summer season (July-September, 2018). Significantly lower populations of generic *E. coli* (0.9-2.6 log CFU/100 mL) were recovered in water samples collected in winter season (January-March, 2019) than in summer season. While *E. coli* O157:H7 was not detected in any of the water samples, 9 out of 228 samples were confirmed as *Salmonella* positive. Higher prevalence of *Salmonella* was found during July-September, 2018 (7%) as compared to 0% in January-March, 2019 (P < 0.05). Prevalence of Shiga-toxigenic *E. coli* virulence genes was not affected by the season; the *stx1* and *stx2* genes were detected in 16% and 7% of the water samples, respectively.

Significance: Results of the current study suggest a potential seasonal effect on the populations of generic *E. coli* and the prevalence of *Salmonella* in Conococheague creek, PA.

P1-236 Evaluation of *Escherichia coli* and Coliforms in Water Used in a Decoupled Aquaponics System

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❖ ***Developing Scientist Entrant***

Introduction: There is a concern of foodborne pathogens, specifically *Escherichia coli*, transferring in an aquaponic system from water containing Nile tilapia to the plants.

Purpose: The purpose of this one-year study was to identify the introductory points of *E. coli* and understand whether *E. coli* populations were within the limits of the FDA Produce Safety Rules for irrigation water.

Methods: Throughout the experiment, four rounds of 14 cucumber plants and three rounds of 14 tomato plants were planted in perlite with automatic watering (3 min) every 1 h of aquaculture effluent. Two hundred and fifty mL water samples were collected every two weeks in triplicates from six locations within the system, for a total of 598 samples. One hundred mL samples were membrane filtered for microbial analysis using EPA Method 1604 with modifications on MI agar and VRBA for *E. coli* and coliform identification, respectively.

Results: Temperature was measured from each sample immediately after collection and ranged between 12.6°C and 32.8°C with an average of 24.24°C. The Produce Safety Rules require a geometric mean (GM) of ≤126 and a statistical threshold (STV) of ≤410 CFU of generic *E. coli*/100 mL of water. The GM and STV were calculated based on the irrigation source *E. coli* populations. From February 1 to May 31, 2019, the GM and STV were within the FDA limits. From June 1 to July 31, 2019, the GM was below the limit and the STV was above the limit and from August 1, 2019 to January 31, 2020, the GM and STV were above the limit. Coliform population remained around the same throughout the entire year.

Significance: This study showed that there are needs to monitor *E. coli* populations more closely from June to January in a decoupled aquaponics system and correct the microbial quality of irrigation water if necessary.

P1-237 Comparison of Multiple Test Methods for the Assessment of Retort Cooling Water

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Introduction: 21 CFR 113.60 (b) and 9 CFR 431.6 (h) (2 and 3) require disinfection of certain cooling water systems to control bacteria levels in cooling water. Microbiological testing of water is a best practice to verify that implemented disinfection systems are working effectively. Use of scientifically valid methods to recover bacteria in cooling water can help reduce the post-process contamination risk.

Purpose: This study compared five different methods against U.S. Food and Drug Administration (FDA) approved benchmark methods in the recovery of indicator bacteria commonly found in retort cooling water.

Methods: *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Bacillus subtilis* were used as indicator organisms for the study, using FDA approve methods for culture preparation. Water samples were inoculated separately (~10^2 CFU/mL) with the three microorganisms. Samples were enumerated using: 3M™ Petrifilm™ (with and without sodium thiosulfate), Millipore® HPC Total Count Sampler™, Millipore® MC-media pad™ (24- and 48-hour incubation). Pour plates using aerobic plate count agar was used as the benchmark method. A minimum of 10 replications were used for each method. One-Way ANOVA was performed to determine if the mean recovery of organisms significantly differed between the different methods ($\alpha = 0.05$).

Results: Overall, the Millipore® HPC Total Count Sampler™ and the Millipore® MC-media pad™ (24- and 48-hour incubation) showed equal or better recovery ($P > 0.05$) of indicator bacteria when compared with the benchmark method. 3M™ Petrifilm™ (with and without sodium thiosulfate) showed significantly ($P < 0.05$) less recovery of indicator bacteria when compared to other methods (including the benchmark method).

Significance: By combining microbial testing with the necessary chemical treatment for cooling water systems, manufacturers are better able to control the quality of the water and reduce the risk to thermally processed containers. The data generated in this study provide insights on the methods currently available for use for testing of retort cooling water.

P1-238 Simultaneous Enumeration of Total Coliform and *Escherichia coli* in Drinking Water Using ColitagTM MPN-Plate and MPN-Tray

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Introduction: Total coliform and *E. coli* are indicators for environmental and fecal contamination. The testing kit which contains ready-to-use dehydrated medium has been approved by the US Environmental Protection Agency (EPA) for qualitative detection of total coliform and *E. coli* simultaneously in drinking water. The detections are based on the presence of two enzymes, β-galactosidase and β-glucuronidase, which are characteristic of total coliform and *E. coli*. Yellow color development represents the presence of total coliform, and blue fluorescence under a UV light along with yellow color indicates the presence of *E. coli*.

Purpose: Quantitative detection of total coliform and *E. coli* in drinking water using easy-to-use MPN methods were evaluated and compared to reference method.

Methods: The drinking water was contaminated with wastewater effluent collected from three different wastewater treatment plants (three samples). After chlorination, total coliforms and *E. coli* were enumerated with two most probable number (MPN) based platforms, the MPNPlate (16-well) and MPNTray (97-well) and compared with EPA reference method 1604. Twenty replicates were tested for each sample and each MPN method. The numbers of positive wells for total coliform and *E. coli* were recorded at 16, 18, 24, and 48 h. An ANOVA test was conducted to compare the easy-to-use MPN methods to the EPA method.

Results: No significant differences ($P > 0.05$) were found between MPNTray and EPA method for quantitative detection of both total coliform and *E. coli*, regardless of the incubation time. The MPNPlate also showed similar ($P > 0.05$) results at 18 and 24 h compared to reference method. The sensitivities of the MPN methods tested were ≥98.25%, and the specificities were ≥ 95.16%.

Significance: The Colitag MPNTray and MPNPlate can simultaneously enumerate total coliforms and *E. coli* in drinking water. These methods are easy to use, have flexible detection times and show accurate results compared to reference method.

P2-01 Research Priorities Identified by the United States Department of Agriculture Food Safety and Inspection Service

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Introduction: The US Department of Agriculture's Food Safety and Inspection Service (FSIS) is a science-based food safety regulatory agency which strives to promote research in areas important our mission. To that end, FSIS maintains and shares a list of research priorities.

Purpose: FSIS communicates our research priorities to encourage researchers to address these, and to encourage funding agencies to consider FSIS priorities when developing funding opportunities.

Methods: FSIS reviews and updates our research priorities on an annual basis, using stakeholder input via the Research Priorities Review Panel (RPRP). RPRP members represent each Program Area in FSIS, including the laboratories.

Results: Research priorities include detection methods and mitigation strategies for both microorganisms and chemicals in foods. Specific areas of interest include:

- Improved screening technologies and confirmatory tests for both microorganisms and chemicals in FSIS regulated products (meat, poultry, egg products, fish of the order siluriformes)
- Testing methods for quantifying pathogens in meat, poultry and egg products
- Occurrence of potential emerging pathogens that may present a public health risk to consumers
- Presence of antimicrobial resistant strains of bacteria in FSIS-regulated products
- Effectiveness of pre-harvest and post-harvest interventions on finished products
- Physiologically Based Pharmacokinetic (PBPK) models to estimate chemical concentrations in beef, pork and chicken tissues
- Unique attributes of pathogen outbreak strains that may increase the probability of foodborne illness

Significance: By communicating our research priorities and associated food safety research studies, we seek to encourage researchers to apply their expertise to address FSIS priorities, and encourage research funding agencies to consider FSIS priorities when developing research opportunities. To date, research has been initiated on 80% of FSIS Research Priorities, with $60+ M in funding allocated to research related to FSIS Research Priorities within the USA.

P2-02 *Salmonella* Transmission Associated with Live Poultry in Tennessee: The Need for a One Health Approach

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Introduction: *Salmonella* outbreaks linked to live poultry (LP) reoccur annually and have increased with the popularity of backyard flocks. Since 2000, a total of 76 LP multistate outbreaks were reported in the U.S. During 2019, Tennessee (TN) reported the highest number of LP-associated cases on record. We describe the 2019 LP outbreak in TN and recommend approaches to prevent future illnesses.

Purpose: One health approach to combat the morbidity caused by LP outbreaks.

Methods: Cases were interviewed with a standard case report form and LP Exposure Questionnaire for *Salmonella*. Questions included contact with LP, length of poultry ownership, and purchase locations. Whole-genome sequencing was performed. TN shared epidemiological and laboratory data with CDC. Feed store locations reported by cases were contacted for traceback information.

Results: Seventy LP-associated cases were included in 10 multi-state LP *Salmonella* clusters in 2019. Of 60 (86%) cases interviewed, twenty-five (42%) cases were ≤5 years old. Six (10%) were ≥65 years old. Twenty-two (37%) were hospitalized and 38 (63%) reported exposure to LP. Among 21 cases with supplemental data, 90% reported owning the LP for <6 months, 50% purchased LP from retail feed stores, and 59% reported touching, snuggling or feeding the LP. Only 14% reported gathering eggs. Traceback efforts identified 2 out-of-state hatcheries as sources of LP among TN cases. Educational materials on proper handling of LP and food/egg hygiene were used to educate the public and employees at the points of purchase. Next steps to prevent transmission of *Salmonella* from LP were discussed with the TN One Health Committee.

Significance: *Salmonella* transmission associated with LP is predictable annually and appears to be an increasing cause of morbidity. Environmental sampling could assist in identifying cases linked with LP's environment. One Health strategies for communication between animal and public health should be prioritized to educate the public on proper handling of LP and their environment to reduce morbidity.

P2-03 Application of "Hypocrisy" Strategy in Food Safety Practices

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❖ ***Developing Scientist Entrant***

Introduction: Hypocrisy is the cognitive dissonance-related technique, which has been used to motivate individuals to change their behaviors.

Purpose: The study was to (1) assess food service employees' attitudes and practices toward handwashing and glove behaviors, (2) to use the hypocrisy approach to influence attitudes and behavioral intentions related to such behaviors, and (3) to provide recommendations on employees' handwashing and glove behaviors.

Methods: An online questionnaire was developed based on literature review, including direct attitude measures, implicit measures, behaviors intention of handwashing and glove use behaviors and respondents' demographics. All participants were randomly and equally divided into four groups. Group 1 was assigned to the commitment-only condition, in which participants were required to sign in a flyer promoting handwashing and glove use. Group 2 was given the mindfulness-only condition, in which participants were required to finish a self-check question of handwashing and glove use. Group 3 experienced the hypocrisy condition (commitment plus mindfulness conditions; finished both signing in the flyer and self-check question), while Group 4 was used as the control group. All participants were asked to answer the attitude and intention survey at the end of the study.

Results: Of 371 participants, 66.3% (n = 246) were male and 33.7% (n = 125) were female. There were no significant differences between the four groups in terms of direct attitude towards handwashing or glove use, but the implicit attitudes of handwashing (2.74 ± .31) and glove use (2.66 ± .46) in the hypocrisy group is significantly better than that in the control group (2.56 ± .52; 2.41 ± .67; $P < 0.005$). Also, there were no significant correlations among four groups in terms of implicit attitude of handwashing and glove use. In addition, the intention of glove use in all groups (6.00 ± 1.13; 5.99 ± .99; 5.86 ± 1.10) was significantly better than that of the control group (5.43 ± 1.57; $P < 0.005$).

Significance: This study shows that the hypocrisy" strategy can influence participants' implicit attitudes toward handwashing glove use and intentions of using gloves, which can improve restaurant employees' food safety practices.

P2-04 Exploring Food Safety Training as a Potential Risk Mitigation Activity: A Pilot Case Study with 4-H Volunteers and Extension Agents in Florida

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Introduction: Limited information is available on food safety knowledge and practices among active adult 4-H volunteers in Florida ($N > 8,000$). Many educational programs and events (i.e., fund raising and concessions) associated with the 4-H in Florida involve food, often handled by volunteers. This could be a potential liability to the organization.

Purpose: To assess retention of knowledge and adoption of key practices by the participants after the training and compare selected results with the 2016 FDA food safety consumer survey data.

Methods: From January to August, 2019, we conducted nine one-day food safety training sessions at multiple locations in Florida. The training material was based on the *Safe Staff*® program which is accepted by the Department of Business and Professional Regulations (DBPR) for professional food handlers. Three months after the training, participants ($n = 131$) were asked to complete a 28-item survey instrument (selected and adapted from the FDA Consumer survey).

Results: Fifty-three participants responded to the survey (40% rate of response). Ages of respondents (39/53) were between 24 to 65 years old with majority as female (41/53). Among major findings: Respondents (43/47[(91%]) reported correct use and cleaning of cutting boards; 97% (46/47) reported proper personal hygiene practices; 93% (44/47) reported proper glove uses; 87% (41/47) reported having thermometers at volunteer sites and 60% always use thermometers when cooking poultry. Majority (42/46); 91% know the correct temperature setting for a refrigerator. While some gaps in specific issues with cooking are identified, respondents exhibited equal or higher food safety knowledge and practices than the 2016 FDA Consumer Food Safety Survey.

Significance: This study revealed that volunteers need some type of food safety training beyond consumer level before handling foods to ensure the mitigation of foodborne illness.

P2-05 Empowering the U.S. Virgin Islands' Food Industry through Food Safety Education

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❖ ***Developing Scientist Entrant***

Introduction: Restaurants and fresh produce contribute the most to foodborne illness outbreaks in the United States. Food safety educational materials designed for specific audiences are needed to ensure safe production and handling of food.

Purpose: The project's purpose was to determine the key food safety educational needs of food handlers in the U.S. Virgin Islands and develop culturally appropriate extension materials to meet these needs.

Methods: Under Human Subject Approval #19-448-00, in-depth interviews were conducted with 28 restaurant managers, 7 key informants and 14 produce farm managers, to identify the main food safety concerns in the U.S. Virgin Islands. Interviews were transcribed and thematically coded based on knowledge and behavioral themes. Educational flip charts, posters, signs and brochures were developed based on the analysis and piloted to 21 persons representing restaurants, farms, and educators in two rounds. Evaluation occurred for nine criteria related to clarity and appeal with a Likert scale of 1 to 5 (1-criteria not met at all, 5-criteria fully met). Materials with total points 40-45 needed no revision, 21-39 points needed revision, and 0-20 points were rejected.

Results: The four food safety concerns identified with restaurants were: 1) poor hygiene and sanitation; 2) time and temperature abuse of food; 3) cross-contamination; and 4) low quality food. The top food safety concerns on produce farms were: 1) inadequate water quality; 2) facility sanitation; 3) hygiene and health of personnel; and 4) insufficient employee food safety training. Photographs and figures in first draft of educational materials were altered for cultural appropriateness. In their second piloted draft, materials scored 44.1±2.2 and 42.4±3.2 points for restaurants and produce grower materials, respectively, indicating no revision was needed.

Significance: Culturally appropriate food safety interventions will aid in promoting safe food handling practices among distinct communities, and protect the public from foodborne illnesses.

P2-06 Experiences and Needs of Virginia Cooperative Extension Educators When Supporting Food Recovery Organizations

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Introduction: Food handlers in food recovery organizations (FROs) have been documented to engage in unsafe food handling behaviors. This may put clients at heightened risk for foodborne illness due to preexisting conditions and insufficient access to healthcare. There are approximately 1,700 FROs in Virginia, including food banks, pantries, and meal kitchens. State Cooperative Extension programs can serve as a resource for FROs and the populations they serve, however, little is known about their engagement with this audience.

Purpose: The objective of this case study was to determine how Virginia Cooperative Extension (VCE) supports these organizations and understand their perception of the food safety education and the general support this audience needs.

Methods: An online survey containing fixed-choice and open-ended questions was distributed through VCE e-mail listservs in Spring 2019. Responses were analyzed using descriptive statistics and an inductive, grounded theory coding method.

Results: Thirty-four VCE educators responded, and of those 41% (n = 14) reported previously supporting FROs. Seventy-one percent (n = 10) of them provided food safety-related content, such as food handling and preservation, to FROs and their clients. They expressed a need for additional resources, including how to create a HACCP plan and best practices for FROs as well as accessible fact sheets for their clients. Of the 20 respondents who have not supported FROs, 55% (n = 11) indicated they were interested but logistical barriers and insufficient information prevented their doing so. Supporting FROs allowed VCE educators to feel altruistic, as well as develop their personal and professional networks.

Significance: As a result, dedicated resources have been compiled to support VCE's work with FROs and their clients as well as educational material for VCE educators to learn more about their operations and how to engage with them. Future research should be conducted to determine their effectiveness and how other state Cooperative Extension Systems engage with these audiences.

P2-07 Implementation of New Instructor Training Approach to Equip Food Safety Educators to Deliver Food Protection Manager Certification Courses

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Introduction: The 2017 FDA Model Food Code mandates that all food establishments have at least one employee who is a certified food protection manager present at all times during operation. As demand for classes increases, food safety educators must prepare to train individuals for certification. With educators coming from a variety of educational backgrounds, instructor training must be robust to properly equip them to teach.

Purpose: In order to increase skill and technical proficiency among food safety educators, a new training approach was implemented to equip candidate instructors for a food protection manager training program using the same adult education methods employed in the curriculum.

Methods: This novel approach utilized a three-step training process including online pre-coursework, two day in-person intensive training, and post course knowledge and skills assessment. The approach also included an implementation process comprised observations and guided planning. Each step was designed to build on the previous to increase confidence and preparedness in the candidate instructors.

Results: The results of this new approach demonstrate that candidate instructors reported beginning in-person training with increased comfortability with technical materials and better prepared to engage. Review of results from the pre-course assessment and in-person training post assessment showed progression of technical fluency and confidence with material. The baseline assessment of the approach provides foundation for future work on differences in exam scores, increase in number of classes offered and quality of instruction upon observation.

Significance: The implementation of this training model suggests that with this approach, instructors can increase competency and skills while simultaneously increasing confidence in instructional ability. Instructors who possess higher technical proficiency have the ability to increase quality of food safety instruction and better equip food establishment employees to understand and implement best practices.

P2-08 Safe Produce for Food Pantries: Regional Impact in Food Safety Education

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Introduction: In 2014, the national network Feeding America estimated more than 4 million people in the upper Midwest were food insecure. For these families, food pantries serve as an important emergency food source. The largest increase in food distributions in recent years has been in fresh produce. Feeding America delivers over 2 million pounds of fresh produce to its member food pantries each week. Increasing fruit and vegetable consumption is a key recommendation of the Dietary Guidelines for Americans (DGA), encouraging all Americans to "make half their plate fruits and vegetables" to promote good health, maintain a healthy weight and reduce risk of chronic disease. Meanwhile, foodborne illness outbreaks linked to consumption of fresh produce has increased, representing a significant public health risk.

Purpose: Educators in the upper Midwest delivered food safety training to gardeners and food pantry staff to help ensure safe produce is distributed to food pantry guests.

Methods: Educators in Iowa, Michigan, North Dakota, and Wisconsin trained gardeners and food pantry staff using lessons from *The **Safe Produce for Food Pantries Project*** (https://safeproduce4foodpantries.org) developed by educators at the University of Wisconsin and Michigan State University. Gardeners donating fresh produce learned to ***Make Your Donation Count*** and food pantry staff and volunteers learned to ***Keep Produce Fresh and Healthy***.

Results: End of session evaluation (n = 167) showed the training

- increased understanding of why safe produce is important for food pantry guests (92%)
- increased understanding of steps to ensure safe produce is available to food pantry guests (94%)
- increased understanding of the importance of safe produce donations (100%) or a clean and sanitary food pantry environment (92%)

Overall the training was rated as 'good' to 'excellent' across all participants.

Significance: Food safety educators in the North Central U.S. have taken a regional approach to food safety education that has demonstrated impact across the region.

P2-09 Why People are Risky: Qualitative Analysis of Food Handling Practices

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Introduction: Food safety practices in the home have been shown to contribute to the overall public health burden of foodborne pathogens. While the literature of self-reported and observed food safety behaviors continues to be developed, the understanding of motivations and antecedents behind certain practices are not well described.

Purpose: The aim of this study was to investigate the role that experiences and past behaviors play in both self-reported and observed food handling behaviors in household kitchens. A secondary objective of this study was to better understand how consumers perceive risk and what contributes to their decision-making during meal preparation.

Methods: A sample of 1,081 participants was recruited from various communities in North Carolina. Participants were asked to prepare a simple meal in a consumer-style kitchen while being observed by researchers. At the end of the meal preparation, researchers conducted semi-structured interviews with each participant. Interview questions included probing the reasons behind observed behaviors; typical behaviors when cooking at home and how personal risk assessment impacted self-reported practices. Interviews were recorded, transcribed, and coded using Nvivo software to identify common themes.

Results: Qualitative analysis of interviews led to the discovery of a series of themes. Personal confidence leading to an underestimation of risk was common. Participants reported feeling confident with subjective measures of doneness when cooking familiar products, both from raw and from pre-packaged, frozen because of a history of no illnesses. Participants also underestimated risk of behaviors such as chicken washing due to previous positive experiences including family tradition.

Significance: Understanding the precursors to behaviors can help food safety communicators develop more targeted messages. These results suggest that food safety messages should address consumer perceptions and previous experiences in order to increase personal relevance and effectiveness of food safety messages.

P2-10 Call to Action: What Information Do U.S. Consumers Need to Respond to Food Recall Notices and Public Health Alerts?

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Introduction: USDA, FSIS issues food recall notices and public health alerts to inform consumers about food recalls for regulated products and foodborne illness outbreaks so that consumers can take appropriate action to protect themselves and their families from foodborne illness by not consuming the affected food.

Purpose: We conducted this study to better understand how consumers currently respond to food recalls and how they would like to receive information from FSIS on food recalls and foodborne illness outbreaks.

Methods: We administered a survey to 2,586 adult home food preparers that were part of a nationally representative web-enabled panel.

Results: Respondents identified the following as the most important information they need to know to respond to a food recall: type of food (71%), where the food was distributed (69%), affected brands of the food (65%), and date and establishment or lot number on the product packaging (59%). About 3% of respondents currently receive government email/text message alerts about food recalls, and about one-third said they would be very or somewhat likely to sign up to receive emails/text messages. About 64% of respondents would like to receive such alerts immediately after a recall and 21% would like to receive a weekly summary. About 67% would like to receive alerts targeted to the state they live in or their state and surrounding states. Respondents believe the government should first announce to the public that a foodborne illness outbreak has occurred whenever there is an outbreak, even if the food source has not been identified (35%) or only after the food source has been identified (41%).

Significance: FSIS can use the survey findings to refine their outreach and communication with consumers to help improve consumers' response to food recalls and foodborne illness outbreaks.

P2-12 Raw Milk Legalization – What Do Consumers Think? Willingness of Purchasing or Consuming Raw Milk Products among Consumers in Louisiana

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Introduction: Over the last two decades, we have witnessed a legislative trend which favors the legalization of raw milk sales despite the justified safety concerns from the scientific community. In 2004, it was legal to sell raw milk in 22 states. By 2011, this had increased to 30 states. This movement towards the legalization of raw milk distribution in the United States has continued to expand. Although it is currently illegal to sell raw milk in the state of Louisiana, this legislation has been challenged in recent years.

Purpose: 1) To evaluate consumers' awareness of potential risk of raw milk, and 2) to evaluate consumers' willingness of purchasing or consuming raw milk products.

Methods: A questionnaire was designed to assess the participants' awareness of potential risk of raw milk and their willingness of purchasing or consuming raw milk products in the state of Louisiana. The survey was carried out throughout the state. Data were analyzed using descriptive statistics.

Results: Three hundred and one questionnaires were collected. All participants were Louisiana residents. Fourteen out of 301 participants reported not consuming dairy products. Fifty-five people consumed raw milk products. Most participants considered raw milk as natural (86.4%), healthy (62.8%), or harmless (60.1%), while less people consider raw milk as safe (50.8%). Participants showed poor awareness of potential risk of raw milk but reported that they would not consume raw milk if they know that it can cause food poisoning. Participants did not show support towards sale of raw milk. They also reported that they were less likely to purchase raw milk if its price is higher than commercial pasteurized milk.

Significance: Louisiana is at a pivotal point in its legislative process of legalizing raw milk sales. It is timely and critical to evaluate consumers support for raw milk sale and consumption.

P2-13 Enhancement of PSA Grower Training Curriculum through Activities That Increase Participant Engagement

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Introduction: The standardized Produce Safety Alliance (PSA) grower training curriculum, developed to train produce growers covered under the Food Safety Modernization Act (FSMA) Produce Safety Rule, is based on an 8-hour, single-day lecture delivery, with limited participant engagement and attendee networking, and no real-time feedback on the understanding of the delivered curriculum.

Purpose: To modify the PSA curriculum with diverse activities that will increase participant engagement while maintaining or improving participant knowledge gain.

Methods: A 1.5-day modified PSA training course was developed and tested in four different training sessions in Oregon (Aurora, Corvallis, Hillsboro, Hood River; 70 participants). Modifications included add-ons of fictional farm scenarios for assessing compliance status, real-time testing and interpretation of agricultural water samples, and photograph collections to evaluate wildlife intrusion and cleaning and sanitizing strategies. The pre- and post-training assessment scores from the modified trainings were compared to four standard PSA courses (Canby, Central Point, Hermiston, and Ontario; 85 participants; *t*-test, $P < 0.05$). Participant feedback was collected through evaluation forms and trainer observations.

Results: Assessment scores significantly increased ($P < 0.05$) between the pre- and post-test in all 8 PSA trainings, demonstrating that both training formats were effective in increasing the food safety knowledge of participants. Training format impacted the post-assessment scores in 3/25 questions (topics of biosolids, information guiding risk management actions, and wildlife control), with the standard training yielding higher knowledge gain. Notably, for both training formats, when asked what should guide risk management actions, the percent of participants answering correctly decreased between pre- and post-assessments by 91%/74% (standard) and 91%/84% (modified). Observations and evaluations from trainings indicated that the modified training enhanced participant engagement.

Significance: The modified PSA training format was equivalent to the standard training in overall food safety knowledge gain while enhancing participant engagement and improving instructor experience.

P2-14 A Review of Food Safety Education Programs for Produce Growers

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❖ ***Developing Scientist Entrant***

Introduction: Produce growers play an essential role on managing produce food safety. Farmer food safety education has become a hot topic in research and extension. An increasing number of publications report growers food safety programs, but the content and delivery methods have not been thoroughly reviewed.

Purpose: Evaluate produce growers' food safety knowledge and attitudes and the effectiveness of previous food safety education programs for growers.

Methods: A search of online databases, journal achieves, conference abstracts, and reference lists of relevant studies was conducted to locate published peer-reviewed artifacts on the topic of food safety knowledge and behavioral change amongst produce growers. Study selection criteria include (1) in English, (2) published between 2000 and 2019, (3) targeted at food safety topics for produce growers. The studies were summarized in six topics: agriculture water, soil amendment, animal management, worker health and hygiene, food safety plan and record-keeping, cleaning and sanitation, and general food safety information.

Results: Forty-two studies were reviewed, with 33 peer-reviewed journal articles, 2 government reports, and 7 IAFP conference abstracts. The majority of studies were published in the United States and in the past 5 years. A survey was the most commonly used method to collect data. Agriculture water and soil amendment were the two topics least understood by growers while worker health and hygiene were well understood. Only 13 studies evaluated food safety education interventions. Most studies used in-person workshops to deliver information and most used self-reported pre- and post-test to evaluate. Knowledge and attitude of food safety were reported to increase after the intervention, but many did not conduct a statistical analysis on the difference between pre and post-test.

Significance: Findings provide insights and guidance on the development of future grower food safety education. Gaps in coverage are areas that could benefit from further refinement were identified.

P2-15 How Oregon and the Western Region are Using the Food Safety Resource Clearinghouse

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Introduction: There is a national effort to create resources to support farms in their food safety efforts towards compliance with Food Safety Modernization Act (FSMA) regulations. This effort has been coordinated by federally funded regional centers, including the Western Regional Center to Enhance Food Safety at Oregon State University. The Northeast Center to Enhance Food Safety created the Food Safety Resource (FSR) Clearinghouse (go.uvm.edu/clearinghouse) to assist extension professionals, trainers, and growers with organizing and sharing FSMA-related resources.

Purpose: Use web analytics from the Clearinghouse to identify food safety themes most searched for by stakeholders in the Western Region (WR).

Methods: Google Analytics was used to track and tabulate activity on the Clearinghouse from March 1, 2018 to January 1, 2020, including number of users, page views, and types of documents viewed.

Results: As of January 1st, 2020, unique users (*n* = 1,544) from WR have accessed the Clearinghouse with 4,740 unique page views. California had the most users (*n* = 685) with Washington, Oregon, and Colorado each having 117-209 users. In Oregon, the most common downloaded resources included topics of food safety plan template, employee health and hygiene, container/equipment cleaning and sanitizing, building portable handwashing stations, and Technical Assistance Network (TAN) answers. This is in contrast to common topics from users in California, including: food safety plan template and audit checklist, agricultural water, cannery license program, and dropped produce.

Significance: These findings highlight differences in the educational needs in different states in WR. Using these data will help food safety educators in WR to better prioritize the development of region-specific outreach and resource materials. Additional initiatives are underway to promote the use of the Clearinghouse, increase membership and number of resources available, as well as to develop a peer-review process for materials added to the Clearinghouse. Social media campaigns are being launched to disseminate information.

P2-16 Preparing Oregon Produce Farms for Produce Safety Rule

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Introduction: The Produce Safety Rule (PSR) went into effect in January 2016, setting minimum standards for growing fresh fruits and vegetables. FDA started inspections in the 2019 growing season taking an "educating while regulating" approach. In Oregon, there is an estimate of 1,300 farms that are fully covered by the PSR.

Purpose: Provide education and technical assistance related to PSR to produce growers in Oregon.

Methods: Oregon's Farm Food Safety Team was formed in July 2018 through a cooperative agreement between Oregon Department of Agriculture (ODA; *n* = 4 members) and Oregon State University Extension Service (*n* = 5 members) to provide education and technical assistance to Oregon produce growers.

Results: From July 2018 to December 2019, FFST completed 25 Produce Safety Alliance (PSA) grower trainings, and 77 on-farm (12 in 2018, 65 in 2019) visits using the "On-Farm Readiness Review" (OFRR) approach developed by the National Association of State Departments of Agriculture (NASDA), State, FDA, and Cooperative Extension food safety specialists. Through standardized one-day and modified two-day PSA trainings the team reached >700 people. OFRRs were completed on a variety of farms, including mixed vegetable, berry crops, cherry orchards, and herbs, and ranging in size from very small to large farms. ODA personnel were also able to observe multiple FDA inspections.

Significance: Bringing together ODA and OSU staff as a team enabled both organizations to develop innovative ways to reach growers and assist them with understanding and complying with the PSR. As an outcome of these opportunities, the team has adapted the OFRR process to better align it with the observed focus of FDA inspectors. This includes records review and verification of approved use of antimicrobials in accordance with the label. Other educational efforts around inspections include the development of factsheets, listserv posts, and industry presentations.

P2-17 Impacts of Food Safety Education and Outreach on Florida Growers' Knowledge and Preparedness for PSR Inspections

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Introduction: The University of Florida Institute of Food and Agricultural Sciences (UF IFAS) and the Florida Department of Agriculture and Consumer Services (FDACS) collaborate 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 Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR).

Purpose: The objectives of this evaluation are to determine if PSA trainings and OFRRs are successful in improving the Florida growers' level of knowledge of the PSR, foundational food safety principals, and determine Florida growers' readiness for PSR inspection.

Methods: Trainers and lead trainers for the PSA curriculum, and Assessors for OFRRs have been developed throughout the state to assist in education and serve as resources for Florida growers. Pre- and post-test scores out of 25 points were compared using a *t*-test to determine if there was an increase in knowledge before and after completion of the PSA grower training *(n* = 1043*).* The level of preparedness of Florida growers for a PSR inspection was measured through online anonymous surveys completed by the OFRR team and submitted to National Association of State Departments of Agriculture and Rutgers University.

Results: A total of 107 PSA trainers and 19 PSA lead trainers have been developed geographically across Florida; 7 UF IFAS and 13 FDACS employees are trained as OFRR Assessors. Post-tests means (21.53/25) were significantly higher than pre-test means (18.13/25), indicating an increase in knowledge after the training *(t*=-1.72*, P* < 0.05). Of OFRR assessed farms *(n* = 40*)*, 47.50% met the requirements, 40% needed minor improvements, and 12.50% needed significant improvements to meet the FSMA PSR requirements; areas which required most improvement were preharvest water, sanitation, and worker training.

Significance: PSA Grower Training and OFRR program efforts continue to improve the knowledge and compliance levels of Florida farms regarding the FSMA PSR.

P2-18 Compliance of Farmers Market Vendors with the Produce Safety and Preventive Controls Rules

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❖ ***Developing Scientist Entrant***

Introduction: Farmers markets (FM) in the United States have grown rapidly over the past twenty years. Food producers and processors at these markets are diverse and with varying business scopes and sizes. Often direct market food businesses receive little food safety education or oversight therefore it is unclear if they are required to follow the newly implemented Produce Safety Rule (PSR) and Preventive Controls Rule for Human Foods (PC).

Purpose: The purpose of this study was to understand size and scope of farmers market vendors businesses to determine their regulatory compliance requirements; as well as better understand their educational needs.

Methods: Farmers market vendors were recruited in Virginia and North Carolina through e-mail listservs and asked to participate in a telephone interview to discuss their business practices. Questions were asked about food products produced, annual revenue, and food safety practices on the farm. Interviews are ongoing so data will be collected throughout the year.

Results: Most farms sold a combination of raw agricultural commodities and commodities that are rarely consumed raw. Of the thirty-two FM vendors interviewed, only one was covered by the PSR; no participants were covered by PC, and two exclusively sold fresh meat. The majority of vendors (94%, 30 of 32) sold produce at the FM. Eighteen (60%, 18 of 30) of the produce vendors also sold value-added products. Seventy-five percent (n = 24) of vendors were exempt from the rule based on total sales, and the remaining were qualified exempt. Some common themes related to risky behaviors included not using potable water or comingling livestock among fresh produce. These were identified as key areas requiring further education.

Significance: This study provides much-needed data on regulatory requirement trends associated with FM businesses and their educational needs. These interviews will aid in developing specialized, tailored training opportunities for vendors who do not full under PSR or PC.

P2-19 Evaluation of the Southern Regional Center for FSMA Training, Outreach and Technical Assistance Training Efforts

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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 vegetables wholesale merchants.

Purpose: The Southern Regional Center for FSMA Training, a consortium of 22 institutions, is 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 and FSPCA PCQI courses to assess short-term knowledge gains from September 2018-September 2019 across the South. A qualitative evaluation was conducted with participants of both courses four months after training to evaluate medium-term outcomes of behavior change related to food safety practices.

Results: FSPCA PCQI post-test scores (n = 108) were significantly higher than pre-test scores (T = -6.950, P < 0.001), indicating a significant increase in knowledge after participation in the training. Of seven practices included in the FSPCA PCQI behavior change surveys (n = 14) the most frequent behavior changes were implementation of new food safety plans (64%) and training employees on FSPCA rules (57%). PSA post-test scores (n = 1,303) were significantly higher than pre-test scores (T = -41.615, P < 0.001), indicating a significant increase in knowledge. Of 13 practices included in the PSA surveys (n = 61) the most frequent behavior changes were creation or modification of food safety record-keeping systems (56%) and beginning to write or modify farm food safety plans (52%).

Significance: Trainings conducted by members of the Southern Center have increased food safety knowledge and practices important for compliance with the PSR and PCHF.

P2-20 Comprehensive Agricultural Water Testing Laboratory Database for Texas Growers

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Introduction: Agricultural-water has been identified as a source of contamination in numerous produce-related foodborne illness outbreaks in the USA. Depending on the source, agricultural water can harbor a variety of foodborne pathogens and contaminate fresh produce. Therefore, the FSMA produce safety rule requires produce growers to test agricultural water. Having the necessary information will encourage growers to test agricultural water quality and be better informed.

Purpose: The goal of this project was to develop a comprehensive database for water testing laboratories in Texas to help growers to comply with the agricultural water requirements of the FSMA produce safety rule.

Methods: Water testing laboratory information was collected through personal visits or communication via telephone or email. To this end, data from 72 water testing laboratories was collected. The following information was recorded: lab name, location, type of the tests, website, contact information, and testing days. A survey tool was disseminated to evaluate the efficacy of the resource created at a statewide grower conference. The respondents (70) were provided with a brief description of the project and 10 open-ended questions. After the completion, the responses were collected and analyzed.

Results: The data showed that 93% of participants found this project useful and informative, and 86% of participants responded that the project will provide a huge benefit to growers in terms of information and the location of the labs. Furthermore, when participants asked whether they have limited past experience with water testing, only 29% of participants were familiar with water testing and had limited previous experience of where to test agricultural water.

Significance: This project is highly feasible and effective for growers by providing useful information and knowledge to facilitate water testing. In the long-term, the data generated by this project provides the useful tools to growers for agricultural-water testing and robust water quality data.

P2-21 Evaluate Food Safety Practices at Louisiana Summer Feeding Sites

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❖ ***Developing Scientist Entrant***

Introduction: The United States Department of Agriculture's Summer Food Service Program (SFSP) provided children under the age of 18 in low-income areas 145 million free meals in 2018. Food safety issues arise for the SFSP due to time-temperature controlled foods served, weather patterns in Louisiana, seasonal staff and short operation time, and lack of equipment.

Purpose: The purpose of this study was to perform a needs assessment of food safety at summer feeding sites to identify high-risk behaviors for different settings (institutional and non-institutional) through an observational study

Methods: A rubric following USDA food safety guidelines based on categories – hot holding, cold holding, cross contamination prevention, personal hygiene, and cleaning was developed as a tool for observational study. The rubric was used during observation of randomly selected Louisiana feeding sites to record food safety practices (n = 22). Data obtained during observations were compiled to determine high-risk behaviors and differences between institutional and non-institutional settings.

Results: Sixteen institutional and six non-institutional settings were observed. Non-institutional feeding sites had the most violations per site compared to institutional (s = 8 violations per site vs s = 4 for institutional). Limited staff and training, improper holding and equipment, and improper sanitation were the most common practices found. All non-institutional feeding sites were staffed by volunteers with limited food safety training, while institutional sites had staff trained with health-department approved training. Non-institutional sites all had improper hot and cold holding, mostly due to lack of equipment. The use of insulated coolers were popular with non-institutional sites, which provided little temperature control.

Significance: This study demonstrated that the unique challenges presented by location of summer feeding sites must have specifically targeted food safety education in order to prevent foodborne illness.

Poster

P2-22 A Pilot Evaluation of Two Water Safety Videos for Stakeholders by Individuals Familiar with Food Safety Modernization Act

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Introduction: Food Safety Modernization Act (FSMA) is the most comprehensive legislation related to safety of food products in >80 years. Vast majority of growers of raw agricultural commodities are required to meet new water safety requirements in their operations.

Purpose: The purpose of the current study was to pilot evaluation of two recently developed water safety videos' teaching methods as well as knowledge gain of participants based on six criteria: (A) Explanations in videos were clear; (B) I will change how I conduct this procedure based on videos; (C) I will change how I teach others this procedure based on information in videos; (D) Videos provided sufficient information; (E) The settings in videos helped me understand the topic; (F) Explanations in videos were at appropriate speed.

Methods: After watching videos entitled "Agricultural Water Testing: Sample Collection and Procedures (V1)," and "Production Water Standard Quality (V2)," participants (n = 13) with previous FSMA training rated videos using a 5-point hedonic scale. In addition to descriptive statistics, proportion of individuals strongly agree or somewhat agree (upper two scales) and those strongly disagree or somewhat disagree (lower two scales) were calculated and statistically compared at type I error level of 5%.

Results: For V1 and V2, 84.6 and 61.5% indicated explanations are clear, respectively. For both V1 and V2, 76.9% indicated settings in videos assisted them understand the topic. Responses in upper two scales for above-mentioned categories of A, D, E, and F were statistically higher than responses in lower two scales in V1 ($P < 0.05$), similar trends were also reported for V2.

Significance: Videos generated by collaborative endeavors of Iowa State and Tennessee State Cooperative Extension Programs are addressing issues of critical importance for FSMA stakeholders and could enhance better assimilation and adaption of water safety requirements of the legislation.

P2-23 Evaluation of Pet Owners' Knowledge and Practice of Handling Pet Food

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Introduction: Pet food has been identified as a source of pathogenic bacteria including *Salmonella* and *E. coli*. A recent outbreak linked to *Salmonella* contaminated pet treats infected over 150 people in the U.S. The mechanism by which contaminated pet food leads to human illness has not been elucidated. Pet owners' food safety knowledge and their practice of handling pet food have not been reported.

Purpose: This study evaluated pet owners' food safety knowledge and handling practice of pet foods using an online consumer survey.

Methods: The survey consists of 62 questions and assesses (1) owners' food safety knowledge and handling practices of pet foods; (2) owners' interaction with pets; (3) owners' risk perception related to their own health, their children's health, and their pets' health. The survey was pilot tested among 59 pet owners before distribution to a national consumer panel, managed by Qualtrics Inc. All participants (n = 1,040) were dog and/or cat owners in the U.S.

Results: Almost all pet owners interacted with their pets (93%) and most cuddled, allowed their pets to lick them, and slept with their pets. Less than one-third of pet owners washed their hands with soap after interacting with their pets. About half the owners reported washing their hands after feeding their pets. Most pet owners fed their pets dry pet food and dry pet treats. Some fed their pets raw meat or raw animal product (RAP) diets because they believed these diets to be beneficial to their pet's overall health. Many owners (78%) were unaware of pet food recalls or outbreaks associated with foodborne patho-

gens. Less than 25% considered dry pet foods and treats as a potential source of foodborne pathogens. Pet owners were more concerned when seeing news about pet becoming ill from pet food, and less concerned when seeing news about people becoming ill from pet food.

Significance: The findings will support the development of an effective consumer education program of safe pet-food-handling and the data collected can assist in developing more accurate risk assessment models related to pet food handling.

P2-24 Exploring the Food Safety Perceptions and Practices of Pet Owners and Provision of Hygiene Information Regarding Raw Meat-based Pet Diets

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Introduction: Concerns have been raised regarding potential health risks to pet-owners preparing/providing raw meat-based diets for pets. Pet-owners need to be adequately informed about potential microbiological hazards and proper food-safety practices, in order to minimize risks. With popularity of social media, pet-owners may utilize such opinion/experience-based platforms to obtain information regarding raw meat-based diets for pets.

Purpose: To explore pet-owners' perceptions and self-reported food-safety practices and explore online provision of food-safety information regarding raw meat-based pet diets.

Methods: An online-questionnaire regarding food-safety perceptions and practices was completed by pet-owners reporting to provide raw meat-based diets for pets (*n* = 179). Forum posts (*n* = 308) obtained from 'PetForums Community' archives, were reviewed using a content-analysis approach for emerging themes relating to 'raw-feeding' and 'food-safety'. Manufacturer/supplier websites (*n* = 33) were reviewed for provision of food-safety guidelines for pet-owners on raw meat-based diets for pets.

Results: The questionnaire determined the majority of pet-owners (95%) believed that raw-meat pet food was safe and 90.5% were 'very confident' in the safety of their preparation of raw-meat pet food. Pet-owners did not perceive any risk to themselves or others in their household from the practice. Three-fifths of pet-owners reported seeking information online and were 'very confident' that their online sources were 'reliable'. Online-forums and social media were cited as 'trusted' sources of information. However, the review of pet-owner forum posts indicated potential food-safety malpractices were frequently reported/promoted. Furthermore, 61% of manufacturer/supplier websites did not have food-safety guidelines for pet-owners providing raw meat-based diets for pets.

Significance: There is a need to ensure food-safety practices when pet-owners prepare/provide raw meat-based diets for pets. Although pet-owners indicate awareness of food-safety, potential food-safety malpractices were reported and food-safety information was lacking. It is essential for raw pet food manufacturers/suppliers and pet-owner forums to provide appropriate food-safety information for pet-owners providing raw meat-based diets for pets.

P2-25 A Content Analysis of Professional Food Handler Cognitive and Behavioral Food Safety Research Data

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Introduction: A significant contributing factor to foodborne illness can be the food safety practices of the food handler. Therefore, there is a need to have a clear understanding of professional food handlers' food safety cognition and behavior. This is particularly important for food and drink manufacturing/processing businesses given the quantity and distribution of products.

Purpose: To conduct a content analysis of published research papers detailing professional food-handlers' knowledge, attitudes, self-reported practices and observed behaviors in relation to food safety principles within commercial food environments.

Methods: Published food safety related research studies detailing cognitive and/or behavioral food handler food safety data (*n* = 100) were identified, reviewed and analyzed according to data collection methods, measures, settings and key food safety principles.

Results: One hundred studies spanning a 30-year period (1999-2019) were reviewed. The majority (78%) were published during the last decade (2009-2019). Data were obtained from 44 countries, indicating international interest. Most frequent countries of data collection included Malaysia (11%), Brazil (8%) and India (6%). The most commonly included data collection methods incorporated self-complete questionnaires (54%) and interviews (50%). Consequently, reviewed studies frequently reported data indicating knowledge (75%), self-reported practices (48%) and attitudes (39%). Observation of behavior was less frequently included (29%). Most often, studies focused upon food handlers providing food direct to the consumer; such as food handlers in catering settings (25%) and institutional/high-risk food service settings (e.g., schools, hospitals, nursing homes) (24%), other food handlers included mobile street vendors and retailers. Data specific to food handlers in manufacturing/processing environments were included in only 13 studies.

Significance: Given the volume of food produced by the manufacturing industry internationally, the behavior of food handlers in this setting is critical. However, observed food safety behavioral data of food handlers in manufacturing/processing food environments are particularly lacking. Understanding the complexities of food safety cognition and behavior in food manufacturing is especially critical in assessing, enhancing and embedding food safety culture.

P2-26 Could Educational Actions be a Starting Point to the Transition of Food Safety Culture?

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Introduction: Despite the implementation of food safety systems, foodborne diseases still occur globally. In this context, food safety (FS) culture has emerged as a risk factor as it can influence the behavior of people. FS-culture addresses the social context and experiences that shape the behavior.

Purpose: The purpose of the study was to investigate if educational actions could influence the transition of FS-culture in a proactive way in foodservice establishments.

Methods: A longitudinal design following action research was developed to monitor the FS-culture transition and to implement educational actions in foodservice of an army headquarter in Brazil. The FS-culture was assessed at three times during the study using a mixed-method approach; triangulated data were analyzed using an interpretation grid. From the results of the FS-culture assessment, the educations actions were developed aiming the transition of the FS-culture in the case study. The longitudinal study followed action research, to implement educational actions and to understand the transition of FS-culture. Food handlers (39) and managers (3) participated and were guaranteed by the ethics guidelines.

Results: The educational actions, which were based on the FS-culture assessment, revealed a change from a reactive to a more proactive FS-culture indicating that educational actions can initiate a cultural change. After the FS-culture transition based on the educational actions, the quality manager in the food service performed educational action, and it also sustained the transition of the FS-culture in a proactive way.

Significance: Our results showed that the assessment of FS-culture is a good starting point to initiate the development of educational actions and to change behaviors aiming the transition of a reactive into a more proactive prevailing FS-culture.

P2-27 War on Biofilms – A Joint Task Force Approach

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Introduction: Biofilms are ubiquitous in our environment, including our food factories. Here, their colonization and persistence can have a severe impact on the safety and quality of the products being made and, consequently, on the consumer and on the business operator. Effective control of biofilms is therefore essential to minimize this risk. Biofilms can only form given sufficient moisture and time to develop. Cleaning aids the removal of biofilms but does not inactivate the microbes. Studies have shown that the complex make up of a biofilm can afford it greatly increased resistance to chemical disinfectants. Consequently, an effective biofilm control strategy requires a multi-faceted approach.

Purpose: To produce an educational package that provides key information on biofilm development; their impact on food safety and quality; and the multi-faceted approach required to achieve their effective control.

Methods: Information on the composition and formation of biofilms; their impact on food quality and safety; and ways in which to effectively control them, was gathered, reviewed and summarized.

Results: Using the information gathered a poster presentation and a practical demonstration video were developed. These will be presented.

Significance: Development and application of simple and effective education and training related to food safety is essential. This training package is designed to provide key information relating to biofilms and practical advice on their multi-faceted control in an interesting and enjoyable format.

P2-28 Smart Food Safety: Remote Audit Using Smart Glass in EU Food Industries – Results, Limitations and Opportunities

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Introduction: The IAF MD 4:2018 document introduced a new policy for the use of information and communication technology (ICT) for Remote Audit. In 2019 FDA launched the "new era of smarter food safety." In line with the IAF and FDA suggestions, we decided to test in EU Food Industries Remote Audit using Smart Glass.

Purpose: Our purpose was testing the real usability of this smart tool inside a real working establishment.

Methods: We developed a specific checklist to test the usability on the floor, the critical factors and all possible improvements. We identified different types of food plants to stress the tool usability. We divided a real FSMA PC HF Audit in 2 h block Audit respecting the battery life of the Glass.

Results: We audited 11 different food establishments: Dairy (n = 3), Olive Oil Mill (n = 2), Fresh Produce Consolidator (n = 1), Canners (n = 3), Bakery (n = 1), and Pasta Plant (n = 1). The greatest limitation was noise inside the plant (25%) and the WiFi connection (20%). Photos and quality streaming videos confirm the usability of the tool (55%). We identified improvements like the need for specific training for pilot and auditor and an update version with earphones to mitigate the environment noise.

Significance: Cost reduction, reduction of entry of external people inside the plant, greater safety on workplace, less environmental impact related to travel needs. This audit will be the future considering the possibility of conducting sessions simultaneously, connecting different entities for example Health Authority and clients.

P2-29 Food Safety 2020: New Trend, New Fashion. Smarter Is Better!

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Introduction: Food safety is in a period of full changing, development, evolution and why not, fashion. Considering US FSMA to the Canadian SFCR to the new Chinese food safety e-commerce regulation to the IAF MOD 4:2018 Remote Auditing, what is the feedback from food operators?

Purpose: Understanding food safety is not simple. New opportunities are spreading around the "Smart" definition. We explained to food business operators how to choose the right tools for the right needs avoiding loss of resources in a period of economic instability.

Methods: We started from the new era of smarter food safety an FDA policy, a CFIA goal, an EFSA dream, and an Asian aspiration. We explored all this ocean of new tools from Blockchain to Augmented reality, from Whole-genome sequencing to Big Data, from Artificial intelligence to quantum physics comparing positive and negative effects in real food industries management using EU food industries model.

Results: The EU Commission is increasing its annual investments in AI by 70% under the research and innovation program Horizon 2020. It will reach EUR 1.5 billion, but many food operators are not comfortable with new technology. The European Union has already allocated €180 million to support R&I in Blockchain and in food used by 7% in food manufacturers. Big data are at a starting point. According to quantum physics, whatever food we eat can be transformed into building blocks for a happy, peaceful human being with our thoughts, beliefs, and intentions. There is a lack of awareness about that in EU SMEs (99.9 % of all industries).

Significance: The "perfect food safety project" is right around the corner. Smart tools could effectively help to achieve the goal of food safety, but alone are insufficient without the human factor. Right Behaviors remain a fundamental pillar of food safety.

P2-30 Evaluation of Dietetic Students' Food Safety Knowledge and Attitudes: A Multistate Study

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Introduction: Nutrition and food safety are critical to consumer health, especially high-risk consumers. Registered Dieticians (RD) are regulated by law and are viewed by consumers as a trusted authority for food safety information. Despite incorporation of food safety education in curricula for RDs, deficiencies in RDs' food safety knowledge have been identified.

Purpose: Evaluate and compare food safety knowledge and attitudes of dietetic students enrolled in programs across three programs in the United States.

Methods: Surveys were distributed to students enrolled in accredited dietetics programs. Students from California State University (CSU) (N = 14), The Ohio State University (OSU) (N = 104), and Purdue University (PU) (N = 64) completed the survey. IBM SPSS Statistics (Version 26.0, Armonk, NY: IBM Corp.) was used for statistical analysis. The significance level was 0.05.

Results: Less than half (47%) of dietetic students reported receiving food safety certifications as part of their degree, but most students (66%) reported their dietetics degree prepared them to disseminate food safety information to patients. Seventy-four percent of students were interested in learning more about food safety for at-risk populations. *Campylobacter* (31%) and *Clostridium* (46%) were the least known pathogens among students. Students understood thermometer use is the safest way to determine if meat and poultry are cooked thoroughly (CSU-86%, OSU-96%, PU-86%), but few students knew the recommended cooking temperature for ground beef (CSU-27%, OSU-2%, PU-36%). Knowledge of handwashing recommendations after feeding or handling pets was significantly lower among OSU students (52%) compared to CSU (79%) and PU (83%) students. PU students were least aware that washing raw poultry could lead to cross-contamination (22%) compared to OSU (47%) and CSU students (55%).

Significance: Mastery of food safety knowledge varies widely among dietetic students from different accredited universities. Evaluation of university food safety curriculum and teaching methods is needed to enhance the depth and retention of knowledge among dietetic students.

P2-33 *Salmonella*, *Enterococcus* and Pathotypes of *Escherichia coli* from Irrigation Water on Farms in Mexico.

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❖ ***Developing Scientist Entrant***

Introduction: Irrigation water is recognized as a common source of antibiotic resistant-foodborne pathogen contamination in agriculture

Purpose: To identify foodborne pathogens from irrigation water samples from farms of México (states of Chihuahua, Puebla and Veracruz), and determine their virulence and antibiotic resistance genes.

Methods: A total of 30 irrigation water samples (10 samples of 1 L per state) were collected from farms in Chihuahua, Puebla and Veracruz in Mexico. *Salmonella* spp, *Enterococcus* spp, and *E. coli* (EPEC, EHEC, EAEC, ETEC) were detected by inoculating aliquots of samples in enrichment broths and plating onto selective agars. According to their phenotypic characteristic, five typical colonies were selected from each agar and confirmed by PCR (detecting genes related to toxins, adhesins and invasion virulence factors). Each isolate was analyzed for antibiotic resistance to ampicillin (10 µg/mL), cefoperazone (30 µg/mL), vancomycin (6 µg/mL, erythromycin (30 µg/mL), polymyxin B (30 µg/mL), colistin (4 µg/mL), ciprofloxacin (5 µg/mL), trimethoprim (25 µg/mL), tetracycline (30 µg/mL) and rifampin (30 µg/mL) by plating on agar.

Results: A total of 496 presumptive isolates of generic *E. coli*, *Enterococcus* and *Salmonella* were obtained from irrigation water, and according to the identification of virulence genes, eight pathogenic bacteria were detected: two *Enterococcus* spp (*eda*$^+$, *ccf*$^+$, *efa*$^+$, *gelE*$^-$), two tEPEC (*eae*$^+$, *bfp*$^+$), and one aEPEC (*eae*$^+$, *bfp*$^-$) were isolated from irrigation water from Veracruz, and two aEPEC (*eae*$^+$, *bfp*$^-$) and one ETEC (*lt*$^+$) from Puebla. The *Enterococcus* isolates were resistant to cefoperazone, polymyxin B and colistin. All *E. coli* isolates were resistant to vancomycin. In general, all isolates were susceptible to ciprofloxacin, trimethoprim and rifampin. All isolates were resistant to two or three of the antibiotics tested.

Significance: These results suggest that although at low levels, pathotypes of *E. coli* strains are present in irrigation waters of Mexican states and could represent a health human risk.

P2-36 From Feed to Fork: Characterization of *Salmonella* spp. and *Escherichia coli* from Selected Swine Feed Mills and Their Relatedness to Historic Isolates from the Pork Production Chain

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne pathogens in feed and feed mill environments and their potential transmission and spread into the food production chain have increased attention toward feed safety. Enteric bacteria such as *Salmonella* and *Escherichia coli* have been detected in feed products. These pathogens are also commonly linked to foodborne outbreaks.

Purpose: The objectives of this study were to: i) characterize *Salmonella* and *E. coli* isolates from feed mill environments by Whole Genome Sequencing (WGS); and ii) identify relatedness with historic isolates from the pork production chain.

Methods: Samples (405) were obtained from feed mill facilities located in the US Midwest area. Each sampling site was analyzed for the presence of both *Salmonella* and *E. coli* with culture methods (following FSIS guidelines) and then confirmed by PCR. WGS was carried out on either Illumina MiSeq or NextSeq sequencer. *De novo* genome assemblies were obtained with the Shovill pipeline and serotypes determined with the SeqSero2 v1.0.2 prediction tool for *Salmonella* and Serotype Finder for *E. coli*. Isolates relatedness was determined using NCBI pathogen detection tools.

Results: *Salmonella* confirmed isolates (27) were grouped in 10 clusters with a wide range of other isolates from the database. Strains were matched with environmental, feed, pet food, swine and pork products isolates. Fifty-two % were clustered with clinical samples. None of the confirmed *E. coli* isolates (16) carried *stx1/stx2* genes. The strains were typed into 12 *E. coli* O groups (O8, O18, O25, O34, O36, O54, O71, O104, O133, O156, O159, O160) and 10 H groups. Some of these serogroups were observed in other studies investigating *E. coli* prevalence through the pork chain.

Significance: This study shows the presence of *Salmonella* and *E. coli* in the feed mill environment. Additional research is warranted to determine if their presence indicates potential transmission into the pork production chain.

P2-37 Food Defense – It is Time for Consensus

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Introduction: No fewer than six food standards organizations have specified expectations for food defense programs, yet there is a lack of uniform definitions creating confusion and potentially leading to the inadequate protection of the food supply.

Purpose: This work investigates the imprecise use of terminology and the benefits of utilizing risk assessments to identify nodes at which the implementation of protective measures can diminish the consequences of an intentional adulteration of food products.

Methods: Three food defense assessment methodologies were applied at 90 facilities manufacturing various foods. The findings are evaluated and limitations of the methodologies discussed. Many erroneously accept that threat, vulnerability and risk are interchangeable but this study attempts to demonstrate the necessary distinctions and their roles in risk.

Results: This work evaluated risk and two measures of vulnerability. The difference between the number of risks (RE) and the number of vulnerabilities (VE) is significant, $t(90) = 6.59$, $P < 0.0001$ as is the difference between the number of risks and the number of APSs, $t(90) = -4.92$, $P < 0.00001$. The means for RE (M = 1.73, SD = 3.59), VE (M = 8.80, SD = 9.63), and APSs (M = 10.92, SD = 17.57) show that the risk assessments' more thorough evaluation of the process steps' likelihoods of being exploited in an intentional adulteration results in fewer process steps requiring protection.

Significance: The work described herein can provide public health protection at a level superior to the wide-scale harm level referenced in the Intentional Adulteration regulation. Risk has historically been the preferred measure of when potential health effects are unacceptable and should be utilized in food defense efforts.

P2-38 Food Authenticity: The Use of RT-PCR to Detect Contamination in Halal Meat

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Introduction: The significant growth of the Halal food market presents opportunities to all players in the food industry. The Codex Alimentarius CAC/GL 24-1997 provides the General Guidelines for use of the term Halal regarding lawful "permitted" food, additives .... But trust in halal food is fragile, and smart approaches should be developed to ensure halal authenticity. Halal certification requires that meat samples be free from porcine products.

Purpose: A real-time PCR (RT-PCR) assay was developed to detect porcine DNA using the supplied primer/probe set specific for mitochondrial DNA sequence specific to pork species. The purpose of this study is to determine i) the sensitivity of the method, and ii) the ratio of possible food contamination or fraud on a panel of more than 500 samples.

Methods: Raw and processed meat samples were prepared with various levels of pork meat and analyzed with the RT-PCR method to assess the sensitivity of the methods. Afterwards, a total of 507 samples supposed to be Halal were tested using the developed swine detection method.

Results: The swine method detects both raw and processed pork and provides sensitivity down to 0.0005% porcine DNA, enabling an efficient detection of pork meat in Halal food samples. A significant proportion of 507 meat samples presented traces of pork DNA. Such small amounts of pork DNA might end up adulterating the final products due to accidental contamination during processing, thus rendering it Haram, or non-permitted.

Significance: The developed RT-PCR assay ensures a sensitive detection of porcine species in Halal meat samples. The present study highlights the importance of implementing specific and sensitive surveillance methods to ensure the authenticity of Halal products.

P2-39 Validation of a Lateral Flow Device for the Detection of Abrin in Foods

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Introduction: Abrin is an extremely potent bio-toxin produced from the seed of the tropical plant *Abrus precatorius,* commonly found in the southern U.S. and the Caribbean. Abrin toxin inhibits the synthesis of proteins in the cells of an exposed individual, causing severe illness and cell death. Due to the lethality and ease of dissemination, a rapid screening method for the detection of abrin toxin in foods is necessary for biodefense purposes.

Purpose: This study's objective was to provide a lateral flow screening method, combined with a single optimized extraction for preliminary and confirmatory detection of abrin toxin in foods.

Methods: To determine the LOD, three brands each of hot dogs, liquid infant formula, and liquid eggs were fortified at five levels of abrin in quadruplicate and cold stressed overnight. Samples were homogenized with GBS and the toxin was extracted via centrifugation. Following extraction, a 150 µL aliquot of the aqueous layer was analyzed utilizing commercially available LFDs and read at both 15- and 25-minute intervals. A 100 µL aliquot was removed and analyzed using the FERN Abrin ELISA method for result confirmation. The full-scale validation incorporated replicate sets in three fortification levels and analyzed in duplicate.

Results: Differing matrices exhibited a degree of variance for the limit of detection (5 µg/g-10µg/g). The sensitivity for the matrices evaluated ranged from 83.3% (15/18) to 100% (18/18) dependent on the fortification. All matrices examined demonstrated 100% specificity (9/9).

Significance: The data from this study suggests that the extraction procedure, combined with the LFD detection assay, may offer a suitable screening method for the detection of abrin in foods. This study has been submitted for review as an official FERN screening method and offers a single extraction for both the preliminary and confirmatory test.

P2-40 Luminescence-based Detection of Pathogenic Bacteria Using Engineered Bacteriophage

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Introduction: Novel methods for detection of foodborne pathogens utilizing engineered bacteriophage that are rapid and sensitive can improve on existing PCR and culture counting methods.

Purpose: The objective of this study is to identify bacteriophage with specific affinity to pathogenic *E. coli* O157:H7 and characterize luminescence based detection of viable bacteria.

Methods: Bacteriophage were first isolated from sewage samples by harvesting plaques on standard culture plates. Next the isolated bacteriophages' affinity to *E. coli* O157:H7 was determined by measuring changes in the optical density of culture isolates over time in response to individual bacteriophage. Then down-selected bacteriophage with high affinity were genetically engineered to produce a luminescent reporter enzyme. These engineered phage were combined with bacteria incubated in LB media and diluted in buffer (100 microliter) and a luminol substrate in a microwell. The light intensity of the microwell over time was measured with a luminometer.

Results: From the raw sewage sample, 22 isolates were harvested and characterized through nucleic acid analysis. Down-selection identified 7 isolates with affinity to multiple *E. coli* O157:H7 strains in addition to other isolates with affinity for *Shigella* and *Salmonella* sp. The lower limit of detection was defined as the average of the negative control sample plus 3x the standard deviation ($n \geq 3$). The detection limit of 8, 23, and 1,667 cells at 60, 40, and 20 min, respectively, was determined using a linear fit ($n \geq 3$).

Significance: The results demonstrate successful isolation and genetic engineering of luminescent bacteriophage for rapid and sensitive of viable bacteria. Future work will characterize performance in food matrices spiked with pathogenic bacteria to develop novel detection methods for food safety and defense.

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P2-42 Antiviral Activity of Chitosan Microparticles Against Bacteriophage MS2, a Human Norovirus Surrogate

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❖ ***Developing Scientist Entrant***

Introduction: Human norovirus, the leading cause of foodborne illnesses worldwide, has a low infectious dose, and is environmentally persistent. Therefore, effective decontamination of norovirus in water is necessary to control its transmission to food. Chitosan microparticles (CM), derived from chitin, may be a potential chemical antiviral treatment.

Purpose: To investigate the antiviral activity of CM against bacteriophage MS2, a cultivable human norovirus surrogate.

Methods: CM suspension in water was prepared through the ionic gelation of acidified chitosan solution by cross-linking particles using sodium sulfate. CM was quality assessed against the MS2 host *E. coli* using the macrodilution broth method and size-characterized with dynamic light scattering. The efficacy of CM (diluted in PBS, pH 7.2) against MS2 infectivity in suspension was assessed with plaque assay using an initial viral titer of 7 log PFU/mL. Genome integrity of MS2 in suspension was assessed with reverse-transcriptase quantitative PCR (RT-qPCR), with an initial titer of 8 log RT-qPCR units/mL. The experiments were performed in triplicate and utilized MS2 in PBS as a positive control.

Results: The CM preparation had 6% (w/v) dry weight and a particle size of approximately 0.5-1.0 µm. The assessment of CM revealed a minimum inhibitory concentration of 0.006% (w/v) and no noticeable interference with the infectivity assay. Within a 15-min contact time with 0.3% CM, the infectious titer of MS2 decreased to limit of detection 1.85-log PFU/mL ($P < 0.05$). Similarly, the viral genome of MS2 treated with up to 0.01% CM decreased to limit of detection 3.3 log RT-qPCR units/mL ($P < 0.05$). Further research focuses on antiviral mechanistic studies and assessing the impact of CM on Tulane virus (another surrogate) and human norovirus.

Significance: CM appears to be effective on viral genome and virus infectivity and shows promising potential as an antiviral treatment for improving the safety of food and water.

P2-43 Regulatory Frameworks and the Role of Land Grant Institutions in the Legalized Cannabis Edibles Market

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❖ ***Developing Scientist Entrant***

Introduction: Foods infused with marijuana (or 'edibles') carry particular public health concerns (i.e. microbial contamination, consistent dosing, etc.). Edible formulation and analysis are challenging due to a lack of standard methods and variability in composition. The industry is growing faster than researchers and regulatory bodies can monitor and oversee it.

Purpose: This study investigates state institutions' interactions with the legalized recreational marijuana industry.

Methods: Appropriate representatives from Land Grant universities, Cooperative Extensions, and state Departments of Agriculture were contacted in the ten states and Washington D.C. where recreational marijuana is legal (27 institutions in total). Representatives were asked to participate in 20-minute phone interviews comprising standard questions regarding the marijuana industry and their agency's interaction with businesses. Written policies were obtained via representatives or websites. Questions, confidentiality, and data handling procedures were approved by the University of Maine's Institutional Review Board for the Protection of Human Subjects.

Results: One interview was obtained conducted, three representatives declined an interview but provided written policies, and 23 declined participation or did not respond. Of the 27 institutions, 18 demonstrated little-to-no interaction with the marijuana industry. Seven institutions offer limited support and two provide extensive support. Regulatory bodies are generally more forthcoming with policies, while other institutions are hesitant to discuss positions. There is significant variability in the departments that oversee regulation in legalized states, (i.e. Commerce vs. Agriculture), which may contribute to continued uncertainty regarding aspects of edible production.

Significance: The focus of research and regulation to date has been on smokable marijuana, leaving gaps regarding other ingestion vehicles and compounding public health effects concerns. Additional clarity is needed regarding appropriate resources for stakeholders in areas such as food production and safety. The extent to which Land Grant researchers and Cooperative Extension agents can provide this support remains unclear.

P2-44 Supporting FSMA Compliance for California's Regional Food Hubs through Training and Technical Assistance

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Introduction: Food hubs aggregate locally grown produce and facilitate access to restaurants, schools, retailers and other institutions. The Food Safety Modernization Act (FSMA) has created a need to support food safety program development for food hubs.

Purpose: The goal of this study was to develop FSMA training materials and food safety plan development resources for food hubs.

Methods: We developed and delivered three webinars explaining FSMA status for food hubs, Preventive Controls for Human Food (PCHF) Rule exemptions, and steps to take if fully covered by the PCHF Rule. Additionally, we delivered PCHF Qualified Individual training to food hub staff members. In-person technical assistance was offered to food hub managers to develop a food safety plan. Site visits for 10 individual food hubs were conducted with the project food safety specialists to verify flow diagrams and hazard analysis.

Results: Learning outcomes for the webinars and PCQI training were determined by retrospective post-test and participants were asked to rate their knowledge of the PCHF Rule and implications for their food hub as 1) no knowledge, 2) somewhat knowledgeable, or 3) very knowledgeable. Prior to training all attendees identified as either somewhat knowledgeable (67%) or having no knowledge of FSMA (33%) (n = 12). Following the training 50% felt very knowl-

edgeable and 50% were somewhat knowledgeable. A pre- and post-test was utilized to determine individual understanding of GMP and Preventive Control implementation as a result of the site visit with food safety specialists. Products of the project include two model food plans for food hubs, model sanitation standard operating procedures, and a list of common food safety hazards for food hubs.

Significance: Food hubs represent a unique model for aggregation and distribution of local foods and supporting the success of these small businesses includes ensuring implementation of a food safety system.

P2-45 Contamination Concerns Drive Local Illinois Health Inspectors Interpretation of the Food Code as It Relates to Share Table Food Recovery

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❖ ***Developing Scientist Entrant***

Introduction: Food recovery methods such as share tables, a place where children place unconsumed food and beverage items that they choose not to eat, provide new opportunities for food redistribution through donation, re-service, or use as a cooked ingredient for future meals.

Purpose: The purpose of this qualitative study is to understand what drives differences in health inspector interpretations of the food code as it relates to share tables.

Methods: A snowball sampling technique was used to identify local Illinois health inspectors engaged in share table inspections. Interview questions were developed using a review of the literature and feedback from key stakeholders, including administrators from state and local health departments and school nutrition administrators. Telephone interviews were audio-recorded and transcribed verbatim. Interview transcripts were coded independently by two trained researchers using inductive content analysis.

Results: Our findings show that the common documents used by health inspectors (n = 13) to guide share table inspections are the food code (state, n = 3; federal, n = 3; scope unspecified, n = 4), Illinois state inspection sheet (n = 3), The Use of Share Tables in Child Nutrition Programs USDA Memorandum (n = 4), and the 2013 Illinois Department of Health "Sharing Tables" at Schools Memorandum (n = 4). Many of the interviewees (n = 7) were not aware of that Illinois Department of Health memo. Reported restrictive and lenient enforcement of the food code as it relates to share table inspections were linked to the health inspector's views on contamination and interpretation of the food code. Although all interviewees were concerned with temperature, contamination was the primary factor that inspectors thought that may pose a risk for foodborne illness. Our findings suggest health inspector's concerns are driven by food safety rather than quality.

Significance: While inspectors are primarily focused on preventing foodborne illness from share table food recovery, specific policy interpretation varies across health inspectors.

P2-46 Inactivation of Shiga Toxin-producing *Escherichia coli* and *Listeria monocytogenes* within Plant Versus Beef Burgers Using High Pressure Processing

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Poster

Introduction: As it is for raw beef, pathogenic bacteria may be present in plant-based proteins if such products were manufactured with contaminated raw materials and/or due to post-processing contamination. Thus, interventions are needed to enhance product safety and extend shelf life of burgers made with plant proteins.

Purpose: Quantify inactivation of Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* (Lm) in plant burger samples compared to beef burger samples following exposure to high pressure (HPP).

Methods: Plant burgers or ground beef were separately inoculated with the STEC or Lm cocktails (ca. 7.0 log CFU/g) and hand mixed. Refrigerated (i.e., 4°C) or frozen (i.e., -20°C) samples (25 g each) were subsequently subjected to pressure levels of 350 MPa for up to 9 or 18 min or 600 MPa for up to 4.5 or 12 min, respectively (N = 3, n = 3).

Results: When refrigerated samples were treated at 350 MPa for up to 9 min, levels of STEC were reduced by ca. 0.7 to 1.3 log CFU/g; however, levels of Lm remained unchanged (ca. ≤0.3 log CFU/g decrease) in plant samples and were reduced by ca. 0.3 to 2.0 log CFU/g in ground beef. For refrigerated plant and beef burger samples treated at 600 MPa for up 4.5 min, levels of STEC and Lm were reduced by ca. 0.3 to 5.6 log CFU/g. Similarly, when frozen plant and beef burger samples were treated at 350 MPa up to 18 min or to 600 MPa for up to 12 min, reductions of ca. 0.6 to 3.6 log or ca. 1.8 to 4.4 log CFU/g, respectively, were observed in STEC and Lm numbers.

Significance: These data established the efficacy of HPP as an effective post-processing intervention for inactivating cells of STEC and Lm in refrigerated or frozen plant burger and ground beef samples without causing untoward effects on product color.

P2-47 Impact of Fat Content on Resistance of Escherichia coli to High Pressure Processing of Beef and Yogurt

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❖ ***Developing Scientist Entrant***

Introduction: High pressure processing (HPP) is used to reduce or eliminate pathogens and spoilage bacteria on food to maintain safety and extend shelf life. The inactivation of *Escherichia coli* by HPP is dependent on several factors including the food matrix.

Purpose: The aim of this study was to investigate impact of fat on pressure resistance of *E. coli* in beef and yogurt.

Methods: Four ground beef (10 g) and five yogurt (10 mL) samples with different fat content were inoculated with 10^8 CFU/g of pressure-sensitive or -resistant strains of *E. coli* and treated at 600 MPa, 20°C or 30°C for 3 min. To eliminate the effects of adiabatic heating, samples temperatures were adjusted prior to pressure treatment. Cells were recovered on Luria-Bertani agar and data were subjected to ANOVA to determine differences among means. Experiments were performed in triplicate.

Results: The impact of HPP was dependent on strain and fat content. In beef with >15% fat, resistance of all strains decreased (P < 0.05). At 20°C, an increase in fat from 15.5 to 24.4% increased treatment lethality from 3.2 to 9.3 ± 0.6 log CFU/g for pressure sensitive *E. coli* MG 1655. In ground beef with 35% fat, pressure resistant *E. coli* DM 18.3 was reduced by only 4.0 log CFU/g when processed at 20°C but reduced by 9.0 log CFU/g when processed at 30°C. In yogurt, the impact of the fat content on treatment lethality was limited. The reduction of cell counts ranged from less than 2 to more than 8 CFU/g, depending on strain.

Significance: The pressure resistance of *E. coli* is dependent on strain, fat content, food matrix and adiabatic temperature changes. Adiabatic temperature changes are important to consider when investigating the pressure resistance of *E. coli* in a food matrix.

P2-48 High-Pressure Thawing (HPT) and Inactivation of Salmonella spp. in Raw Ground Chicken

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❖ ***Developing Scientist Entrant***

Introduction: Conventional thawing of meats requires tempering products at refrigerated temperatures for a few hours or days. Ambient conditions and the rate at which thawing takes place have an impact on the safety, structure, and quality of the meat. The longer it takes to thaw frozen meats, the greater the chance for microbial growth and quality losses. Therefore, a quick thawing method is needed by meat processors to mitigate these safety and quality losses.

Purpose: The purpose of this study was to determine the effects of high-pressure thawing (HPT) at 220 MPa on the inactivation of *Salmonella* spp.

Methods: A five-strain cocktail of *Salmonella* spp. was inoculated into raw ground chicken prior to freezing at -20°C. Samples were HPT at 220 MPa, either at 4 or 25°C, for 5, 7 or 10 min of pressure holding time, 3 samples were used for each hold time and the experiments were run in triplicate. Surviving cell were recovered from 10 g of the treated sample after 24 h of incubation at 35°C on TSA at room temperature for 2 h and overlaid with XLD. Differences in survival counts before and after each process (freezing, HPT) were used to determine log reduction of *Salmonella* spp.

Results: Preliminary results showed that freezing reduces microbial loads by 0.5 log CFU/g. When combined with HPT at 4°C, microbial loads in frozen ground chicken (log CFU/g) were reduced further: 2.56 ± 0.10, 2.41 ± 0.11, and 2.59 ± 0.22 for 5, 7 and 10 min, respectively. When fluid temperature was increased to 25°C, log reductions (log CFU/g) were 2.10 ± 0.08, 2.40 ± 0.1, and 2.25 ± 0.20 for 5, 7, and 10 min, respectively. More tests are underway to confirm whether effect of fluid temperature is significant.

Significance: HPT can be used to reduce the microbial load in frozen ground chicken. Future research will evaluate whether applying HPT in multiple cycles enhances *Salmonella* spp reduction.

P2-49 Thermal, Pressure and Shear on the Inactivation of *Lactobacillus brevis* and *Bacillus cereus*

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❖ ***Developing Scientist Entrant***

Introduction: Ultra-shear technology (UST) exposes bacterial cells to lethal factors including pressure, shear and heat. Limited studies evaluated the sensitivity of bacteria to these lethal factors.

Purpose: The aim of this study is to investigate the inactivation of two bacteria (*Lactobacillus brevis* and *Bacillus cereus*) by ultrashear applied at 400 MPa and 40°C and 70°C.

Methods: Cultures of *L. brevis* (1.6×10^{10} CFU/mL), a non-spore former, and *B. cereus* (3.2×10^{8} CFU/mL) a spore former, were suspended in HEPES buffer (pH 7.3) and tested in this study. Bacterial suspension was processed using a bench scale ultra-shear equipment at 400 MPa at 40°C (temperature contribution to microbial lethal effect ignored) or 70°C (temperature contributes to microbial lethal effect). The product exits through a shear valve at two different shear flow rates (0.41 ± 0.10 g/s and 1.05 ± 0.06 g/s). UST treatment time was varied with or without addition of 1.5 m long holding tube. The number of survivors was enumerated on lactobacilli MRS agar (*L. brevis*) and nutrient agar (*B. cereus*). Additional isothermal (40°C/70°C) and high-pressure processing (HPP; 400 MPa, 40°C or 70°C) experiments were carried out to evaluate thermal only, pressure only and combined pressure-thermal effects. Treatments employed two independent process and biological replications.

Results: The process come-up time for thermal (70°C, 0.1 MPa), HPP (70°C, 400 MPa) and UST (70°C, 400 MPa) were 10, 30, and 3-10 (depending on flow rate) seconds, respectively. This resulted in respective maximum reductions of 0.7, 8.4, 7.1 log for *L. brevis* and 0.6, 2.3, 2.1 log for *B. cereus*. Within the study conditions, shear flow rate and residence time, did not show significant ($P > 0.05$) influence on microbial inactivation for both strains.

Significance: This study evaluated the influence of pressure, temperature, and shear on two bacterial strains using batch and continuous high-pressure treatment. The study contributes to the development of safe harbor UST process conditions for beverages.

P2-50 Sensitivity of *Staphylococcus aureus* to Mild Elevated Hydrostatic Pressure and Nisin in HEPES Buffer

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❖ ***Developing Scientist Entrant***

Introduction: *Staphylococcus aureus* is one of the leading causes of healthcare-associated infections in the United States and is an important cause of foodborne diseases in community. Food products are one of main reservoirs of this pathogen.

Purpose: Current study investigated effects of exposure to mild elevated hydrostatic pressure and hydrostatic pressure with nisin for inactivation of *Staphylococcus aureus* in buffered environment.

Methods: Hydrostatic pressure at 450 MPa and 350 MPa with addition of nisin (5,000 IU/mL) were applied for reduction of a four-strain mixture of *Staphylococcus aureus* in HEPES buffer at 4 and 40°C for 0 (control), 1, 3, 5, and 7 minutes. Results were statistically analyzed using Proc GLM of SAS by Tukey-adjusted ANOVA. *D*-values were additionally calculated using best fitted (maximum R^2) linear model. The study was a randomized block design with two biologically independent repetitions.

Results: Prior to exposure to treatments at 4°C, counts of the pathogen were 7.95 ± 0.4 log CFU/mL and were reduced ($P < 0.05$) to 6.44 ± 0.3 log CFU/mL after 7 minutes of treatment at 450 MPa. The *D*-value associated with this treatment was 5.34 ($R^2 = 0.72$). At 40°C, counts were 8.21 ± 0.7 and 5.77 ± 0.3 log CFU/mL before and after the 7-min treatments, respectively. The *D*-value associated with 40°C treatment was 3.30 ($R^2 = 0.62$). Addition of nisin at 350 MPa lead to similar pathogen reductions and inactivation indices indicating that a lower pressure augmented with a bacteriocin could be as efficacious as a more intense treatment.

Significance: Results of this study could be incorporated as a part of predictive public health microbiology modeling and risk assessment analyses for prevention of foodborne infection and toxicoinfection associated with *Staphylococcus aureus* and for meeting the regulatory requirements such as Food Code, HACCP, and Preventive Control for Human Foods rule of FSMA.

P2-51 Novel Processing of Dried Beef Products (Biltong) without Antimicrobial Intervention to Achieve USDA-FSIS Validation of *Salmonella* (5-log Reduction)

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❖ ***Developing Scientist Entrant***

Introduction: Biltong, a traditional South African style dried beef product, is manufactured without a heat lethality step, yet it must still achieve USDA-FSIS required 5-log reduction of *Salmonella* for sale in the USA.

Purpose: Our objective was to examine a process for the manufacture of biltong that achieves a 5-log reduction of *Salmonella* without a heat kill step or the use of antimicrobial interventions.

Methods: Beef obtained through a broker was sliced thick (1 in x 2 in x 3 in) and inoculated with a 5-serovar mixture of *Salmonella*. The beef was processed by vacuum or manual tumbling, with spice, salt, and vinegar, and marinated for either 30 min or overnight (16-18 h). The beef was then dried in a temperature controlled humidity oven (73-77°F/23-25°C; 55% RH) for 6-8 days.

Results: Both short-term and overnight marination regimens, using only a spice/vinegar marinade and drying at the specified temperature/humidity levels, provided >5-log reduction of *Salmonella*. Water activity levels of < 0.85 or below were achieved within 4-7 days of drying. All trials were performed in triplicate replication and repeated measures one-way ANOVA to determine significant differences ($P < 0.05$) among the various treatments.

Significance: This work provides the first published validation of a biltong process demonstrating a > 5-log reduction of *Salmonella* spp. without a heat-lethality step or the need of additional antimicrobials. This should be beneficial to processors who cannot manufacture and sell biltong unless they use a 5-log validated process.

P2-52 Efficacy of Liquid Smoke Fractions to Control Infestation of the Ham Mite, *Tyrophagus putrescentiae* (Schrank), in Semi-moist Pet Food

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❖ ***Developing Scientist Entrant***

Introduction: Ham mite, *Tyrophagus putrescentiae,* is an important mite species that contaminates stored foods, including pet food. Control of this mite depends on chemical methods such as fumigation and spraying with organo-phosphorus compounds. Liquid smoke is a naturally derived flavor component and preservative with known antimicrobial properties.

Purpose: To investigate the effects of liquid smoke fractions on *T. putrescentiae* survivability and orientation behavior in semi-moist pet food.

Methods: Semi-moist pet food was formulated with 0.3% inclusion of liquid smoke fractions namely, P-1720 (phenols+carbonyls+≤10% acetic acid), S-5 (carbonyls+<3% acetic acid), or C-100 (concentrated carbonyls+0% acetic acid). Survivability of *T. putrescentiae* on treated and untreated (0% liquid smoke) pet food samples was determined by enumerating their population at 7, 14, and 28 days. In another experiment, untreated semi-moist pet food pieces were dipped in liquid smoke fractions at 100%, 50%, 25%, 10%, 5%, 1% or 0.3% concentration, and used in two-choice behavioral assays to determine orientation of mites. The attraction or repellency of mites towards treatments was determined by enumerating them at 2 h, 8 h and 24 h, and the repellency index (RI) was calculated.

Results: At 14 days, the average mite population ($n = 18$) among smoke treatments ranged from 207 to 244 when compared to the untreated (212 mites), positive control (propylene glycol treatment, no mite growth) and negative control (standard mite culture diet, 454 mites). There was no significant difference among treatments ($P > 0.05$), therefore, liquid smoke did not kill or inhibit mite population growth. Two-choice behavioral assays among treatments ($P < 0.05$) indicated that, on an average ($n = 84$), P-1720 attracted mites the most (+20 to -50% RI), while C-100 repelled them (-10 to +60% RI). Hence, C-100 has the potential to act as a mite repellent in pet food.

Significance: Liquid smoke will not replace chemical fumigants, but may provide repellency to control mite infestation in stored foods.

P2-53 Plasma-activated Water as a Novel Disinfectant: Effectiveness Against Selected Bacteria and Application to Produce and Egg Washing

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Introduction: Plasma-activated water (PAW) is gaining interest as a novel disinfectant, however, the microbial inactivation efficacy of PAW against different species of bacteria is less investigated and its application to food products needs further efforts.

Purpose: This study focused on 1) the inactivating efficacy of PAW against four selected Gram-positive and Gram-negative bacteria, and 2) the effects of PAW on microbial inactivation and quality of tomatoes and eggs.

Methods: PAW was prepared by exposing deionized (DI) water to an atmospheric air plasma jet. PAW5 and PAW10 denote water treated by plasma for 5 min and 10 min, respectively. Stationary phase *Escherichia coli* DH5α, *Salmonella* Typhimurium, *Listeria innocua*, and vegetative cells of *Bacillus subtilis* were selected as target bacteria for the analysis of microbial inactivation by PAW or DI water as control in either a planktonic system or on the surface of grape tomatoes and chicken shell eggs. Bacterial populations, color and firmness of the food products after washing were analyzed ($n = 3$).

Results: The inactivation activity of PAW varied among the different bacterial agents. PAW5 reduced planktonic *Escherichia coli*, *Salmonella* Typhimurium, *Listeria innocua*, and *Bacillus subtilis* by >5 log CFU/mL, 2.2 ± 0.2 log CFU/mL, 1.0 ± 0.4 log CFU/mL, and >5 log CFU/mL, respectively. The inactivation efficacy increased with increasing plasma activation duration or bacterial incubation time in PAW. In addition to planktonic cells, PAW5 inactivated *S.* Typhimurium attached on tomatoes by 1.7 ± 0.4 log while PAW10 resulted in >4-log reduction. PAW10-washed eggs with a gentle massaging treatment resulted in >5-log reduction, with no detectable surviving cells in the spent wash solution. No significant ($P > 0.05$) change were observed in either color or firmness of the food.

Significance: PAW is a promising disinfectant for food safety and preservation. However, the resistance towards PAW among various food pathogens differs and needs further investigation.

P2-54 Blend Uniformity and Vitamin Stability in Dairy-based Foods Fortified with Lipid-encapsulated Ferrous Sulfate

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Introduction: Homogeneity of powder blends is an important metric for industrial applications in fortified dairy foods. Studies evaluating physical properties and nutrient stability performance are reliant on parameters that deliver a uniform powder. Quantities of individual micronutrients in finished products are particularly critical for formulated infant foods.

Purpose: We developed a simple, efficient method, specifically for a pilot scale double ribbon blender, in which fortified dairy-based infant formula powder blends were repeatedly produced and analytically verified as uniform or homogenous complex fortified mixtures of macro- and micronutrients.

Methods: Operating parameters were determined for blending using the response surface method (RSM). Using 16 independent blends, a comparison study of nutrient stability in fortified model non-agglomerated powder infant formula (PIF) and agglomerated whey protein concentrate 80 (WPC) blends was executed in duplicate to evaluate the effect of microencapsulated ferrous sulfate (MFS) with an encapsulating composition of 60% stearic acid vs. unencapsulated ferrous sulfate (UFS), a novel form of iron used in infant formula fortification applications.

Results: The stability of vitamins C, A, and E in micronutrient-fortified dairy powders containing encapsulated vs. unencapsulated ferrous sulfate held at 37°C/75% RH for 8 weeks was determined. Most of the vitamin degradation rates, when comparing similar base blends only differing in the ferrous sulfate ingredient (microencapsulated vs unencapsulated) during eight weeks of accelerated shelf-life storage conditions were not statistically significant. Vitamin A degradation was accelerated in samples of agglomerated WPC containing unencapsulated ferrous sulfate ($P = 0.016$) when comparing time zero and week 8. Non-agglomerated infant formula base with unencapsulated ferrous sulfate showed suggestive statistical significance of higher rates of degradation for vitamins A and E ($P = 0.072$ and 0.073, respectively).

Significance: RSM can be used to qualify a pilot ribbon blender for evaluating vitamin degradation rates in fortified dairy powders.

P2-55 Evaluate the Stability of Water Droplets in Margarines and Spreads

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Introduction: The microbial stability of margarines and spreads depends largely on water droplets that are dispersed in oil. Although many studies have shown that microorganisms are not likely to grow in the products with water droplets smaller than 15 µm, it is still important to evaluate the stability of water droplets during its product shelf life.

Purpose: The purpose of this study was to evaluate the stability of water droplets in margarines and low-fat spreads stored at different temperatures.

Methods: Pilot-scaled samples were prepared using a model water-in-oil emulsion containing 20 and 45% (w/w) water with target water droplet sizes ranging between 5 µm to 15 µm. The emulsions were crystallized by rapid cooling in a scrapped surface heat exchanger to a fill temperature of 55°F. Each batch of sample variable was divided into three containers equally and stored at 40°F, 45°F and 55°F for up to 60 days. Samples were collected in triplicate at various intervals and analyzed for water droplet size using Pulse-field gradient NMR technique. Temperature-stressed samples were also analyzed to understand the behavior of water droplets after exposing the samples at 80°F for 6 h.

Results: Samples were produced with a droplet size ranging from 5 µm to 15 µm. Results showed no statistical differences on samples collected between Day-1 and Day-60 at each temperature. However, results of temperature-stressed samples showed significant differences due to droplet size increased by a factor of 1.5.

Significance: This study indicates that water droplets are stable during its product shelf life for up to 60 days, regardless of product variables. It can be inferred that storage temperatures up to 55°F does not play a role in modifying or altering the water droplet size. However, temperature-stressed test results demonstrate the importance of optimizing the emulsifier system to withstand extreme temperature variations due to potential temperature abuse.

P2-57 Efficacy of Ozone Against *Salmonella* Newport in Recycled and Non-recycled Spinach Wash Water

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✦ ***Undergraduate Student Award Entrant***

Introduction: Recycling the water used in agricultural production may be essential in regions where drought conditions are likely to occur in the future. The safety of such practice of recycling agricultural water is unknown. Appropriate treatment technologies are needed to decontaminate the water prior to recycling, especially for use in food crops.

Purpose: The objective was to investigate the efficacy of ozone against *Salmonella* Newport in recycled and non-recycled spinach wash water.

Methods: The non-recycled spinach wash water was prepared by adding spinach (25 g) and tap water (500 mL) in a stomacher bag, shaking gently for 2 min, then removing the spinach. Recycled wash waters were prepared by washing either 2 or 3 batches of spinach (25 g each) subsequently in the same tap water (500 mL) in separate stomacher bags. For the 3 batch stomacher bags, 0.1 g soil was also added to simulate soil getting into the flume tank from field crops. Each wash water sample was inoculated with 500 µL of *Salmonella* (approximately 6 log CFU/mL) and mixed thoroughly. The different spinach wash waters (6 samples per repeat; 3 repeats) were treated with ozone (approximately 5.5 mg/L) for 30 s, 1 min, and/or 2 min. Untreated wash waters were also included as controls. Wash waters were serially diluted in 0.1% peptone water and plated on xylose lysine desoxycholate agar for enumeration of surviving *Salmonella*.

Results: Overall, there was a reduction (approximately 6 log CFU/mL) in *Salmonella* population to below detection levels (<1 log CFU/mL) with different ozone treatment times, both in recycled and non-recycled spinach wash waters. No survivors were detected in any of the treatments. The pre-treatment *Salmonella* population ranged from 5.4 – 6.7 log CFU/mL. The amount of ozone produced at a flow rate of 5.5 was 5.49 ± 0.17 mg/L.

Significance: The results from this study indicate that ozone can potentially be used as a treatment for effectively decontaminating recycled spinach wash water.

P2-59 Investigation of the Ability of Butyl-Parahydroxybenzoate in Selected Dry Food Matrices to Enhance Thermal Inactivation of *Cronobacter sakazakii*

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❖ ***Developing Scientist Entrant***

Introduction: Parabens have shown to enhance the thermal inactivation of foodborne pathogens in model system, including *Cronobacter sakazakii*. However, there have been few studies looking at this phenomenon in actual food systems.

Purpose: Evaluate the potential enhancement of thermal inactivation of *C. sakazakii* by butyl-parahydroxybenzoate (BPB) in powdered infant formula (PIF) and non-fat dry milk (NFDM) before and after rehydration.

Methods: *C. sakazakii* 607 was grown to early stationary phase, concentrated by centrifugation, and then re-suspended with 1 mL of 0.1% peptone water to inoculate 500 g PIF and NFDM supplemented with 0 and 125 ppm BPB. The PIF was rehydrated with water at designated temperatures in baby bottles, and sampled at 30, 300 and 600 s. Rehydrated NFDM and lactose solutions with BPB were inoculated and heated at 58°C to study thermal kinetics. Inoculated dry NFDM was also stored at 24°C, 55°C, and room temperature, respectively, for 14 days and sampled daily. All samples were plated on Trypticase Soy Agar, incubated at 37°C, and enumerated. Survival curves were plotted, and D-values were calculated. Fluorescence tests were conducted to investigate the interaction between BPB and PIF.

Results: In PIF, BPB did not enhance the thermal inactivation when rehydrated with ≤70°C water. In rehydrated NFDM, the enhancement of thermal inactivation was suppressed in a positive dose-dependent manner, whereas equivalent levels of lactose displayed clear thermal enhancement. When added to NFDM under 55°C dry storage conditions, 125 ppm BPB did not enhance inactivation. The fluorescence test results suggested that protein in PIF and NFDM interacted with BPB, possibly reducing its efficacy.

Significance: This study suggests that BPB is not likely to enhance mild thermal inactivation treatments in foods that have appreciable amounts of protein. Other FDA approved compounds will be investigated to evaluate their ability to enhance thermal inactivation for proof of concept.

P2-60 Evaluation of Synergistic Effect of Butyl-Parahydroxybenzoate on the Thermal Inactivation of *Cronobacter sakazakii* in Apple Juice as a Function of pH

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❖ ***Developing Scientist Entrant***

Introduction: After studies with powdered infant formula indicated that the enhancement of thermal inactivation of *Cronobacter sakazakii* by butyl-parahydroxybenzoate (BPB) was blocked by the presence of high protein levels, we hypothesized that BPB would retain its synergistic activity in food with limited protein content. Apple juice was chosen as the food to test this hypothesis.

Purpose: This study examined the ability of BPB to enhance the thermal inactivation of *Cronobacter sakazakii* 607 at 58°C in commercial apple juice, including the effect of pH and its possible synergistic effects with malic acid in apple juice.

Methods: Apple juice was adjusted to designated pH values between 3.8 and 9.0 using 1N NaOH. *C. sakazakii* was inoculated in brain and heart infusion broth and incubated at 37°C to early stationary phase, concentrated by centrifugation, and transferred into prepared apple juice with selected concentrations of BPB (≤ 125 ppm) prior to thermal treatment (58°C) for 15 min using submerged coil apparatus. The same methods were used to study the enhancement of thermal inactivation by malic acid. Samples were plated on Trypticase Soy Agar for recovery and enumeration. Survival curves were plotted, and D-values were calculated and compared using ANOVA.

Results: BPB significantly enhanced thermal inactivation in a concentration dependent manner, with D-values of a few seconds at the original pH (3.8). The enhancement of thermal inactivation was pH dependent in the range of pH 3.4 to 9.0. Malic acid enhanced thermal activation as the pH was decreased from 3.8 to 3.0. The effects of malic acid and BPB on thermal inaction were additive.

Significance: BPB enhances thermal inactivation of *C. sakazakii* in this low protein food. This is a proof of concept study to explore of processing aid compounds to enhance thermal inactivation.

P2-61 Efficacy of Residual Ozone on Surrogate Microorganisms for Waterborne Pathogens in Bottled Water

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Introduction: Ozone is a powerful disinfectant that is widely used in the bottled water (BW) industry. Primary ozone disinfection occurs in a reaction tank with specific contact time; however, residual ozone in the water that is bottled may still possess disinfection activity.

Purpose: The purpose of this study was to evaluate the efficacy of residual ozone in BW in reducing the populations of surrogate microorganisms for waterborne pathogens, *Escherichia coli* (BAA-1427), *Enterococcus faecalis* (ATCC 19433) and *Burkholderia cepacia* (ATCC 25416).

Methods: A pilot scale ozone delivery system and filler were assembled to allow filling of 0.5 L polyethylene terephthalate plastic water bottles with ozonated (0.1, 0.2, 0.3 and 0.4 mg/L) water. The microorganisms were inoculated in TSB (50 mL) and incubated for 24 h at 35°C. Ozonated water was inoculated with individual microorganism to attain 6 log CFU/mL population in water and populations were determined after 5, 30, 60 and 180 min at 25°C. The samples (100 mL) were filtered through Neogrid membrane filters, the filters were placed on tryptic soy agar, incubated for 48 h at 37°C and enumerated. Ozone dissipation from the bottled water was also measured with or without biological load (log 6 CFU/mL) at 4.4, 21.1 and 37.7°C for 6 h. Three independent replications were performed for each treatment, on a separate day using freshly prepared inoculum. Non-ozonated water bottle inoculated with each organism was used as positive control with each trial.

Results: Greater reductions ($P \leq 0.05$) in *E. faecalis* (4.61 vs. 3.68 log CFU/mL) and *B. cepacia* (5.24 vs. 4.12 log CFU/mL) were observed at 0.4 vs 0.1 mg/L ozone in BW, respectively. Extension of storage time did not result greater reductions ($P > 0.05$) in microbial populations. Faster ozone dissipation ($P < 0.05$) was observed at 37°C and dissipation rate increased with biological load.

Significance: The residual ozone in the bottled water (0.1 to 0.4 mg/L) can provide ≥ 4-log reductions in *E. coli*, *E. faecalis* and *B. cepacia*, providing an additional measure of microbiological safety for bottled water.

P2-62 Use of Surrogate Bacteria for Validation and Verification of High-Pressure Processes (HPP)

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Introduction: High Pressure Processing (HPP) has gained an increasing level of traction as an alternative pathogen control measure for heat-sensitive food products. Validation studies for HPP systems are currently mostly being conducted at pilot scale using the actual pathogen(s). The surrogate microorganism methodology, by using non-pathogenic organisms that mimic the resistance of pathogens when exposed to a defined "stress," offers the food industry a complementary method to test HPP performance in real industrial conditions.

Purpose: The objective of the study was to evaluate the appropriateness of a surrogate candidate for use in validation/verification of High-Pressure Processing (HPP) systems.

Methods: Three plant-based matrices (avocado puree, hummus spread, and zucchini puree) and two matrices of animal origin (dairy cream and ground beef) were independently inoculated with SurroNov®18, a Novolyze dry ready-to-use surrogate preparation derived from *Enterococcus faecium*, and three pathogenic organisms (*Salmonella*, *Listeria* and *E. coli 0157:H7*). Inoculated foods were processed through an industrial high-pressure equipment for 5 minutes at 400 MPa (4,000 bar) to achieve partial survival of microorganisms. For each condition, 3 samples of 15 g were collected and enumerated in order to compare the inactivation kinetics.

Results: In the conditions of the test, *Listeria monocytogenes* was confirmed to be the highest resistant pathogen in all matrices. The resistance of *Enterococcus faecium* surrogate preparation steadily showed a slightly higher/similar resistance compared with *Listeria monocytogenes*.

Significance: These findings show promising results for using surrogate methodology to validate and verify lethality of high-pressure processing systems in real, industrial conditions, offering HPP processors a robust tool to test new HPP applications as well as implement surrogate-based, routine verification programs of HPP systems. In order to confirm these results, the recovery of the microorganisms could be investigated during the expected shelf life of each product.

P2-63 Use of Surrogate Bacteria for Validation and Verification of Thermal Treatments of Fruits and Vegetables

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Introduction: Use of surrogate bacteria for process validation and verification is a well-established methodology to estimate the lethality of industrial processes against pathogenic microorganisms. To answer food safety issues, Novolyze has developed a range of bacterial surrogates and continuously performs intensive R&D trials to confirm the compatibility of its SurroNov® surrogates for new applications. Although thermal processing is commonly applied for low-moisture foods decontamination, high-moisture foods like fruits and vegetables may also be submitted to different industrial heat treatments, which need methods to be qualified and validated.

Purpose: The objective of the study was to evaluate the appropriateness of an *Enterococcus faecium* derived surrogate preparation for fresh mangos dehydration process and potatoes blanching process.

Methods: Slices of fresh mangos (10 g) and potatoes (5 g) were independently inoculated with *Salmonella*, *Listeria monocytogenes* or a Novolyze dry ready-to-use surrogate preparation derived from *Enterococcus faecium*. To simulate a dehydration process, inoculated mangos were placed into a ventilated oven for 16 h at 60°C (140°F). To simulate a blanching process, inoculated potato slices were immersed for 2 minutes in a water bath at 70°C (158°F). Samples were then enumerated using non-selective medium to estimate the microbial lethality. For each experiment, two independent replicates were performed.

Results: The *Enterococcus faecium* derived surrogate preparation showed higher resistance to thermal treatments than *Salmonella* and *Listeria monocytogenes*, for both experiments. For mango dehydration, no significant difference in the pH of the mangos before and after drying was observed.

Significance: These data show that Novolyze surrogate preparation appears to be an appropriate surrogate for *Salmonella* and *Listeria monocytogenes* for potato blanching and mango dehydration. Further tests should be conducted in different matrices to confirm these findings, but these results are already promising for the development of a surrogate microorganism application in validation and verification of thermal treatments on fruits and vegetables.

P2-64 Compatibility of Plastics and Elastomers Typically Used in Food Equipment with Chlorine Dioxide Gas

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Introduction: Food safety is a concern for all individuals involved in the food supply chain. Besides controlling the food product itself, to improve safety, washing and sanitizing surfaces and equipment are critical. Chlorine dioxide (ClO_2) is a green-yellow gas, known as a strong antimicrobial agent against multiple pathogenic microorganisms and biofilms. Plastics such as polypropylene (PP), polyester (PET), cast nylon, ultra-high-molecular-weight polyethylene (UHMW), polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF) and polyoxymethylene (Delrin®); and elastomers like ethylene propylene diene monomer (EPDM, Shore 50A) and Nitrile butadiene rubber (BUNA-N, Shore 50A) are widely used in food contact surfaces and equipment. Although chlorine dioxide gas is approved as a sanitizer (and even sterilant), changes in the physical and mechanical properties due to gas exposure over time is a concern for plastics and elastomers.

Purpose: To determine effects of ClO_2 gas exposure on mechanical properties of plastics and elastomers.

Methods: Samples (in triplicate) were exposed to ClO_2 gas (2 mg/L) for 15 days inside a chamber at 23°C and 75% relative humidity following ASTM International Standard D543-14. After exposure, mechanical property changes, (tensile strength and shear strength) was determined according to ASTM D638-14 and D732-02, respectively. Changes due to gas exposure were compared within the same material using ANOVA.

Results: Two plastics showed a significant reduction ($P < 0.05$) in peak stress under tension, cast nylon 67.3-47.4 (MPa), and Delrin® 74.4-33.8 (MPa), while only Delrin® showed significant reduction in shear strength (65-59.3 MPa) after ClO_2 gas exposure. Both elastomers showed a significant reduction ($P < 0.05$) in peak stress under tension EPDM (11.1-7.7 MPa) and BUNA-N (4.1-1.2 MPa).

Significance: Some common food contact materials react with ClO_2 gas at typical sanitizer concentration levels and for the first time, changes in mechanical properties were quantified for engineering design and lifetime analysis purposes to maintain the hygienic design of equipment.

P2-65 Examination of the Use of Failure Mode and Effects Analysis (FMEA) to Improve the Risk Assessment of Biological Hazards of a Fresh-cut Produce Processing Plant

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Introduction: Fresh-cut produce is a source of foodborne outbreaks, especially when fresh-cut processors fail to recognize when their biological hazards are not adequately controlled because of errors in risk assessment, thus leading to Type-2 errors. It is suggested that these outbreaks continue to occur because the risk associated with processing is not being comprehensively portrayed by a conventional Hazard Analysis (HA).

Purpose: This study compares FMEA, a risk assessment methodology employed by many other non-food manufacturing sectors, with a conventional HA to assess the relative risks of biological hazards on a fresh-cut carrot processing line.

Methods: A conventional HA was completed on the process and required key control points were determined for each hazard. This was compared to a FMEA in which potential failure modes were identified at each process step after which risk priority was calculated from the severity, occurrence and detection ratings associated with each potential FMEA failure mode and attendant process controls. This information was then used to determine residual risk and prioritize the process steps requiring further corrective actions.

Results: The HA suggested that properly peeling and washing carrots from approved suppliers will adequately control the pathogen load while the FMEA results suggested there remains some residual risk in fresh-cut carrot processing largely because there are currently no realistic methods for detecting pathogens in the incoming carrots.

Significance: FMEA, in contrast with HA, provides a more precise picture of the residual risk associated with a fresh-cut produce processing line. This information can then be used by the processor to focus on continuous improvement activities that will lead to decreased risk, thereby reducing the likelihood of a foodborne outbreak.

P2-66 Evaluation of Innovative Food Safety and Technical Support Delivered to Welsh Food Sector SMEs through Project Helix 2016–2019

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Introduction: Since 2016, a Pan-Wales partnership between Welsh Government/European Union, Small-to-Medium-Enterprise (SME) partners and Food Innovation Wales was established and using a collaborative approach, has implemented 'Project HELIX' across Wales, UK. This has facilitated knowledge transfer of reportedly lacking and required technical and food safety/science skills to the FDMP (food-drink-manufacturing-and-processing) sector.

Purpose: This study aimed to undertake an analysis of Project HELIX cumulative outputs/outcomes and key-performance-indicators, (KPIs) impacting food safety management and technical innovation.

Methods: A quantitative and qualitative mixed methods analysis was carried out to ascertain the projects' process and output data based on FDMP business participation in product delivery programs between 2016-2019. Welsh Government and Project HELIX documentation, reports and media articles (n => 150) were reviewed and analyzed using a content analysis approach.

Results: During Project HELIX implementation (3 years) considerable outputs have been delivered, for example, food safety and technical expertise has been delivered to 279 Welsh food-sector businesses and 353 training days have been provided to enable upskilling of 382 participants. Since implementation, and as a result of strengthened technical performance and food safety culture and management, Project HELIX has resulted in outputs including £110million impact to the Welsh FDMP industry, creation of 298 new jobs and safeguarding 1302 jobs. In addition, 156 'new' businesses have been supported with 241 new markets accessed and 366 new food products developed. All such factors, combined with increased third-party certifications within supported businesses have enabled increased business sustainability and potential growth of the sector.

Significance: To date, Project HELIX has facilitated improved food safety/technical knowledge and compliance in Welsh FDMP SMEs, positively impacting the security of the food supply. In addition, outputs have significantly contributed to The Welsh Government's 2014-2020 strategic economic and ecological needs for the Welsh Food and Drink sector.

P2-67 Identification of a Large-scale Inoculation Method for On-site Validations of Wheat Milling Facilities with a Surrogate for STEC and *Enterococcus faecium* NRRL B-2354

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Introduction: Previous lab-scale studies replicating addition of a peracetic acid and hydrogen peroxide-based sanitizing solution to tempering water during wheat milling for pathogen control found: i) *Enterococcus faecium* NRRL B-2354 is a suitable surrogate for STEC and ii) the solution applied under representative mill tempering conditions gave a >2-CFU/g log reduction in *E. faecium*.

Purpose: Investigations were conducted to determine suitability of the inoculation method for validating wheat milling facilities, including: i) stability of *E. faecium* populations under refrigerated and ambient storage and ii) scale-up of the inoculation method.

Methods: For inoculum stability testing, wheat was inoculated (60 mL/kg, overnight culture of *E. faecium*), dried back to original % moisture content (MC) in a fluidized bed dryer (ambient temperature) then stored under refrigerated (5°C) or ambient temperatures for 7 weeks, during which 5 x 45 g samples were enumerated from both storage conditions every week and compared (single factor ANOVA).

For inoculation scale up, 5,000 kg of wheat was inoculated (60 mL/kg, overnight culture of *E. faecium*), using an industrial-scale applicator to apply inoculum to the wheat and an industrial-scale dryer (100°F, 20 250 CFM) to dry the wheat back to original MC. As the inoculated wheat exited the dryer, samples were taken for enumeration and MC analysis every 10 minutes for the run duration (60 min; total of 6 samples).

Results: Inoculated wheat samples stored under ambient conditions were as stable as samples stored in refrigerated conditions and retained similar levels of *E. faecium* during a 7-week storage period ($P \geq 0.05$). The level of inoculum remained > 6 log CFU/g for the study duration.

For inoculation scale-up, similar levels of inoculation ($P \geq 0.05$) were achieved compared to the lab-scale method (> 6 log CFU/g).

Significance: An inoculation method, shown to provide stable inoculation levels over 7 weeks, was scaled-up for validating wheat milling facilities.

P2-68 Survival Abilities of *Lactobacillus* Strains in Fermented Milk Product Co-cultured with Selected Foodborne Pathogens

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Introduction: The study was aimed at isolating *Lactobacillus* strains with probiotic potentials from fermented cow and goat milk, and the inhibitory effect against selected foodborne pathogens in a co-culture fermentation.

Purpose: To investigate the survival abilities of probiotic *Lactobacillus* strains in a co-culture with *Shigella dysenteriae* and *Salmonella* Typhi.

Methods: The LAB strains were assessed for probiotic potentials and safety assessments. The potential probiotic strains were co-cultured in fermented milk product with *Salmonella* Typhi and *Shigella dysenteriae*.

Results: Twelve were *L. plantarum*, six were *L. fermentum*, five were *L. brevis* and two were *L. casei* strains. *Lactobacillus plantarum* and *L. fermentum* strains were fast acidifiers with a change in pH of 2 units after 6 h and 12 h. To 0.3% and 1.0% bile salts concentration, *L. plantarum* G14 and *L. casei* C10 had the highest and least values of 92.0±0.4% and 39.3±0.2%, respectively. *Lactobacillus plantarum* G14 displayed the highest value of 94.3±0.5% and *L. casei* C10 had the least value of 50.2±0.7% tolerance to gastric acidity, while *L. plantarum* G14 exhibited highest value of 90.5±0.3% and *L. casei* C10 had the least value of 43.1±0.7% tolerance to intestinal juice comparable to reference strain *L. acidophilus* CNRZ 1923. *Lactobacillus plantarum* G14 produced the highest value of 0.25g/L, 6.5g/L and 4.5g/L of hydrogen peroxide, diacetyl and lactic acid at 72 h fermentation time, respectively. All the strains were negative to hemolysis and gelatinase activities. The strains displayed antagonistic activities against *Salmonella* Typhi, *Listeria monocytogenes* and *Shigella dysenteriae*. The highest inhibitory activities were demonstrated by combination of *L. brevis* C17 and *L. plantarum* G14 co-cultured with *Shigella dysenteriae* after 24 h ($P < 0.05$).

Significance: The probiotic potentials and metabolites produced have shown strain specific variance among *Lactobacillus* strains isolated from fermented milk. The inhibitory activities of the strains make them interesting candidates for selection as functional starter cultures

P2-70 Evaluation of GENE-UP® New Markers EHEC for Detection of Shiga Toxin-producing *Escherichia coli* in MicroTally Sheets Collected from Beef Carcasses

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Introduction: Detection of Shiga toxin-producing *Escherichia coli* (STEC) based on the *stx*, *eae* and Top7 serogroup-associated genes may result in false positives. The GENE-UP® New Markers EHEC kit is designed to target virulence genes (*espK, espV* and *CRISPR_O26E*) for more accurate screening of pathogenic STEC.

Purpose: To evaluate the GENE-UP® New Markers EHEC (NM-EHEC) kit using a selected panel of STEC strains and *E.coli*-inoculated MicroTally sheets (MT).

Methods: A total of 96 STEC strains from diverse origins, including feces, beef products and carcasses, ground beef and humans, were used to evaluate the NM-EHEC kit. These strains were also tested for virulence factors, including *ecf1, stx*, *espk1, nleB, eae*, *nleF, subAB* and *ehxA* genes. In addition, 144 beef carcasses were sampled with individual MT, and these, in turn, were either inoculated with selected *E.coli* strains or were left uninoculated. All samples were enriched (42°C, 10 h) in 200 mL of buffered peptone water and then tested using GENE-UP® kits (NM-EHEC, STEC *stx/eae* [EH1], STEC-Top 6 [EH2], and *E. coli* O157:H7 [ECO]) following the manufacturer's instructions. Presumptive positives were plated onto ChromID™ Coli and CHROMagar™ STEC plates for cultural confirmation.

Results: The NM-EHEC kit results for the 96 STEC strains corresponded 100% with the virulence profiles of the strains. For the 144 MT samples evaluated, 55 positives were obtained with EH1, 49 positives with both EH1 and NM-EHEC, and 43 presumptive positives with a combination of EH1, NM-EHEC, EH2 and ECO. Among the 43 presumptive positives, 41 were confirmed as true positives by culture and two were false positives. Therefore, the positive predictive value for NM-EHEC was 95.3%.

Significance: The GENE-UP® New Markers EHEC kit can be used to screen for pathogenic STEC that are more relevant to public health and therefore, reduce presumptive positives that require cultural confirmation.

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P2-71 Survival of *Cronobacter sakazakii* in Powdered Infant Formula in a Dynamic *In Vitro* Newborn Gastric Model

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❖ ***Developing Scientist Entrant***

Introduction: *Cronobacter sakazakii* (*Cs*) is a foodborne pathogen that causes lethal necrotizing enterocolitis in newborns and immunocompromised infants. Powdered infant formula (PIF) is a vehicle of infection as recontamination can occur after pasteurization through handling and the addition of heat-sensitive vitamins, therefore posing health risks once reconstituted. Low gastric acidity in newborns can promote the growth of *Cs* if ingested. Current research on survival of *Cs* in newborns' stomachs uses static *in vitro* models which cannot mimic the digestive system as precisely as dynamic models such as the Simulator of the Human Intestinal Microbial Ecosystem (SHIME).

Purpose: This research investigates the survival of *Cs* in the stomach of newborns by using SHIME to simulate the gradual decrease in gastric pH over the course of newborns' digestion time (4 h).

Methods: In SHIME bioreactors, reconstituted PIF, pepsin, and simulated gastric fluid were inoculated with 2 strains of *Cs* exhibiting different acid sensitivities. SHIME monitored the gradual changes in pH mimicking newborn digestion from an initial pH of 6.45 to fasting pH values of 4.00, 5.00, and 6.00. Samples were collected at 0, 2, 3, 4 h and plated onto R&F agar ($n = 6$).

Results: Compared to the acid-resistant strain, the sensitive strain grew 10% less at pH 4 and 5% less at pH 5 and 6 within the first 2 h ($P < 0.05$). However, in the last 2 h, the sensitive strain grew 8%, 20%, and 35% more than the resistant strain at pH 4, 5, and 6, respectively ($P < 0.05$).

Significance: The findings suggest that *Cs* growth in newborns' stomachs may be attributed to low gastric acidity, lengthy digestion time, and potentially upregulation of stress response genes which will be explored in this research. A better understanding of *Cs* stress mechanisms is needed to develop better preventive controls for *Cs* in PIF.

P2-72 A Rapid Response to Seek and Destroy *Listeria monocytogenes* in a Ready-to-Eat Manufacturer Supplying the UK Retail Sector

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Introduction: A ready-to-eat chilled manufacturer supplying the UK retail sector identified presence of *L. monocytogenes* in finished product and environmental isolation for *Listeria* spp.

Purpose: To implement a rapid response approach to enable identification of potential source and implement actions to reduce the risk of further product contamination and potential product recall.

Methods: The six-week intensive rapid response, included reviewing historic environmental monitoring and product testing results (n = 2566) to identify *Listeria* spp. 'hotspots' in the facility. A multidisciplinary Listeria-Action-Team was created to conduct site inspections and factory floor observation (n = 30) to determine 'root cause' and implement and monitor appropriate interventions.

Results: Despite procedures being in place, staff in the structurally segregated processing area were not sufficiently supervised, consequently, non-compliant manufacturing and hygiene practices were determined. Design of the decontamination process was not robust or implemented rigorously. Structural defects further complicated the issue; which included inadequate storage of ingredients and contact packaging, lack of air flow along with inappropriate flooring exasperated the potential for listeria niches to establish in the area. The Listeria-Action-Team developed corrective actions, which included; bespoke listeria awareness training, detailed inspections of the facility with staff to identify non-compliance in manufacturing and hygiene practices, and the optimization of processes in the contained processing area. Increased environmental monitoring was implemented to evaluate the efficacy of the actions. Following implementation of the actions, environmental isolation and end-product contamination of listeria was reduced. No further issues were reported following the implementation of the Listeria-Action-Team's intervention.

Significance: Consequently, the rapid response approach, which considered multiple influencing factors including the organizational culture, the manufacturing process and the design of equipment, successfully identified potential sources of the pathogen and opportunities for product contamination. The bespoke actions that were implemented as part of the rapid response improved the hygienic status of the manufacturer and enhanced product safety.

P2-73 The Formation of *Listeria monocytogenes* Persister Cells in Fresh Produce Processing Environment

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Introduction: *Listeria monocytogenes (LM)* can survive in food processing environment and proliferate in food matrices at low temperatures. Under harsh environment, bacterial cells may enter into the persister state and become phenotypically resistant to lethal doses of antibiotics. In consequence, the presence of persister cells becomes a severe public health threat.

Purpose: The aim of this study was to evaluate the ability of *LM* to form persister cells under simulated food processing environment and conditions.

Methods: Thirty-six *LM* strains isolated from produce processing plants were used for this study and were first characterized based on their adherence capacity by using the microplate adherence assay. Five strains with the strongest adherence capacity were chosen for the following studies. The formation of persister cells were evaluated by washing and resuspending overnight fresh *LM* cultures in Brain Heart Infusion broth (BHI) (control) and fresh produce juice (PJ). Resuspended cells were then inoculated onto stainless steel coupons and dried at an ambient temperature for overnight. Inoculated coupons were then stored at 4°C for 2 weeks. The percentages of persister cells formed during storage were investigated by exposing the total surviving cells to different concentrations of gentamicin (0, 10, 25, 50 and 100 ppm) for 4 hours and counting the gentamicin-resistant cells.

Results: Fresh stationary-phase *LM* cultures (~ 8.5 log CFU/mL) generated 0.1408 to 0.0072% persister cells when exposed to 25 to 100 ppm of gentamicin. *LM* in BHI was also able to form persister cells after desiccation on stainless steel coupons although in lower percentages (0.1119-0.0002%). The determination of persister cells in PJ was limited by the low survival ratio in the coupons.

Significance: *LM* could form persister cells when survived on stainless steel coupons. The enhanced antimicrobial resistance of persister *LM* cells highlights the importance of efficient environment cleaning, sanitizing and monitoring programs.

P2-74 Antilisterial Activity of Microencapsulated *Lactobacillus paraplantarum* FT-259 in a Brazilian Fresh Cheese Model

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Introduction: Microencapsulation can improve the survival and viability of lactic acid bacteria (LAB) under adverse environmental conditions, being interesting for use for food biopreservation. *Lactobacillus paraplantarum* FT-259, isolated from Brazilian semi-hard Minas type cheese, is a LAB with bioprotective and probiotic characteristics.

Purpose: To evaluate the antilisterial activity of *L. paraplantarum* FT-259, entrapped in casein-pectin particles, in a Brazilian fresh Minas cheese (FMC) laboratory model.

Methods: Microencapsulated *L. paraplantarum* (5.9 log CFU/mL), was co-inoculated with *Listeria monocytogenes* ATCC 7644 (3.4 log CFU/mL) in FMC stored for up to 21 days at abuse temperature (8°C). Controls were run in FMC containing free *L. paraplantarum* (5.2 log CFU/mL) co-inoculated with *L. monocytogenes* (3.4 log CFU/mL). At selected time points, colony counts were performed in MRS and Oxford agar, respectively, for LAB and *L. monocytogenes*.

Results: After 21 days of incubation, monocultures of *L. monocytogenes* in FMC reached 8.2 log CFU/mL. In the presence of free LAB the pathogen population achieved 7.3 log CFU/mL. However, at the end of the experiment, listerial growth (5.5 log CFU/mL) was significantly affected ($P < 0.05$) by the presence of *L. paraplantarum.*

Significance: These results suggest that the use of *L. paraplantarum* FT-259 microencapsulated in casein-pectin matrix may be an interesting approach to improve food safety of dairy products.

P2-77 Survival of Foodborne Pathogens in Citrus Storage and Finishing Waxes

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Introduction: Although waxes are widely used in the citrus industry to coat fruit, information about the behavior of foodborne pathogens in the waxes is very limited.

Purpose: The purpose of this study was to evaluate the chemical and microbial properties of commercially-available citrus waxes and the survivability of human pathogens within them.

Methods: Citrus storage and citrus finishing waxes were obtained from commercial sources. The pH of each wax was measured upon receipt and total aerobic bacteria were determined by plating appropriate dilutions of the wax samples onto plate count agar. Five-strain cocktails of *Listeria monocytogenes* (*LM*) and *Salmonella* were inoculated into separate samples of each wax at a final concentration of ~6.0 log CFU/mL. Surviving pathogens were enumerated immediately after inoculation and at 0.5, 1, 4, and 24 h during post-inoculation storage at 4°C or at ambient temperature.

Results: The pH of storage and finishing waxes ranged from 8.0 to 8.5, and 8.8 to 10.1, respectively. Total bacteria counts of storage waxes were 2.6 to 5.8 log CFU/mL while those of finishing waxes ranged from below the limit of detection (1 CFU/mL) to 4.4 log CFU/mL. Populations of *LM* and *Salmonella* slightly but not significantly ($P > 0.05$) decreased in the storage waxes after 24 h regardless of storage temperature. In the finishing waxes, significant rapid reductions of culturable pathogens were observed; after 24 h, *Salmonella* could not be detected by plating (<1 CFU/mL) while *LM* populations ranged from <1 CFU/mL to 2.0 log CFU/mL.

Significance: *Salmonella* and *LM* can survive in some types of commercially-used citrus waxes but further research is needed to determine survival persists after application to fruit.

P2-78 Developing and Maintaining Food Safety Culture Through Implementation of GFSI Benchmarked Standards: A Success Story

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Introduction: Access to safe and quality food is of paramount importance and essential requirement for consumers to maintain their health and wellbeing. The meticulous efforts of food producers to demonstrate their commitments to food safety and fulfill customers' preferences and expectations can gain more attention if organizations demonstrate well-established quality and food safety cultures. Top management commitment and involvement is mandatory to imbed positive food safety culture at all levels in the organizations

Purpose: The purpose of this study was to depict adoption of innovative ideas and reflection of collective attitude, beliefs and behaviors of organizations' top management, managers, supervisors and food handlers towards resolving food safety and hygiene issues and setting contemporary trends which leads towards transforming existing food safety practices into more sophisticated and regimented food safety culture.

Methods: In the present study, a survey of food manufacturing units and distribution centers of Mawarid Food Company was conducted on quarterly basis in the kingdom of Saudi Arabia

Results: Results of this study showed that appropriate trainings, empowering the employees to share their ideas, motivations, strong top management commitments are ways which lead towards transforming existing food safety practices into a more sophisticated and regimented food safety culture.

Significance: This study is quite helpful for food producers and retailers as to how they can turn their dreams into reality when they successfully attain certification of their food facilities against FSSC 22000 benchmarked standards by a prestigious GFSI-approved certification body.

P2-79 From Top Floor to Shop Floor: Exploring the Food Safety Culture Communication Concept

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Introduction: It is irrefutable that food safety culture exists in every food business whether it is acknowledged, celebrated or ignored. From an academic perspective, it has been described as everything from a 'shared value system' to a 'fuzzy concept,' however, translating terminologies into practice can prove difficult. Before food safety culture strategies are communicated company-wide, managers must be confident that they understand the concept so that realistic direction and sustainable expectations are shared.

Purpose: To explore manager attitudes and perceptions towards food safety culture and review company documentation and communication mechanisms to identify gaps and inconsistencies.

Methods: Semi-structured interviews ($n = 23$) were conducted with managers at three manufacturing facilities of a multi-site food business. Interview transcripts were analyzed following a pre-defined coding structure developed from the Global Food Safety Initiative's position paper on food safety culture. Company documentation and communication mechanisms were analyzed using the same structure.

Results: Food safety culture investment was perceived as non-committal by junior managers with suggestions that senior management were too distant from production and so efforts were "ticking a box." In company documentation, the 'customer' was implied as the catering establishment to which food products were supplied and all managers overwhelmingly agreed that "customer service" was the "number one priority." Contrary to this, communication mechanisms raising food safety culture awareness included signage portraying the 'customer' as the end consumer. No documented vision, mission or communication strategy existed, and few managers were able to clarify how food safety culture shaped the organization beyond accrediting body requirements.

Significance: A positive food safety culture is led by a consistent, credible communication strategy, underpinned by a vision and mission that is relevant and relatable to encourage sustained engagement. Identifying and remedying gaps in management knowledge and addressing inconsistent messaging may enhance food safety culture endeavors.

P2-80 Gender Analysis of Food Safety Practices in the Dairy and Meat Value Chains in Two Locations of Ethiopia

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❖ ***Developing Scientist Entrant***

Introduction: Women in low and middle income countries (LMIC) such as Ethiopia carry a disproportionate responsibility for food safety because their traditional roles place them in charge of food production, handling and preparation. Examining gender roles in the meat and dairy value chains allows for the creation of interventions for those who are more likely to be at risk of foodborne diseases.

Purpose: The purpose of this analysis was to examine the gender roles in the meat and dairy value chains in the Kersa and Walmera Woredas of Ethiopia in order to more fully understand the implications of gender issues on foodborne disease transmission.

Methods: Semi-structured questionnaires were used to gather data on gender roles pertaining to food safety practices in the meat and dairy value chains. Twenty-two interviews were conducted in the Walmera and Kersa Woredas. Male farmers were interviewed by males, and female farmers were interviewed by women. Interviews were conducted in the Afaan Oromoo or Amaharic languages for both Woredas. Results were translated into English by native speakers.

Results: In both regions, adult males and male children were responsible for slaughtering and cutting beef and other livestock for consumption. Adult women and female children were responsible for milking, boiling and processing of milk, butter and cheese, handling of cut meats and cleaning of dairy utensils and containers. Fumigating milk utensils with smoke was viewed as an adequate milk preservation technique by 41.7% of the respondents. Many of the participants consume raw milk and meat, and no preference was indicated between raw milk and boiling milk by 33.3% of participants.

Significance: Gender roles and food safety are intrinsically connected in the dairy and meat value chains in LMIC. Future food safety trainings need to understand gender roles to target the correct audiences in order to be effective in reducing exposure to foodborne pathogens.

P2-81 Lethality of Ultraviolet-C Irradiation Against Foodborne Pathogens as Affected by Types of Abiotic Surfaces

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Introduction: Ultraviolet C (UV-C) irradiation (wavelength: 200~280 nm) has been known to effectively kill microorganisms, but the antimicrobial activities of UV-C irradiation against foodborne pathogens as affected by types of abiotic surfaces has not been intensively studied yet.

Purpose: This study was performed to investigate the influences of types of abiotic surfaces on the antimicrobial activities of UV-C irradiation generated from LED against *Escherichia coli* O157:H7 and *Bacillus cereus* spores.

Methods: *E.coli* O157:H7 or *B. cereus* spores were spot-inoculated (*ca*. 6 log CFU/coupon) on the surfaces of abiotic coupons (glass, plastic, stainless steel and wood; 5 cm×2 cm) followed by drying for 1 hour. After drying, the coupons were exposed to UV-C irradiation (distance: 5 cm) for up to 10 or 45 min, respectively. The remaining populations of microorganisms on abiotic surfaces were determined by a direct plating method.

Results: The initial populations of *E. coli* O157:H7 and *B. cereus* spores inoculated on various coupons were *ca*. 6.2 log CFU/coupon. When coupons containing *E. coli* O157:H7 were treated with UV-C irradiation for 10 min, *E. coli* O157:H7 inoculated on plastic, and stainless steel surfaces was completely inactivated but the populations of *E. coli* O157:H7 on the wooden surface or glass surface were reduced to 3.2 or <1.5 log CFU/coupon, respectively. When coupons containing *B. cereus* spores with UV-C irradiation for 45 min, the populations of *B. cereus* spores on glass, plastic, stainless steel, or wooden surfaces were decreased only by 1.3, 0.8, 1.0, or 1.5 log CFU/coupon, respectively.

Significance: This is the first study that investigated the influence of types of abiotic surfaces on the lethality of UV-C irradiation generated from UV-C LED. These results may provide basic information in developing decontamination program of foodborne pathogens using UV-C irradiation.

P2-82 Cross-border Safety and Regulatory Implications of Clean Label: Stats, Trends, Challenges and Lessons Learned

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Introduction: The Clean Label movement has spread around the globe to fundamentally change how some foods and food ingredients are produced, handled, and consumed. Varying country and regional perspectives of what's "clean" and "legally allowed" are casting a shadow of concern on the relevance and validity of some regulations and food safety guidelines and also on trade opportunities.

Purpose: Identify similarities and differences in the approach of leading international regulatory agencies for categorizing emerging ingredients and processing technologies used to produce Clean Label foods in the international trade.

Methods: This presentation will review cross-country trends in published and unpublished data along with case studies on the shelf life and safety of Clean Label products to identify patterns in the classification and regulatory oversight of emerging types of food ingredients and novel food processing technologies against the backdrop of various prevailing food safety regulations and guidelines.

Results: Quantitative and qualitative data from food manufacturing sectors from around the world will illustrate the importance of a common and harmonized defining what constitutes a Clean Label product or manufacturing process in terms relevant to shelf life and food safety. This presentation will provide a practical science-based pathway for categorizing Clean Label ingredients and food processing technologies for a systematic safety monitoring and eventually, a harmonized regulatory oversight around the globe.

Significance: Understanding the fundamentals of Clean Label formulation, reformulation, and processing in terms relevant to categorization, shelf life and food safety will not only be reassuring to consumers and the industry but also help regulatory agencies with a framework for oversight and possibly, certification to help level the playing field for international trade.

P2-83 Validation of the Baking Process as a Kill-step for Controlling *Salmonella* in Brownies

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* can survive under dry environment of flour for long periods of time. *Salmonella* can thrive when flour is hydrated while preparing batter or dough, and could cause serious foodborne illnesses if the product is improperly cooked or baked.

Purpose: To validate a simulated commercial brownie baking process as a kill-step for controlling *Salmonella*.

Methods: This study was conducted in a randomized complete block design with three replications. All-purpose flour was spray inoculated with a 5-serovar *Salmonella* cocktail, and dried back to original pre-inoculation water activity (a_w). The *Salmonella* serovars used in this study were Enteritidis, Montevideo, Newport, Senftenberg and Typhimurium. Brownie batter was prepared from inoculated flour and baked in a conventional oven at 176.7°C for 40 min in a 12"x12" pan, followed by 15 min ambient-air cooling. Samples were taken at five-minute intervals during baking and after the 15 min cooling period for microbial enumeration, pH and a_w analyses. *Salmonella* populations were enumerated using injury-recovery media (brain heart infusion agar overlaid with xylose lysine deoxycholate agar). At each sampling point, a_w and pH of brownies were measured separately for the crust and crumb portions.

Results: The initial *Salmonella* population in brownie batter was 6.9 log CFU/g. The *Salmonella* population was below the detection limit (0.60 log CFU/g) after 30 min of baking. The *Salmonella* population decreased significantly at 15, 20, 25, 30, 35, 40 minutes and after cooling compared to the initial population. The pH was similar throughout the baking process, and the a_w significantly decreased over the entire baking process.

Significance: This study validated that the typical commercial brownie baking process utilizing an oven temperature of 176.7°C and baking for at least 40 min would achieve >5-log reductions in *Salmonella* populations. However, validation studies should be individually conducted for brownie products with different baking parameters and drastically different ingredients.

P2-84 Comparison of Thermal Resistances of Nonproteolytic C*lostridium botulinum* Types B and F and Psychrotrophic *Bacillus cereus*

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Introduction: Many Extended Shelf-Life (ESL) refrigerated foods are given a "nonprot bot cook" (i.e., 90°C for 10 min) to yield a 6-log reduction of nonproteolytic *Clostridium botulinum* spores and ensure food safety. Spores of certain psychrotrophic strains of *Bacillus cereus* have been found to be more heat resistant than those of nonproteolytic *C. botulinum*.

Purpose: This study evaluated and compared the thermal resistance of seven psychrotrophic *B. cereus* strains with ten nonproteolytic *C. botulinum* type B and F strains.

Methods: Spores of 10 nonproteolytic *C. botulinum* strains were prepared using biphasic media. Seven *B. cereus* strains were prepared using a Nutrient Agar (NA) method. Spores were diluted in ACES buffer (0.05 M, pH 7) to 10^5-10^6 CFU/mL, heat sealed in sterile NMR tubes and subjected to temperatures of 80-91°C in an oil bath. *C. botulinum* survivors were determined by a 5-tube MPN method using TPGY broth after incubation for 10 weeks and *B. cereus* survivors by plate count using NA after 48-hour incubation.

Results: Thermal *D*-values of nonproteolytic *C. botulinum* and *B. cereus* strains decreased as process temperature increased from 80 to 91°C, with highest being obtained at 80°C. *D*-values at 80°C ranged from 0.74 to 8.28 minutes for nonproteolytic *C. botulinum* and 183 to 454 minutes for *B. cereus* strains. *D*-values of nonproteolytic *C. botulinum* decreased to < 1.0 minute at 87°C, while all *B. cereus* strains had higher *D*-values, (52 to 133 minutes) at this temperature. *B. cereus* 6A16 had the highest *D*-values at any given temperature among the *B. cereus* strains tested.

Significance: The *B. cereus* strains tested in this study are more resistant to thermal processing than nonproteolytic *C. botulinum* spores. Therefore, the data may suggest the use of *B. cereus* spores as a potential target pathogen and non-select agent surrogate for establishing thermal processes for ESL foods.

P2-85 Thermal Inactivation of *Escherichia coli, Listeria monocytogenes, Salmonella* and *Enterococcus faecium* in Grains

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Introduction: *Salmonella, Escherichia coli* and *Listeria monocytogenes* may contaminate grains under unsanitary handling and processing conditions. *Enterococcus faecium* NRRL B-2354 is used as a surrogate in low moisture products.

Purpose: The objective of this study was to determine the thermal death time characteristics of *E. coli*, *L. monocytogenes, Salmonella* and *E. faecium* in groats and wheat.

Methods: Three independent replicate samples were inoculated with freeze-dried *E. faecium* or a cocktail of *E. coli*, *L. monocytogenes,* and *Salmonella* to achieve approximately 7 log CFU/g, and acclimated at 25°C for at least 24 h. Inoculated samples were aseptically dispensed in boil-in pouches, vacuum sealed, heat treated in water bath at temperatures of 85 to 99°C for 50 s to 12.5 min. Samples were pulled at predetermined intervals and plated for bacteria using appropriate media. Surviving organisms were counted, averaged, then transformed to log CFU/g.

Results: *E. faecium* was significantly more heat resistant in groats and wheat compared to *E. coli*, *Salmonella* and *L. monocytogenes.* Among the pathogens*, Salmonella* was the most heat resistant, followed by *L. monocytogenes* and *E. coli*. *E. faecium* demonstrated an average *D*-value of 2.04 and 1.45 min at 90°C, 1.01 min and 0.61 min at 94°C, and 0.57 min and 0.32 at 98°C in groats and wheat, respectively. S*almonella* demonstrated an average *D*-value of 1.42 and 1.57 min at 85°C, 0.61 min and 0.65 min at 92°C, and 0.44 min and 0.32 at 99°C in groats and wheat, respectively.

Significance: *D*-values of *E. coli*, *Salmonella*, *L. monocytogenes* and *E. faecium* were greater in groats compared to wheat. Thermal inactivation data showed that *E. faecium* would be a suitable surrogate for in-plant validation studies of groats and wheat in the range of 85 to 99°C. These data can be used as a scientific basis for thermal validation of similar grains.

P2-86 Sampling and Analysis of Food Industry Biofilms

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Introduction: A study of the composition of biofilms occurring on food contact and non-food contact surfaces within 7 food production facilities was conducted in order to determine species composition and levels of chemical constituents of biofilm extracellular polymeric matrix.

Purpose: To understand the microbiota associated with various food production environment biofilms and the composition of the EPS (extracellular polymeric substances) structures of the biofilms.

Methods: Environmental swab/scraper samples were taken from 7 food factories, including meat, dairy, ready meals, RTE fish and ambient sauce processing facilities in the UK. Biofilms were located at 20 areas within each factory using a commercial indicator spray (Itram Biofinder) and tested for aerobic total viable counts. Species composition of each biofilm swab was determined using both MALDI-TOF and 16s rDNA metagenomic analysis. Swabs were also analyzed to determine levels of carbohydrate, proteins & uronic acids using microplate assays.

Results: Microorganisms isolated from factory swabs identified by MALDI were predominantly pseudomonads and gram positive cocci, and to a lesser extent, *Enterobacteriaceae*. The organisms isolated most frequently from across the 7 sites were *Kocuria rhizophila*, *Acinetobacter junii* and *Micrococcus luteus*.

Metagenomic analysis revealed that food industry biofilm samples tested were mainly composed of the phyla Actinobacteria and Proteobacteria. Microbial diversity (ACE) varied between samples with ACE values of between 300 and 1,700. Amongst the most common genera across all sites tested were *Kocuria*, *Micrococcus, Rhodococcus, Pseudomonas* and *Acinetobacter*.

Carbohydrate levels tested from the swab biofilms ranged from 0 - 0.95 µmol/mL, protein levels tested from the swab biofilms ranged from 0.10 - 62.2 µg/mL and uronic acid levels tested ranged from 1.6 - 3,077 µmol/mL.

Significance: This study provides insights into the species composition of food industry biofilms across a diverse range of processing facilities.

P2-87 Rate of Inactivation Affects the Enumeration and Culturability of Shiga Toxin-producing *E. coli* Persisters Exposed to Ciprofloxacin

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❖ ***Developing Scientist Entrant***

Introduction: Bacteria, including Shiga toxin-producing *E. coli* (STEC), can enter a dormant state known as persistence. Although persisters have hitherto been linked to temporal antibiotic tolerance, it is possible that dormancy enhances survival in food systems and delays growth during enumeration. The traditional method to recover persisters is to add antibiotic to susceptible populations which inactivates growing and tolerant cells leaving residual dormant cells. There is debate whether antibiotics just select for persisters or actually induce formation. This is of direct significance when studying persisters in food or environmental systems.

Purpose: To determine whether antibiotic exposure selects for or induces persistence in STEC.

Methods: STEC O103:H3 was grown to mid-exponential phase in tryptone soy broth, treated with ciprofloxacin at or above the minimum bactericidal concentration (MBC), and incubated 12 hours at 37°C. Bacteria were enumerated before treatment, and up to 12 hours post-antibiotic addition. The number of culturable cells was determined by plating on TSA, and incubating 16 hours at 37°C. Rate constants (k) for log-linear inactivation were determined by first kinetics where: $k = (-\log(N/N_0)*2.303)/(t-t_0)$; N and N_0 are the CFU·mL^{-1} at times t and t_0. Persistent proportion is reported as $\log(N/N_0)$ after populations had stabilized. At least three replicates were performed. Significance was established by Welch's *t*-test at $P < 0.05$.

Results: Populations became persistent at the same time (2 hours) post-exposure regardless of antibiotic concentration. Rate constants correlated to the number of persisters recovered (k = 4.08 vs. 6.87 and $\log(N/N_0)$ = -2.67 ± 0.64 vs. -5.25 ± 0.60 at 1 and 10 × MBC). This indicated antibiotic exposure likely induced persister formation.

Significance: Persisters can pose a significant threat to food safety. This work highlights the need for alternative methods to enumerate persisters for research purposes given that antibiotic treatment induces dormancy rather than selects against susceptible cells.

P2-88 Sensitivity of Non-pathogenic LT2 and Pathogenic *Salmonella enterica* Serovars to Elevated Hydrostatic Pressure and Citricidal Under Controlled Temperature

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✦ ***Undergraduate Student Award Entrant***

Introduction: As the leading cause of foodborne hospitalization and death episodes, *Salmonella enterica* serovars are also responsible for very high (32,900) disability adjusted life year (DALY) annually in the United States. While the private food manufacturing relies heavily on results of validations studies for inactivation and decontamination of this pathogen from an array of products, availability of such studies conducted in realistic environments of manufacturing and processing is limited due to pathogenic nature of the organism.

Purpose: Our objective was to compare the sensitivity of a non-pathogenic strain of *Salmonella enterica* (LT2) to a five-strain mixture of the pathogenic non-typhoidal *Salmonella enterica* serovars.

Methods: A single strain of non-pathogenic *Salmonella enterica* LT2 and a five-strain mixture of wild-type pathogenic *Salmonella enterica*, were exposed to i) hydrostatic pressure at 500 MPa; ii) hydrostatic pressure at 300 MPa; iii) hydrostatic pressure at 300 MPa with 1% citricidal for 0 (untreated control), 1, 3, and 5 minutes at 25°C. Samples were neutralized using D/E broth prior to enumeration. The experiments were statistically analyzed using LSD-based ANOVA.

Results: Counts of the pathogen mixture and the non-pathogen isolate were similarly reduced ($P < 0.05$) for >5 log CFU/mL when the samples exposed to 500 MPa treatments for 3 and 5 minutes. Similar reductions (i.e., >99.999%) were also reported when samples treated at 350 MPa in presence of 1% citricidal for both phenotypes. Reductions were less pronounced for treatments at 300 MPa without the antimicrobial but were similar ($P \geq 0.05$) comparing the two phenotypes.

Significance: Results of our study indicates both pathogenic and non-pathogenic phenotypes have comparable ($P > 0.05$) sensitivity to heat- and pressure-based pasteurization in presence or absence of an antimicrobial. This indicates that both phenotypes could be used interchangeably for decontamination and validation studies that could increase the external validity of studies associated with this pathogen of public health concern.

P2-89 Reduction of *Enterococcus faecium* and *Salmonella* in Fried Potato-based Snacks at Various Moisture Levels

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Introduction: *Salmonella* is a pathogen of concern in low moisture ingredients that can be associated with potato-based snack pellets. *Enterococcus faecium* NRRL B-2354 is commonly used as a surrogate for foodborne pathogens in low moisture products.

Purpose: This study investigated the fate of *Salmonella* and *E. faecium* in potato-based pellets at 5.4% and 14.4% moisture levels when subjected to thermal treatment in oil.

Methods: Samples (25 g) were inoculated with *E. faecium* or a cocktail of *Salmonella* to achieve about 7 log CFU/g, and stored overnight for culture adaptation at 4°C. Three independent replicates of *E. faecium* and *Salmonella* samples were subjected to oil frying from 80 to 108°C. Samples were pulled at predetermined intervals and plated for *E. faecium* or *Salmonella* using appropriate media. Surviving organisms were counted, averaged, then transformed to log CFU/g.

Results: *E. faecium* demonstrated an average *D*-value of 1.06 min at 98°C, 0.65 min at 103°C, and 0.31 min at 108°C for 5.4% moisture, and 1.93 min at 88°C, 0.85 min at 92°C, and 0.45 min at 96°C for 14.4% moisture. *Salmonella* had an average *D*-value of 2.38 min at 89°C, 1.2 min at 92°C, and 0.72 min at 98°C for 5.4% moisture, and 1.05 min at 80°C, 0.47 min at 85°C, and 0.24 min at 90°C for 14.4% moisture. It was observed that *E. faecium* was approximately 1.6 times more heat resistant than *Salmonella* at the 5.4% moisture level, and 2.8 to 5.4 times more heat resistant at the 14.4% moisture level in the range of 90 to 110°C.

Significance: Thermal inactivation data showed that *E. faecium* would be a suitable surrogate for in-plant validation studies of oil fried, low moisture products, such as potato-based pellets. This data can also be used as a scientific basis for validation of similar fried low moisture products.

P2-90 Thermal Assisted High-pressure Processing of Three Microbial Spores in Presence of Nisin, Lysozyme, Lactic Acid, and Citricidal in Deionized Water and a Food Vehicle

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❖ ***Developing Scientist Entrant***

Introduction: Validation studies for inactivation of microbial spores using pressure-based interventions could enhance the competitiveness and assure shelf-stability of pressure-treated commodities.

Purpose: The current study investigated synergism of heat and four antimicrobials for enhancing efficacy of elevated hydrostatic pressure treatments in deionized water and carrot juice for inactivation of three microbial spores currently considered as one of the most pressure-resistant natural isolates, the biological indicator for heat-based sterilization, and indicator for heat- and chemical-based decontamination interventions.

Methods: Various times (0, 3, 7, and 11 minutes) at pressure intensity level of 650 MPa (94,000 PSI) of elevated hydrostatic pressure (Hub880 Explorer, Pressure BioScience Inc.), were investigated at 50°C for spore reduction of *Bacillus atrophaeus* (ATCC 9372), *Geobacillus stearothermophilus* (ATCC 7953), and a strain of *Bacillus amyloliquefaciens.* Harvested spore suspensions were exposed to treatments at the above-mentioned intensity with or without the presence of lysozyme (22.4 mL/L), lactic acid (1% v/v), citricidal (1% v/v), and nisin (5000 IU/mL) in deionized water and carrot juice. Data statistically analyzed using Tukey-adjusted ANOVA.

Results: Pressure treatments alone at 650 MPa had no ($P \geq 0.05$) or minor ($P < 0.05$) effects on reduction of *Bacillus amyloliquefaciens, Bacillus atrophaeus,* and *Geobacillus stearothermophilus.* The addition of the bacteriocin (nisin, 5000 IU/mL) enhanced ($P < 0.05$) the decontamination efficacy of the treatment against *Bacillus amyloliquefaciens,* and *Bacillus atrophaeus*. Comparable reductions were also associated with the use of lactic acid and citricidal. Counts of *Geobacillus stearothermophilus* were consistently lower than the other strains prior and after the treatments that could be attributed to the growth temperature requirements of the isolate.

Significance: Although spore suspensions were considerably more fastidious than microbial pathogens in planktonic stage in response to elevated hydrostatic pressure, up to >99% inactivation of the selected strains were observed when treatments were synergized with mild heat and a bactericidal and/or bacteriocin compound.

P2-92 Attachment of GFP-producing *Escherichia coli* O103 on Beef Tissues Over Time

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Escherichia coli* O103 is a Shiga toxin-producing bacterium associated with foodborne illness outbreaks in beef. An avirulent *E. coli* O103 strain possessing green fluorescent protein (GFP) may be an effective surrogate simulating Shiga toxin-producing *Escherichia coli* (STEC) behavior in beef; however, attachment characteristics must be investigated prior to use as a STEC surrogate.

Purpose: This study investigated attachment of an avirulent, GFP-*E. coli* O103 to lean and adipose beef tissues over time.

Methods: Beef brisket was purchased from a local grocer, cut into 50 cm2 (lean or adipose) tissue samples, inoculated with ca. 5 log (CFU/mL) GFP-*E. coli* O103 prepared in Tryptic Soy Broth [TSB] or Purge, stored at 4°C, and enumerated at 0, 3, 5, and 20, 60, 180, 480, 720, 1,440, and 2,880 minutes. Inoculated brisket was homogenized for 1 min at 230 rpm in 250 mL 0.1% peptone water (PW) and shaken at 200 rpm for 90 s in an orbital shaker (loose); immediately following, the same brisket was transferred to a second stomacher bag with 250 mL PW and homogenized a second time for 60 s at 230 rpm (firm). Loose and firm samples were enumerated using MacConkey Agar, and then incubated at 37°C for 18-24 h.

Results: The main effects of sample type (loose or firm) and time were statistically significant (P<0.0001). The sample type*tissue (lean or adipose; P=0.0004), sample type*diluent liquid (TSB or Purge; P=0.0228), and sample type*time (P=0.0039) interactions were all significant. GFP-*E. coli* O103 more firmly attached to adipose beef tissue and loosely attached populations decreased throughout storage.

Significance: Observed attachment of GFP-*E. coli* O103 to beef tissues was impacted by fat content and time. Similar research conducted with STEC suggests GFP-*E. coli* O103 attachment to beef tissues is comparable to STEC, demonstrating possible STEC surrogate potential; however, further investigation is needed.

P2-93 Selenite Cystine Agar as a Selective Enumeration Media for *Salmonella* Serovars Used in Antimicrobial Intervention Studies Incurring Conditions of Metabolic Stress

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❖ ***Developing Scientist Entrant***

Introduction: Food processing often introduces various stressors on inoculated challenge microorganisms such that traditional selective media are too harsh to recover and enumerate the remaining viable and injured population quantitatively.

Purpose: Our objective was to examine additional selective media that could be used with our inoculum of 5 *Salmonella* serovars for quantitative enumeration after stressful conditions incurred during food processing.

Methods: *Salmonella* Thompson 120, *Salmonella* Heidelberg F5038BG1, *Salmonella* Hadar MF60404, *Salmonella* Enteritidis H3527, and *Salmonella* Typhimurium H3380 were characterized for antibiotic resistance and acid adaptation in TS broth with 0%, 0.25%, or 1.0% glucose. Selenite cystine (SC) broth, traditionally used as a selective enrichment broth, was used as the basis of a selective agar (SCA) in combination with three antibiotics to which our inoculum strains are resistant. Serovars of *Salmonella*, both individually and in mixtures, were enumerated on TSA, SCA, XLD, and HE selective agars (all containing the same antibiotics) after conditions of nutrient starvation, and acid-, desiccation-, and thermal-stress.

Results: The data show that quantitative enumeration on SCA was not significantly different ($P > 0.05$) than achieved on TSA for all tested stress categories, whereas levels of *Salmonella* enumerated on XLD and/or HE were often more than 1-1.5 log lower. All trials were performed in triplicate replication and analyzed by one-way ANOVA with the Holm-Sidak test for pairwise multiple comparisons to determine significant differences ($P < 0.05$).

Significance: The data confirm that SCA (+antibiotics) is a suitable selective medium for enumeration of these *Salmonella* serovars after various conditions of stress and that acid adaptation of cultures alleviates the need for injury supplements such as sodium pyruvate or yeast extract.

P2-94 Evaluation of the Impact of Different Monosaccharides on *Listeria monocytogenes* and Potential Competitors Growth in a Chemically Defined Medium

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Introduction: *Listeria monocytogenes* (*Lm*) is ubiquitous, leading to challenges in food processing environments and foods lacking a kill step. Customer interest in foods for dietary or health needs can result in newer compositions with limited understanding of microbial effects. Recent changes to food ingredient notifications regarding allulose and the development of *Listeria* enrichment media utilizing allose raised the concern that sugars could alter *Lm* growth relative to competing organisms.

Purpose: This study evaluated whether *Lm* exhibits growth changes in the presence of different carbon sources that could lead to competitive changes.

Methods: Using the Bioscreen C system, several monosaccharides were evaluated for their ability to support the growth of *Lm* (n = 5) and potential competitors (n = 11), such as *Enterococcus.* Growth was evaluated in a chemically defined medium in independent trials with six replicates. Cultures were incubated for 23.5 h at 37°C, and the area under the curve was calculated for each. The fold-change was calculated against the respective media control.

Results: All strains were able to grow when glucose was the provided carbon source. Non-*Listeria* (n = 6) showed a 1.67- to 6.36-fold increase in fructose, glucose, mannose, and trehalose, averaging a 3-fold increase. *Listeria* also showed an average 3-fold change in growth but with less variation, as expected given their relatedness. When evaluating allose and allulose, a stark reduction in growth was observed for the non-*Listeria* group with several strains unable to grow in allose. In allulose, these strains showed a 1.87 average fold increase. Conversely, *Lm* showed a limited growth reduction with a 2.31 and 2.44 average fold increase with allose and allulose, respectively.

Significance: A reduction in relative growth ability could result in reduced competitive inhibition in foods containing these sugars. Further work is needed to determine if this shift is observed with other pathogens and to directly assess competition.

P2-95 Homologous Stress Adaptive Response in Eight Strains of *Listeria monocytogenes* after Gradual Exposure to Increasing Sublethal Concentration of Quaternary Ammonium Compound

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Introduction: There are well-developed stress mechanisms in *Listeria monocytogenes* that can induce adaptation to harsh environmental conditions.

Purpose: The objective of this study is to determine the survival of eight strains of *L. monocytogenes* in lethal quaternary ammonium compound (QAC) after sublethal adaptation.

Methods: Eight strains of *L. monocytogenes* were exposed to gradually increasing sublethal concentrations by spiking at hourly intervals for 5 h to reach sublethal concentration of QAC (0.5 to 2.5 µg/mL) and then the cells were allowed to grow at that sublethal concentrations for 19 h until OD_{600nm} reaches 0.9 at the end of each day for 5 daily cycles at 22°C. Three methods were used for observing differences in QAC adapted and non-adapted cells for changes in growth rate and survival patterns in lethal concentration of QAC: (1) Short-range MIC of QAC was determined for adapted and non-adapted cells by broth dilution assay; (2) Changes in growth rate and lag time of adapted and non-adapted cells was determined by OD_{600nm} in 2 µg/Ll of QAC; and (3) Survival of adapted and non-adapted cells was determined by concentration-to-kill assay in lethal QAC (5 µg/mL) in agar model. All experiments were repeated thrice. Logs-transformed counts were analyzed using One way ANOVA in a completely randomized block design and means were separated by Fisher's protected LSD when $P < 0.05$.

Results: Four main findings were observed: (1) Short-range MIC of QAC was significantly increased for all eight strains by 1-4 µg/mL ($P < 0.05$). A two-fold increase in MIC of QAC was observed for one QAC adapted strain of *L. monocytogenes;* (2) Growth rate of QAC adapted cells was faster for 6 out of 8 strains in 2 µg/mL of QAC by OD_{600nm} which was significantly higher than the non-adapted cells ($P < 0.05$); (3) Lag phase of *L. monocytogenes* was decreased by 6-8 h in 2 µg/mL of QAC for QAC-adapted cells compared to non-adapted cells for 6 out of 8 strains; and (4) Survival of QAC-adapted cells was increased significantly by 2-5 log CFU/mL ($P < 0.05$) for 6 out of 8 strains in concentration-kill-assay in lethal QAC (5 µg/mL) in TSAYE compared to non-adapted cells.

Significance: These findings illustrate the potential for emergence of QAC tolerant phenotypes of *L. monocytogenes* after sublethal adaptive response where QAC may be widely used in the food processing environments.

P2-96 Changes in Susceptibility to Trimethoprim in *Listeria monocytogenes* Strains after Exposure to Gradually Increasing Sublethal Concentrations of Quaternary Ammonium Compound

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Introduction: The rise of antibiotic-resistant bacteria represents a serious threat to public health and the economy.

Purpose: The objective of this study is to determine the heterologous stress adaptive response in *Listeria monocytogenes* strains against trimethoprim in broth and agar models after adaption to sublethal quaternary ammonium compound (QAC).

Methods: Eight strains of *L. monocytogenes* at 10^7 CFU/mL were exposed to gradually increasing sublethal QAC concentrations by spiking at hourly intervals for 5 h to a reach a final concentration of 2.5 µg/mL QAC in TSBYE and then cells were allowed to grow at the sublethal concentration for 19 h until OD_{600nm} reaches 0.9 at the end of each day for 5 cycles at 22°C. Three methods were used for observing differences in QAC-adapted and non-adapted cells for changes in survival patterns in trimethoprim: (1) Short-range MIC of trimethoprim was determined for QAC-adapted and non-adapted cells by broth dilution assay; (2) Changes in growth rate and lag time of QAC adapted and non-adapted cells was determined by OD_{600nm} in trimethoprim (0.125 µg/mL) in broth model; and (3) Survival of QAC-adapted and non-adapted cells was determined against trimethoprim (0.125 µg/mL) in agar model. All experiments were repeated thrice. Logs transformed counts were analyzed using one-way ANOVA in a completely randomized block design and means were separated by Fisher's protected LSD when $P<0.05$.

Results: Four main findings were observed: (1) The MIC of trimethoprim against *L. monocytogenes* was increased by 0.2 to 0.5 µg/mL for QAC-adapted cells compared to non-adapted cells for 6 out of 8 strains. Following adaptation, a two-fold increase in MIC of trimethoprim was observed for two strains of *L. monocytogenes* adapted to QAC; (2) The growth rate ($OD_{600\ nm}$) of QAC-adapted cells was significantly higher for up to 5-8 h than non-adapted cells in TSBYE containing trimethoprim (0.125 µg/mL) for 5 out of 8 strains; (3) The lag phase of *L. monocytogenes* was decreased in 0.125 µg/mL of trimethoprim for QAC-adapted cells compared to non-adapted cells for 6 out of 8 strains; and (4) Six QAC-adapted *L. monocytogenes* strains showed significantly increased survival by 1.5 to 2.5 logs CFU/mL against trimethoprim (0.125 µg/mL) in agar model as comparison to non-adapted cells.

Significance: These findings illustrate the potential for formation of *L. monocytogenes* tolerant phenotypes to trimethoprim in some environments as a result of gradual sublethal adaptive response to QAC in conditions where QAC may be used widely.

P2-98 Comparison of Aqueous Chlorine Dioxide Generated with Different Acids on Reducing Foodborne Pathogenic Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: Chlorine dioxide (ClO_2) is an FDA approved antimicrobial agent. It is commonly produced by mixing $NaClO_2$ with HCl. ClO_2 generated with other food-grade acids may have different antimicrobial efficacy.

Purpose: This study aimed to investigate the effect of acid on the production of ClO_2 and their antimicrobial activities against foodborne pathogenic bacteria.

Methods: Four percent $NaClO_2$ and 1 M HCl, citric acid, lactic acid, malic acid, or $NaHSO_4$ were mixed and stored at 7°C. The release of ClO_2 was measured daily for two weeks. Oxidation reduction potential (ORP) and pH of the aqueous ClO_2 were also determined. Approximately 7 log CFU of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* suspensions were treated with 1, 2.5, and 5 ppm generated ClO_2 for 3 or 5 min, and then analyzed for bacteria counts. Three grams of lettuce inoculated with 9 log CFU of the pathogenic bacteria were treated with 2.5 and 5 ppm ClO_2 for 5 min. Bacterial reduction after the treatments was determined.

Results: Stronger acids released ClO_2 faster, but they had shorter shelf lives. In pure cultures, longer time ClO_2 treatment resulted in 1 to 2 log CFU higher reduction for *E. coli* and *L. monocytogenes*. However, there was no difference for *Salmonella*. 2.5 ppm of ClO_2 made with citric acid, lactic acid, and malic acid showed higher reductions for all three bacteria. 5 ppm of ClO_2 reduced the bacteria to an undetectable level for most treatments. The bacteria reduction on lettuce showed a similar trend. 5 ppm of ClO_2 produced with malic acid resulted in the highest reduction, which was consistent with the result of malic acid made ClO_2 having the highest ORP.

Significance: Food-grade organic acids produced aqueous ClO_2 with stronger antimicrobial properties than inorganic acids. Organic acids and ClO_2 may have synergistic effects on pathogenic bacteria.

P2-99 High Prevalence of Extremely Heat-resistant *Escherichia coli* in Finished Beef Products

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Introduction: Thermal processing is commonly used to reduce pathogens and spoilage bacteria in foods, including beef. Extremely heat resistant (XHR) *E. coli* possess a molecular determinant, the Locus of Heat Resistance (LHR), that allows them to survive processing treatments and potentially contaminate finished products. In recent separate studies, the prevalence of XHR *E. coli* appeared higher in finished meat products compared to meat animals.

Purpose: Determine the prevalence of XHR *E. coli* through the beef production continuum.

Methods: For three lots of fed beef cattle, up to 12 generic *E. coli* (232 total) were isolated from each of the following: feces and hides at feedlot, feces and hides at abattoir, pre-evisceration carcasses, final carcasses, and strip loins (2 lots only). Isolates retaining viability following exposure to 60°C for 20 min were judged XHR. LHR presence was determined using a multiplex-PCR that targeted the 5'-, 3'-, and two internal regions of the locus. Prevalences were compared using Fisher's exact test.

Results: Overall, 12.5% of isolates were XHR, represented by 3% of feedlot hide, 46% of final carcass and 62.5% of strip loin isolates. Eleven percent of isolates contained a full or partial LHR. LHR containing isolates were found amongst those from abattoir hides (3%), pre-evisceration carcasses (11%), final carcasses (7%), and strip loins (79%). In both cases, only isolates from strip loin were statistically different ($P < 0.05$) compared to isolates from all other sources, which were not different ($P > 0.05$) from one another. Fifty-nine percent of the XHR isolates possessed the LHR, while 65% of the LHR containing isolates showed the XHR phenotype.

Significance: This study demonstrates that the occurrences of XHR and LHR^{+} *E. coli* increase as beef processing progresses. Further studies are required to identify the sources of XHR and/or $LHR^{+\ E.\ coli}$ contaminating final products.

P2-101 Effect of Goat Diet on the Prevalence of *E. coli*, Total Coliforms and Bacterial Pathogens on the Rumen Fluid and Feces

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❖ ***Developing Scientist Entrant***

Introduction: Ruminant animals are asymptomatic natural reservoirs of several bacterial pathogens. Recent studies are focused on methods that would reduce bacterial populations in food animals before entry to the food chain.

Purpose: This study examined the effect of diets on the prevalence of generic *E. coli*, total coliforms, and bacterial pathogens on the rumen fluid and feces.

Methods: Thirty-nine meat goats consisting of Savanna and Kiko x Savanna breed were divided into three groups with two replications of each treatment, with each one consisting of twelve to fourteen goats. Goats grazing on Bahia hemp pasture only received the mineral supplement; conversely, the remaining goats were fed with either Dried Distillers Grains and Solubles (DDGS) or Soybean Oil. The feeding trial lasted 90 days and feces samples were collected two weeks before and directly after slaughter. Rumen samples were also collected after slaughter. Fecal and rumen samples were examined for generic *E. coli* and total coliforms levels and the presence of *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Spp.

Results: The levels of *E. coli* (6.93 – 7.79 log CFU/g) and total coliforms (7.14 – 7.81 log CFU/g) in feces samples of goats fed with different diets were not significantly different. Similar results were observed from rumen samples with *E. coli* and total coliforms levels of 5.28 – 5.88 log CFU/g and 5.36 – 5.97 log CFU/g, respectively. However, *E. coli* and total coliforms levels in the feces were significantly higher ($P < 0.05$) than the rumen samples for each treatment. No pathogens were present in feces or rumen samples of any of the feed treatments.

Significance: The diet selected in this study did not affect the levels of *E. coli* and total coliforms in rumen fluid and fecal samples, however, the high number of these organisms indicate the potential risk of cross-contamination to meat carcass during slaughtering.

Poster

P2-102 *Staphylococcus aureus* Survival in Color and Sweetener Solutions

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Introduction: *Staphylococcus aureus* is an opportunistic pathogen that will produce toxins once the bacteria concentration increases numerous generations. The toxins can survive subsequent food processing and cause food intoxication to the consumer. It is important to understand the hold time for high water activity foods to ensure toxins will not exist in the final product.

Purpose: *S. aureus* inoculated color and sweetener solutions were held at various temperatures for up to one week to determine if *S. aureus* growth would be achieved at a level that would produce toxins.

Methods: Two color solutions and two aspartame solutions used for confections were inoculated with low levels of a *S. aureus* cocktail (NCC 8205 (SEE), NCC 8202 (SED), and NCC 8231). The solutions were incubated at either 30°C or 37°C with allocates plated from days 1, 2, 3, 4, and 7 on tryptic soy agar and Baird-Parker agar to allow for enumeration.

Results: The pH of the color solutions and the sweetener solutions varied between 6.01 and 6.26 and 4.61 and 4.73, respectively. On day 0, the average starting concentrations of *S. aureus* in the two color solutions and the two sweetener solutions were 2.76 ± 0.22 log CFU/mL and 2.47 ± 0.35 log CFU/mL, respectively. Over the course of the 7-day trial, no *S. aureus* growth occurred in either of the 4 solutions at 30°C and 37°C with no recovery by day 7 (detection limit = 0 log CFU/mL), suggesting toxin production would not have occurred.

Significance: The color and sweetener solutions did not support *S. aureus* growth, thus would not have led to the production of toxins. Therefore, the color and sweetener solutions can safely be held for a week prior to incorporation into a final food product.

P2-103 Isolation, Characterization, and HPLC Quantitation: Nitrate Reducing Bacteria and Their Fermentation of Nitrate to Natural Vegetable Nitrite

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❖ ***Developing Scientist Entrant***

Introduction: Nitrate-reducing bacteria are used to produce vegetable derived nitrite allowing green/clean label status as per USDA-FSIS definition of 'natural nitrite' which is responsible for cured meat color, microbial inhibition of spores, and flavor.

Purpose: The objectives of the study were 1) to isolate and characterize nitrate-reducing bacteria, 2) to optimize the fermentation of nitrate from vegetable extracts to natural nitrite, 3) to use HPLC to quantify nitrate and nitrite.

Methods: An 'on-agar' colony-screening assay was developed using the principle of the 'in-liquid' nitrate reduction assay to detect conversion of nitrate to nitrite on agar plate using M17 agar base plates (1.5%) were spread-plated with samples, overlaid with nitrate M17 agar as source of nitrate. After incubation, nitrite was detected using a thin (plain) overlay agar layer containing sulfanilic acid followed by a second overlay agar layer containing alpha-napthylamine; the appearance of red color zones above colonies indicated the presence of nitrite. Nitrate reducing bacteria were examined in broth extracts derived from vegetables and ability to ferment nitrate to nitrite was quantified by C8 reversed-phase ion-pairing HPLC analysis.

Results: Nitrate-reducing bacteria (NRB) isolated from vegetable extracts (celery, cabbage, lettuce) included strains of *E. coli*, *Staphylococcus capitis*; NRB isolated from animals includes *Streptococcus hyointestinalis* 1336 and 1340. HPLC analysis showed that crude celery extract content approximately 296.54 ppm nitrate. Using *Streptococcus hyointestinalis* 1336 and 1340, we obtained 92.03 ppm and 149.99 ppm nitrite, respectively. One-way ANOVA was used to determine significant differences ($P < 0.05$) of nitrite levels vs those produced with traditional cultures.

Significance: This is the first report of an on-agar colony screening assay for the detection nitrite and isolation of nitrate reducing bacteria allowing NRB to be readily isolated. Examination of new isolates will allow optimization of the fermentation process that can make nitrate reduction to nitrite more efficient.

Poster

P2-104 Optimizing Growth Conditions for Carotenoids-producing Yeasts

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Introduction: Carotenoids represent a group of valuable molecules for the food and feed industries for their coloring, antioxidant and health promoting properties. Agro-food industrial wastes may provide carbon and nitrogen source and other elements for yeasts to produce carotenoids reducing the production costs and avoiding pollution from the wastes to the environment.

Purpose: The objectives of this study were to identify carotenoids-producing yeasts, and optimize growth conditions for yeast growth using agro-food processing wastes as low-cost substrates.

Methods: Different grape cultivars grown at multiple vineyards in Nova Scotia were collected for isolation of yeasts. Total genomic DNA from yeast cells was extracted using commercial kits followed by identification based on DNA sequence analysis. For optimizing yeast growth condition, grape-wine processing waste was used as culture broth (°Brix 1.6 and pH 3.8) with additions of carbon source (sugar at 0.5, 1.0, 2.0, 2.5, or 3.0%), and nitrogen source (ammonia sulphate at 0.3, 0.4, 0.5, 0.6, or 0.7%). The final pH of the culture broth was adjusted to 5.0, 5.5, 6.0, 6.5, or 7.0. Yeast growth was investigated at 25 or 30°C based on the OD values and growth curves generated by a Bioscreen C instrument. The experiment trials were conducted following the central composite designs and data were statistically analyzed.

Results: Twenty-four carotenoids-producing yeasts were identified including *Rhodotorula mucilaginosa* (14211-3), *Rhodotorula glutinis* (115a11-9), *Sporidiobolus pararoseus* (14311-1) and *Sporobolomyces patagonicus*) (13311-5). Our results showed that the pH of culture medium played an important role in carotenoids-producing yeasts' growth. The culture broth with sugar concentration at 2%, ammonia sulphate concentration at 0.5% and pH at 7.0 was demonstrated as a suitable culture medium. Optimal temperature for yeast growth was different depending on the yeast species.

Significance: This study provides useful information on potential use of agro-food industrial wastes for yeasts to produce carotenoids.

P2-105 Enhancing the Growth and Survival of Probiotic *Saccharomyces boulardii* by Prebiotic Supplements

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❖ ***Developing Scientist Entrant***

Introduction: Multistate outbreaks of *Escherichia coli* O157:H7 have been associated with animal originated foods. Prebiotics may increase metabolic activities of probiotic organisms, such as inhibition against colonization by foodborne pathogens in the gut.

Purpose: This study aimed to investigate the prebiotic potential of inulin, fructooligosaccharides (FOS), and breadfruit fiber (BF) for promoting the growth and survival of probiotic *Saccharomyces boulardii*.

Methods: The optimal concentration of tested prebiotics on the growth of *S. boulardii* was determined. Growth curves of *S. boulardii* were developed in yeast nitrogen base with 1.0% to 4.0% (w/v) of inulin, FOS, or BF, at 30°C. The cells were enumerated at 0, 12, 24, and 48 h. Growth curves of *E. coli* O157:H7 were developed in M9 media with 1.0% individual prebiotics in the same manner at 37°C. Simulated digestion of *S. boulardii* was carried out in 10 mL of yeast extract broth containing 2.0% of inulin or BF. Probiotic yeast cells were enumerated via plate count on yeast extract agar at 0, 2, 4, 6, 9, 12, and 24 h.

Results: At 48 h, *S. boulardii* exhibited an optimal increase of 3.13, 3.14, and 3.00 log CFU/mL with 3.5% inulin, 2.0% FOS, and 4.0% breadfruit fiber, respectively. *E. coli* O157:H7 exhibited an increase of 1.26, 1.33, and 1.23 log CFU/mL with inulin, FOS, and BF, respectively. At 24 h under simulated digestion, BF resulted in the highest improvement in the survival of *S. boulardii*. The final count was 6.28 log CFU/mL, compared to 2.30 log CFU/mL of the negative control and 4.00 log CFU/mL of the inulin treatment.

Significance: Prebiotics have the potential to protect probiotic *S. boulardii* from harsh acidic conditions of the stomach and stimulate its growth in the colon, which could reduce the proliferation of pathogenic *E. coli* in farm animals.

P2-106 Antimicrobial Resistance of *Pseudomonas* spp., *Stenotrophomonas* spp., and *Burkholderia* spp., Isolated from Agricultural Farms in Mexico

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Introduction: *Pseudomonas* spp., *Stenotrophomonas* spp. and *Burkholderia* spp. are usually found in an agricultural environment, and have been considered reservoirs of antimicrobial resistance genes.

Purpose: To determine the presence of *Pseudomonas* spp., *Stenotrophomonas* spp., and *Burkholderia* spp., in soils from agricultural farms in Mexico and determine their resistance to antibiotics.

Methods: Thirty 100-g soil samples were collected in various farms distributed in Mexico. Samples were processed to isolate *Pseudomonas* spp., *Stenotrophomonas* spp., and *Burkholderia* spp., using enrichment media and identified by test biochemical then streaked in culture media with and without antibiotics (µg/mL): cefoperazone, erythromycin, tetracycline and polymyxin B (30 µg/mL), trimethoprim (25 µg/mL), vancomycin (6 µg/mL), ciprofloxacin (5 µg/mL).

Results: Forty-four isolates (16 *Pseudomonas* spp., 20 *Stenotrophomonas* spp., and 10 *Burkholderia* spp.) were obtained. All the *Stenotrophomonas* spp., and *Burkholderia* spp., isolates exhibited antimicrobial resistance against at least one antibiotic. The isolates with more resistance were observed against polymyxin B (76% of isolates), tetracycline (62% of isolates), cefoperazone (62% of isolates), vancomycin (56% of isolates), erythromycin (50% of isolates), trimethoprim (44% of isolates), rifampin (38 % of isolates) and ciprofloxacin (32% of isolates).

Significance: The presence of microorganisms with resistance against multiple antibiotics in the agricultural environment could be a hazard since horizontal transfer of antibiotic resistance could occur among these and foodborne bacterial pathogens

P2-107 Influence of Hippophae Rhamnoides *L.* Polysaccharides on the Human Gut Microbiota in Vitro

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❖ ***Developing Scientist Entrant***

Introduction: Human gut microbiota plays a critical role in maintaining gut homeostasis, and be thought to affect host physiological by preventing pathogen colonization and stimulating immune responses. *Hippophae Rhamnoides L.* is one of the medicinal and edible foods designated by the Ministry of Health of China, which is also recommended by the World Health Organization (W.H.O.) as a health product. *Hippophae Rhamnoides* polysaccharide (HP) has many biological activities such as improving immunity, anti-oxidation, anti-tumor, antiviral and anticoagulant. Howevre, there are few reports about the effects of HP on intestinal flora. Therefore, this study for the first time determined the regulation of gut microbiota functions of WSGP in the simulator of the human intestinal microbial ecosystem model (SHIME).

Purpose: This study investigated the fermentation behaviors of polysaccharides isolated *Hippophae Rhamnoides L.* berries using a SHIME system.

Methods: Polysaccharide (HP-S1) was purified from HP berry. Itsimpact on the microbiota was evaluated using the simulator of the human intestinal microbial ecosystem model (SHIME). The microbiome composition and the short chain fatty acids produced in SHIME were detected.

Results: HP-S1 solely consisted of glucose and its molecular weight was about 51645Da. Comparing with glucose and sugar-free groups, HP-S1 could up-regulate the ratio of Bacteroidetes to Firmicutes (FBR) (p <0.05). Also, the results of RT-PCR and NGS simultaneously observed the significant promotion of Lactobacillus, Bacteroides and Prevotella and the inhibition of Escherchia-Shigella (p <0.05). Furthermore, the concentration of propionate, isobutyrate, butyrate, valerate and hexanoate significantly increased during 48h fermentation (p <0.05).

Significance: The mechanism underlying the regulation of gut microbiota ability of HP has been uncovered. Additionally, this study can provide important evidence for the evaluation of the bioactive properties of *Hippophae Rhamnoides*.

P2-108 Determination of Thermal Inactivation Kinetics of *Salmonella* in Brownie Batter

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* can be introduced into bakery products through ingredients such as flour, milk cocoa powders, and eggs. Studies have shown that *Salmonella* can survive under adverse environmental conditions over a period of time; therefore, it is vital to study the thermal inactivation parameters of *Salmonella* in bakery products such as brownie batter.

Purpose: To determine the D- and *z*-values of a 5-serovar *Salmonella* cocktail in brownie batter.

Methods: All-purpose flour was inoculated with a 5-serovar *Salmonella* cocktail (Enteritidis, Senftenberg, Typhimurium, Montevideo, and Newport) and used to prepare the brownie batter. This study was designed as a randomized complete block design with three replications as blocks. The batter was subjected to heat treatment using thermal-death-time disks in a hot water bath at 64, 68, 72 and 76°C for 0, 3, 6, 9 and 12 min, respectively. The *Salmonella* population was enumerated using injury-recovery media (brain heart infusion agar overlaid with xylose lysine deoxycholate agar). The graphs for calculating D- and *z*-values were plotted using Microsoft Excel, and D- and *z*-values were calculated as absolute values of the inverse of slopes.

Results: The calculated D-values of 5-serovar *Salmonella* cocktail in brownie batter were 53.4 ± 5.38, 27.2 ± 2.04, 10.7 ± 0.72 and 4.6 ± 0.49 min at 64, 68, 72 and 76°C, respectively; whereas, *z*-values of the *Salmonella* cocktail was 11.1 ± 0.71°C.

Significance: The ability of *Salmonella* to survive in brownie ingredients such as flour over an extended period of time could create a public health risk if the product is improperly baked. D-values from this study provide basic information about the thermal resistance of *Salmonella* in brownie batter at the start of baking and could help the bakery industry to optimize cooking parameters ensuring the safety of brownies. However, further research should be conducted to understand thermal resistance of *Salmonella* with change in product water activity during baking.

P2-109 Heat Resistance of Foodborne Pathogens and Surrogates of Interest in Raw Wheat Flour

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Introduction: Thermotolerance data on pathogens and potential surrogates is critical information for the validation and verification of food safety plans for firms globally. Due to survival, heat resistance, and past outbreaks, *Salmonella* is the target organism of concern in most low-moisture foods and ingredients including raw wheat flour.

Purpose: We determined the thermotolerance of *Salmonella* ssp., *Enterococcus faecium*, and *Listeria* strains in a model low-moisture food matrix. In addition, we examined the suitability of a novel surrogate native to wheat flour, *Pantoea dispersa*.

Methods: Six-strain cocktails of both *Salmonella* ssp. and *Listeria monocytogenes*, as well as *Enterococcus faecium*, and *Pantoea dispersa* were cultured at high levels before dry-inoculating raw wheat flour at a water activity of 0.45. Inoculated samples were independently heat-treated at 70°C, 75°C, and 80°C and removed at regular intervals for enumeration on Tryptic Soy Agar. Each run was performed in triplicate.

Results: All of the thermal death time data was characterized using the linear log model. The average $D_{75°C}$-values were calculated as *L. monocytogenes* < *Salmonella* ssp. < *P. dispersa* < *E. faecium*. ANOVA tests on $D_{75°C}$- and $D_{80°C}$-values representing the thermotolerance of *Salmonella* ssp. and *E. faecium* were determined to be statistically significantly different from each other ($P < 0.05$). Notably, *L. monocytogenes* had a significantly lower $D_{75°C}$-value than the other three bacterial strains of interest ($P < 0.05$) whereas at 70°C, the thermotolerance was not significantly different than *Salmonella* ssp. ($P = 0.76$).

Significance: Consistent with findings in other low-moisture foods, *Salmonella* is the pathogen of concern in wheat flour due to thermotolerance and survival. Additional investigation of *L. monocytogenes* in wheat flour is warranted. *P. dispersa* should be considered as a viable non-pathogenic the alternative surrogate to *E. faecium* in this matrix.

P2-110 Comparison of Thermal Resistance of *Salmonella* in Wheat Flour Inoculated Via Glass Bead Transfer and Liquid Inoculation

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Introduction: Methods for inoculating low-moisture foods include use of inert carriers (e.g., talc) and direct addition of liquid cultures. However, these methods may lead to changes to the product (e.g., water activity, clumping/caking, composition). While a bead transfer method minimizes these effects, the thermal resistance of bacteria added to food using this inoculation method has not been characterized.

Purpose: To compare the thermal resistance of *Salmonella* in wheat flour inoculated via bead transfer and liquid inoculation.

Methods: *Salmonella enterica* serotypes (*S.* Agona 447967, Montevideo 988275, Mbandaka 698578, Tennessee K4043 and Enteritidis PT30) were grown on trypticase soy agar with 0.6% yeast extract. Cultures were harvested in buffered peptone water and mixed to form a cocktail. Wheat flour, 100 g, was inoculated directly with 1 mL liquid cocktail or by adding 10 g of inoculated borosilicate glass beads prepared by drying 0.1 mL liquid cocktail onto them. Flour was mixed and held to re-equilibrate water activity as necessary. Beads were removed prior to testing. Flour was enumerated to assess homogeneity and initial inoculation level. Aluminum test cells were aseptically filled with inoculated flour and immersed in a water bath at 80°C. Three subsamples were enumerated at 0, 3, 6, 9, and 12 min after the come up time (CUT) was observed.

Results: Populations, initial and after CUT of 109-110 s, were similar, ~8.1-8.3 log CFU/g, regardless of inoculation method. However, the thermal resistance of *Salmonella* inoculated into wheat flour via borosilicate bead transfer, $D_{80°C}$ = 2.31 ± 0.14 min, was significantly different than direct liquid addition, $D_{80°C}$=5.14 ± 0.24 min ($P < 0.05$).

Significance: Thus, despite the small differences in observed D-values for the two inoculation methods, transfer via glass beads appears to be a suitable method for inoculating low moisture foods and may be advantageous when other inoculation methods may affect the product. This should be confirmed in other matrices.

P2-111 Evaluation of Drying Conditions to Inactivate *Salmonella* in Minimally Processed Apple Products

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Introduction: Hot air drying processes are used to provide specific quality attributes of certain products, such as dehydrated apple pieces. To comply with FSMA regulations there is a need to understand microbial lethality during these processes. Depending on product and process conditions, case hardening may occur which could affect microbial inactivation.

Purpose: To determine the level of inactivation provided by hot air drying on a *Salmonella* cocktail inoculated onto apple cubes.

Methods: *Salmonella enterica* serovars (*S.* Agona, *S.* Tennessee, *S.* Montevideo, *S.* Mbandaka and *S.* Reading) were grown on 0.6% tryptic soy agar with yeast extract (TSAYE). Cultures were harvested in buffered peptone water and combined to form a cocktail. Cored, peeled Gala apple cubes (~6.35 mm) were inoculated at 9.2 ± 0.3 log CFU/sample *Salmonella.* Apple cubes were dried at 104°C or 135°C in ~1.5 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 ($n \geq 5$) from multiple bed depths, on modified TSAYE. Water activity measurements were taken at corresponding time intervals. Studies were carried out in triplicate.

Results: Water activity decreased throughout the duration of the study with samples at 135°C drying faster than 104°C. Samples at the bottom bed depth, closer to the heat source, dried faster than those at the higher bed depth, regardless of temperature. Significant microbial inactivation did not occur during the first 10 min at the bottom bed depth or 40 min of drying at the top bed depth, regardless of temperature ($P < 0.05$). By the end of drying, *Salmonella* inactivation of greater than 5 log CFU/sample was achieved. Case hardening did not inhibit microbial inactivation in the conditions tested.

Significance: Hot air drying under the conditions evaluated may provide a preventive control in the production of dehydrated products, such as apples.

P2-112 Detection of *Salmonella* in 25-g Samples of All-purpose Flour Using the BAX® System

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Introduction: Flour is a raw, minimally processed agricultural ingredient typically sold without a heat treatment step to kill or inactivate bacteria. If present, bacteria can be rendered harmless by further processing to create baked goods and other cooked products. Historically, flour contamination was of little concern because of its low water activity, however in recent years multiple outbreaks of Shiga toxin-producing *E. coli* (STEC) infections have been linked to flour. Now, in 2019, flour has been recalled twice for the potential presence of *Salmonella*.

Purpose: In response to recent recalls, this study was designed to evaluate the BAX® System Real-Time PCR assay for the detection of *Salmonella* in all-purpose flour.

Methods: Flour was divided into 25 g test portions and inoculated with *Salmonella* Agona at levels expected to create low (0.2 - 2 CFU/sample) and high (5 - 10 CFU/sample) spikes after a two-week hold at 25°C to acclimate the target cells. Test method samples were homogenized with 225 mL of pre-warmed (35°C) BPW and incubated at 35°C for 18 - 22 hours before analysis by real-time PCR. For the FDA BAM reference method, samples were homogenized with 225 mL of LB, held at room temperature for 60 minutes, then incubated at 35°C for 18 - 22 hours. All samples were confirmed according to the FDA BAM reference culture method.

Results: The real-time PCR assay returned positive results for 9/20 low level samples and 5/5 high level samples identical to culture. When compared to the reference method, there was no significant difference in results since the 95% confidence interval of the dPOD contains zero.

Significance: The results of this study demonstrates the ability of the BAX® System to accurately detect *Salmonella* in 25 g samples of all-purpose flour at 18 hours equivalent to the reference method.

P2-113 The Natural Antimicrobial Carvacrol and Thymol Disrupt Desiccation Resistance in *Salmonella enterica* serovar Tennessee: Advancing Safety of Low-moisture Foods

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Introduction: *Salmonella enterica* can survive effectively in low-moisture (LM) foods. Consequently, large multi-state salmonellosis outbreaks have been attributed to consumption of foods such as peanut butter, infant milk powder, almonds and others. *S. enterica* employs desiccation resistance mechanisms to survive LM conditions by increasing concentration of intracellular ions (e.g. K^+) and compatible solutes (e.g. trehalose).

Purpose: To overcome the desiccation resistance mechanisms in *S. enterica* serovar Tennessee, a strain isolated from peanut butter, using two membrane-active food ingredients, namely carvacrol and thymol.

Methods: The minimum inhibitory concentration (MIC) of carvacrol or thymol, using broth microdilution method, was determined against *Salmonella* Tennessee before a range of ½ MIC to ≥ 2X MIC concentrations were used in subsequent experiments. Leakage of potassium ions and concentration of intracellular trehalose in *Salmonella* Tennessee, exposed to 0-800 µg/mL carvacrol or thymol, was measured using fluorescence of a potassium-sensitive probe, and trehalase assay, respectively. Luminescence ATP detection assay (Luciferin/Luciferase) estimated total ATP content in treated vs. untreated *Salmonella* Tennessee. Leakage of cellular proteins and DNA along with respiration activity were determined spectrophotometrically. Transcriptional analysis of desiccation-related genes was conducted using RT-qPCR.

Results: Carvacrol and thymol at ≥ 100 µg/mL caused leakage of cellular k^+, in a concentration-dependent manner ($P < 0.05$), from *Salmonella* Tennessee compared to the untreated control. Carvacrol or thymol at MIC sensitized *Salmonella* Tennessee (1.5-log CFU reduction) to desiccation stress. Range of 100 to 400 or 50 to 200 µg/mL of carvacrol or thymol, respectively, inhibited trehalose biosynthesis, decreased ATP content (12-59% decrease), arrested cellular respiration and caused leakage of proteins from *Salmonella* Tennessee, compared to the untreated control. Transcriptional analysis revealed that carvacrol at 100-200 µg/mL down-regulated (> - 2-fold change) some of the desiccation-related genes.

Significance: Having GRAS status, carvacrol and thymol could be exploited to design inactivation strategy to enhance safety of LM foods.

P2-114 Survival of *Salmonella* and Shiga Toxin-producing *Escherichia coli* during Tempering of Wheat Berries

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Introduction: Wheat flour contaminated with *Salmonella* or Shiga toxin-producing *Escherichia coli* (STEC) has been linked to foodborne outbreaks. The behavior of these organisms on wheat berries during tempering (addition of water) or subsequent milling is not known.

Purpose: This study assessed growth or survival of *Salmonella* and STEC during tempering of wheat berries with water or lactic acid (LA).

Methods: Wheat berries were inoculated with a five-strain cocktail of rifampicin-resistant *Salmonella* or STEC at a target of 3.5 log CFU/g and dried at 23°C for 24 h. Sterile water (control) or LA (~2 to 4 mL/kg) was applied at 3% (by weight) to inoculated wheat and then held at 15, 23 and 30°C for up to 12 h. Samples were stomached in 0.01 M phosphate buffered saline and plated onto tryptic soy agar and CHROMagar STEC or CHROMagar Salmonella supplemented with rifampicin and cycloheximide.

Results: After 12 h, populations of *Salmonella* and STEC declined by 0.34 and 0.62 log CFU/g, respectively, in inoculated wheat tempered with water and by 1.84 and 1.60 log, respectively, when 2 mL/kg LA was applied. Most of the reduction was observed immediately after application of LA. Increasing the concentration of LA to 4 mL/kg resulted in a significantly greater (1 log) reduction of STEC upon application, however, this difference was reduced to < 0.5 log after 12 h. Declines at 15, 23 and 30°C were not significantly different ($P > 0.05$). Populations of native microbiota and coliforms in uninoculated wheat increased by 0.5 to 1 log within 6 h of tempering with water but not with LA.

Significance: Addition of moisture during tempering of wheat does not support the growth of *Salmonella* or STEC. Addition of LA at this step may provide a means to reduce these pathogens in wheat.

P2-115 Desiccation and Acid Tolerance of Shiga Toxin-producing *Escherichia coli Associated* with Low-moisture Foods

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) gastroenteritis has been linked to consumption of low-moisture foods but little is known about the sensitivity of these isolates to desiccation and exposure to acid.

Purpose: This study evaluated the desiccation and acid tolerance of selected STEC strains in a model system (wheat berries) during storage and after exposure to lactic acid (LA).

Methods: Five individual strains of STEC, isolated from flour (O121 and O26), cookie dough (CDO157:H7), soy nut butter (O157:NM), and a clinical obtained from a walnut-associated outbreak (WO157:H7), were used to inoculate wheat berries at a target of ~3.5 log CFU/g prior to drying at 23°C for 24 h. Survival of STEC strains were evaluated during storage of inoculated wheat at 23°C for up to 27 days and after addition of 3% by weight LA (final concentration: ~4 mL/kg).

Results: Populations of individual STEC strains decreased by 0.34 (WO157:H7) to 0.93 (CDO157:H7) log CFU/g during 24 h of drying. After 27 days of storage, declines of 1.45 (CDO157:H7) to 1.06 (WO157:H7) log CFU/g were observed. Reductions of CDO157:H7 were significantly greater ($P < 0.01$) than any other strain. Declines in populations over 12 h after exposure to LA ranged from 1.73 (WO157:H7) to 2.14 (CDO157:H7) log CFU/g but were not significantly different ($P > 0.05$) among isolates. Most of the reduction was observed immediately after initial LA application.

Significance: These findings should be useful in assessing risk of STEC in low-moisture food processing.

P2-116 Effect of Water Activity on Thermal Inactivation of *Salmonella* spp. and *Enterococcus faecium* Nrrl B-2354 in Basil Leaves

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❖ ***Developing Scientist Entrant***

Introduction: The recalls/outbreaks of low-moisture foods due to their association with *Salmonella* have gained the attention of the public, the scientific community, and regulations. The survival of *Salmonella* due to enhanced heat resistance developed at low water activity poses a serious challenge in eliminating them during thermal processing.

Purpose: The research aims to: (1) Investigate the effect of water activity on thermal inactivation of *Salmonella* in basil leaves, and (2) Evaluate *E. faecium* NRRL B-2354 as an appropriate surrogate for *Salmonella* in basil leaves.

Methods: Basil leaves, inoculated with a 5-strain *Salmonella* cocktail and *E. faecium* NRRL B-2354 separately, were equilibrated to different water activities (a_w: 0.40, 0.55, 0.70) in a humidity-controlled chamber. During this time, homogeneity and stability tests were conducted for 15 days to ensure that the inoculum was stable and homogeneously distributed in the sample. Basil samples were packed (1.6 ± 0.1 g) in aluminum pouches, hermetically sealed, and thermally treated at 70, 75, and 80°C for various times to obtain the thermal death curve for *Salmonella* and *E. faecium*. Post-treatment, the samples were immersed in ice water bath and spread-plated on differential media: m-TSAYE (*Salmonella*) and e-TSAYE (*E. faecium*).

Results: The inoculation method used provided a stable (after 5 days) and homogeneous population of bacteria in the basil leaves. Therefore, the inoculated sample was equilibrated for at least 5 days before the thermal resistance study was conducted. The thermal resistance of *Salmonella* and *E. faecium* increased as the a_w of the sample decreased. At 70, 75, and 80°C, the *D*-value ($a_w = 0.55$) for *Salmonella* was 15.02 ± 1.57, 6.64 ± 0.78, and 1.86 ± 0.11 and for *E. faecium* was 26.84 ± 1.49, 9.57 ± 0.35, and 5.09 ± 0.26, respectively.

Significance: The spice industry may benefit from this study by developing the thermal processes for improving the safety of basil leaves. Also, *E. faecium* can be used as a conservative surrogate for the validation of thermal pasteurization processes.

P2-117 Fate of Foodborne Pathogens in Commercial Ready-to-eat (RTE) Chocolate Chip Cookie Dough Held at 4, 10, 15, and 23°C

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Introduction: Numerous ingredients used in the production of RTE chocolate chip cookie dough are potential sources of foodborne pathogens; the behavior of foodborne pathogens in commercially available RTE cookie dough remains unexplored.

Purpose: Our objective is to determine the fate of *Salmonella* spp., Shiga toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes* and *Staphylococcus aureus* in commercially available RTE chocolate chip cookie dough held at 4, 10, 15, and 23°C.

Methods: Commercially available RTE chocolate chip cookie dough, supplied by the manufacturer, was weighed (10 ± 0.5 g) into whirl-pak bags and inoculated with a nalidixic acid-resistant five-strain cocktail of either *Salmonella*, STEC, *L. monocytogenes* or a two -train cocktail of *S. aureus* to achieve ca. 5 log CFU/g. Samples were stored at 4, 10, 15, and 23°C and sampled at 0, and 4 hours, and 1, 2, 7, 10, 14, 21, and 30 days. At each sampling point, serial dilutions were plated onto non-selective media supplemented with 50 µg/mL of nalidixic acid, and population numbers calculated. Experiments were replicated 3 times.

Results: All pathogen populations decreased at all temperatures over 30 days; reductions ranged from 0.5 to >4.7 log CFU/g. The greatest decreases occurred during storage at 23°C: 4.0 (*S. aureus*), >4.7 (*L. monocytogenes*), and 4.1 log CFU/g (*E. coli* and *Salmonella* spp.). The smallest population reductions occurred during storage at 4°C: 0.8 (*S. aureus*), 0.5 (*L. monocytogenes*), 2.7 (*E. coli*) and 2.5 log CFU/g (*Salmonella* spp.). Pathogen population declines over 30 days during storage at 10 and 15°C ranged between 1.0 to 3.0 log and 2.4 to 3.6 log CFU/g, respectively. Log reductions of *S. aureus* were less than the other pathogens at all temperatures.

Significance: This study demonstrates that commercially available RTE chocolate chip cookie dough provides suitable conditions for the survival of multiple foodborne pathogens and poses a food safety risk if consumed without baking.

P2-118 Quantifying the Survival of Salmonella during the Long-term Storage of Multiple Sugar Products

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✦ ***Undergraduate Student Award Entrant***

Introduction: Due to the low water activity and high osmotic pressure of sugar, it is generally recognized as presenting a low microbiological risk to human health. However, the role of sugar as a mainstay ingredient in minimally processed food products suggests a need to evaluate the potential for *Salmonella* survival in sugar.

Purpose: The objective of this study was to assess survival of *Salmonella* in multiple sugar products during long-term storage.

Methods: Granulated, powdered, brown, and liquid sugar obtained from a commercial supplier were inoculated with a 5-strain *Salmonella* cocktail. Samples were stored at three temperatures (4, 25, 37°C) in sealed containers, and sampled at 12 times up to ~1,000 days. Triplicate samples for each condition were serially diluted, plated on differential media, and survivors enumerated after incubation for 48 h at 37°C. Data were analyzed using ANOVA to determine the impact of sugar type and temperature on *Salmonella* survival.

Results: *Salmonella* survivors were quantifiable in some sugar products beyond 1,000 days. No significant differences were observed between granulated, powdered, and brown sugar reductions (~1.5-log reductions) at 4°C at ~400 days ($P > 0.05$). Significantly less *Salmonella* survived at 400 days of storage at 25

and 37°C ($P < 0.05$), with average reductions of 2.7-log reductions for granulated and powdered sugar and >5-log reductions for brown sugar. At ~1,000 days, survivors were countable in 8/12, 1/12, and 1/12 of all sugar samples stored at 4, 25, and 37°C, respectively.

Significance: *Salmonella* decline during long-term storage increased with storage temperature, but most samples maintained detectable levels of *Salmonella* after a year. These results suggest that sugar contaminated with *Salmonella* could pose a risk even after long-term storage.

P2-119 Surface Decontamination of Wheat Grain during Simulated Tempering

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Introduction: Wheat flour is a food commodity previously believed to be microbiologically safe. However, during the past decade several outbreaks and recalls were caused by *Salmonella* and enterohemorrhagic *Escherichia coli*. Milling was not designed for microbial decontamination of flour. A possible intervention is the application of antimicrobials during the addition of approximately 4% water to kernels (tempering), that typically lasts between 6 and 24 hours.

Purpose: To determine the efficacy of chemical treatments, to reduce *Salmonella* and *E. coli* viability on wheat kernels under laboratory conditions that resembled tempering.

Methods: *Salmonella* strains (Agona, Tennessee, Enteritidis PT30, Enteritidis 2415, Typhimurium 14028) or *E. coli* O121 (I2016000899, I2016012950, EC-6804, DA-1, MT#18) were grown in tryptic soy agar (TSA) media, mixed, suspended in buffer and inoculated onto wheat kernels (8 log CFU/g). After drying, kernels were mixed at 4% (v/w) with individual chemical solutions and incubated with continuous mixing mimicking tempering for up to 17 h at 4, 23 and 37°C. Kernels were mixed with diluent buffer and plated on TSA to determine viability. Average final counts were compared to controls using Student's t-test.

Results: Treatment of wheat kernels with 3% hydrogen peroxide or 5,000 ppm sodium hypochlorite reduced the viable count less than 0.5 log CFU/g and it was not significant ($P > 0.05$). Wheat kernels treated at 4% with a 0.5 M/0.01% pelargonic acid/quillaja saponin emulsion (PAQSE) at 4, 23 and 37°C for 6 and 17 h reduced the viable count of *Salmonella* and *E. coli* by more than 4 log CFU/g compared to controls ($P < 0.05$). When the PAQSE concentration was reduced to 0.25 M/0.05%, the reduction of *Salmonella* was less than 3 log CFU/g but it was still significant ($P < 0.05$).

Significance: The findings from this study identified a potential chemical treatment in the wheat milling process to mitigate microbial contamination.

P2-120 Intracellular Moisture Retention of Desiccated *Salmonella* in Low-water Activity Environments

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Introduction: The effects of water activity and moisture content on *Salmonella* inactivation in foods have been well-researched. However, previous thermal inactivation studies have shown non-linear kinetics of desiccated *Salmonella*, which has been theorized to be due to further loss of intracellular moisture retained following desiccation.

Purpose: Therefore, the objective of this study was to measure the loss of intracellular moisture retained by desiccated *Salmonella* at elevated temperatures.

Methods: *Salmonella* Anatum 6803 was harvested from tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) using buffered peptone water, then diluted 1:10 with pH-calibrated buffer solution at pH 7. Inoculated ($n = 5$), treated control ($n = 5$), and untreated control ($n = 3$) samples consisted of cellulose acetate filters to which 0.1 mL inoculated buffer solution, 0.1 mL uninoculated buffer solution, and no liquid were applied, respectively. Filters were initially dried at ambient conditions (~25°C/30% relative humidity) for 24 h, after which they were placed in a thermogravimetric analyzer (TGA) capable of measuring micro-scale mass changes during precise heating to determine intracellular water loss. Filters (2.9-4.4 mg) were weighed in the TGA during a 4 min heating treatment from 20-100°C, followed by a 20-min isothermal treatment at 100°C. Initial and final mass measurements were compared using ANOVA.

Results: Significant mass changes during TGA treatment ($P < 0.05$) were observed in the inoculated and treated control samples, but not in the untreated control samples ($P \geq 0.05$). Mass changes during TGA treatment were significantly different ($P < 0.05$) between the inoculated (2.31 ± 0.06% mass reduction), treated control (1.03 ± 0.05%), and untreated control (0.15 ± 0.12%) samples, indicating moisture was retained in cells dried under ambient conditions.

Significance: The observed loss of intracellular moisture may contribute to non-linearity of inactivation kinetics of desiccated *Salmonella* at elevated temperatures.

P2-121 Determination of Thermal Inactivation Parameters of Salmonella in Non-fat Dry Milk Powder and Hydrated Non-fat Milk

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❖ ***Developing Scientist Entrant***

Introduction: Non-fat dry milk (NFDM) powder is widely used as an ingredient in the dairy, confectionery, bakery and chocolate industries. *Salmonella* can withstand dry environment of powders for extended periods of time due to increased adaptability at low water activity (a_w) levels and multiply when powder is hydrated. Therefore, presence of *Salmonella*, even in low numbers in low moisture foods, such as NFDM, can pose significant health risk.

Purpose: To determine survivability and thermal resistance of *Salmonella* in NFDM stored for 210 days.

Methods: This study was designed as completely randomized with three replications. The NFDM was inoculated with 5- serovar *Salmonella* cocktail and dried back to original pre-inoculation a_w. The D- and z-value study was conducted every 30th day, starting day one. Rehydration (13% total solids w/v) was performed on days of performing D- and z-value study. Inoculated NFDM (~5 g) or hydrated inoculated NFDM (~5 mL) were transferred into five thermal-death-time disks, sealed and placed in hot-water baths set at 80, 85 and 90°C for inoculated NFDM, and 59, 62 and 65°C for hydrated inoculated NFDM. The samples were held for 0 to 56 minutes in hot-water baths and quickly transferred to cold-water baths at pre-determined time intervals. The samples were enumerated using injury-recovery media, and D- and z-values were calculated.

Results: D-values of *Salmonella* for day one were 17.9, 9.1, and 4.4 minutes at 80, 85 and 90°C, respectively, in NFDM, and 5.7, 2.3 and 0.6 minutes at 59, 62 and 65°C, respectively, in hydrated NFDM. The z-values of *Salmonella* in NFDM and hydrated NFDM for day one were 16.3 and 6.4°C, respectively. Statistically, D-and z-values were found to be similar throughout the storage period.

Significance: D- and z-values from this study will provide basic information about the effect of storage time on the heat resistance of *Salmonella*.

P2-122 Influence of Water Activity at Elevated Temperature on Thermal Resistance of Freeze-dried *Salmonella* Enteritidis PT30

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❖ ***Developing Scientist Entrant***

Introduction: It has been reported that water activity (a_w) changes in food systems at elevated temperatures influence the thermal resistance (evaluated by D-value in mins) of bacteria. The fundamental reason remains unclear. We hypothesize that bacteria cells as composite biomaterials also have their own intrinsic a_w vs moisture content relationships at different temperatures, referred to as moisture sorption isotherms (MSI).

Purpose: This study aimed to generate MSI for freeze-dried *S.* Enteritidis (FDS) and determine its influence on the thermal resistance of FDS at 80℃.

Methods: Powder of *S*. Enteritidis PT 30 (ATCC-1045) ($a_{w,\ 20℃}$ <0.025) was produced by desiccating at -90℃ for 48 h. The bacterial powders were conditioned for 2 days at 20℃ under eight relative humidity levels. The a_w changes were determined using high-temperature cells (HTC) from 20 to 80℃. The MSI was plotted as a_w against moisture contents (X_w). The thermal resistance of FDS at four $a_{w,\ 20℃}$ (0.2, 0.3, 0.4 and 0.6) were obtained using thermal-death-time (TDT) cells. The $D_{80℃}$ values were calculated and plotted with $a_{w,\ 80℃}$.

Results: The a_w of FDS increased considerably with increasing temperature at fixed X_w. For instance, at X_w of 0.124 g water/g dry solids, the $a_{w,20\ ℃}$ was 0.45 and $a_{w,\ 80℃}$ was increased to 0.64. The MSI of *S.* bacterial cells, which showed a type Ⅱ behavior, was similar to that of carbohydrate-rich or protein-rich foods but differed sharply from that of fat-rich foods. The $D_{80℃}$ values for FDS were 27.69 ± 0.69, 11.93 ± 0.18, 6.50 ± 1.07 and 1.90 ± 0.56 min at $a_{w,\ 80℃}$ of 0.36, 0.49, 0.60 and 0.78, respectively, which were decreased exponentially with increasing $a_{w,\ 80℃}$ or X_w.

Significance: The results suggested that the equilibrated X_w in bacterial cells adjusted by the a_w of low moisture foods is the ultimate determining factor influencing the thermal resistance of bacteria during inactivation.

P2-123 Cell Concentration Dependency of Survival on Drying in *Salmonella* Species

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Introduction: *Salmonella enterica* is well known for its ability to survive drying and persist in low-moisture environments. Growth as sessile cells, quorum sensing, and biofilm formation have been examined for possible links, however, the actual mechanisms involved remain elusive.

Purpose: The purpose of this study was to determine the effect of initial cell concentration or soluble quorum sensing compounds on the desiccation survival of *Salmonella* when grown as sessile cells.

Methods: *Salmonella* was grown on TSBYE with agar and harvested using BPW. Cell suspensions (11.08 ± 0.10 log CFU/mL) were divided into portions and treated as follows: one portion centrifuged and filtered to remove cells to create conditioned BPW (C-BPW) and a second portion heat-treated to kill cells (K-BPW). C-BPW, K-BPW, or control BPW were used to serially dilute the original harvested *Salmonella*. Cells (0.1 mL) were applied to membrane filters at concentrations of approximately 10 (undiluted), 8, 6 or 4 log CFU, dried (ambient) for 1 d, then stored at 25°C/33% RH. Cells were recovered from filters using BPW and cultivated on TSAYE. Data were compared via ANOVA ($P \leq 0.05$).

Results: After 1 and 7 d storage, populations of undiluted *Salmonella* did not significantly change with an overall decrease of 0.25 - 0.50 log. When the initial *Salmonella* population was decreased, survival decreased compared to undiluted when cells were diluted in BPW or C-BPW but not when diluted with K-BPW. At the lowest dilution (4 log CFU), losses at 1 d were 1.97 ± 0.09 for C-BPW, 1.30 ± 0.50 for BPW, but only 0.33 ± 0.06 log CFU for K-BPW. After 7 d, losses increased for cells diluted in C-BPW and BPW (>4 log CFU), whereas those diluted in K-BPW lost only 0.40 ± 0.01 log CFU.

Significance: Survival of *Salmonella* during drying appears cell concentration dependent and may be aided by specific non-soluble components associated with harvested cells.

P2-124 Thermal Inactivation of *Salmonella* on Cocoa Beans during Dry-heat Roasting

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella* is a common bacterial foodborne pathogen that is estimated to cause 1.2 million illnesses and 450 deaths per year in the U.S. *Salmonella* can exhibit high tolerance to heat under dry conditions, which presents a food safety challenge for low water activity food producers, such as the chocolate industry. Cocoa roasting is one of the critical pathogen control points in chocolate processing that is important for *Salmonella* inactivation.

Purpose: The purpose of this study was to characterize the thermal inactivation kinetics of *Salmonella* inoculated onto whole cocoa beans in order to better understand the roasting conditions required to achieve sufficient inactivation of *Salmonella*.

Methods: Cocoa beans inoculated with a strain of *S. enterica* subsp. *enterica* serotype Oranienburg were roasted at different combinations of temperatures (100℃, 110℃, 120℃, 130℃, 140℃ and 150℃) and times (2 to 100 minutes), in minimum of three replicates. After roasting, the beans were resuspended into cold phosphate-buffered saline, homogenized, diluted serially ten-fold and inoculated onto brain heart infusion agar for enumeration. Non-roasted controls were used to calculate a log reduction, and to determine the D- and Z-values for characterizing *Salmonella* thermal inactivation kinetics.

Results: A 5-log reduction was experimentally achieved after 10-min roasting at 150℃. Generally, at higher roasting temperatures, higher levels of *Salmonella* inactivation were achieved, as suggested by the D-values of the log-linear model; 31.98 min, 17.64 min, 12.76 min, 10.49 min, 4.17 min and 1.94 min for 100℃, 110℃, 120℃, 130℃, 140℃ and 150℃, respectively. The Z-value of the dry-heat roasting process was 32.6℃. The thermal inactivation kinetics at lower temperatures were better described by a non-linear model, while the thermal inactivation kinetics at higher temperatures exhibited a linear trend.

Significance: The data produced in this study can inform selection of best temperature-time combinations for cocoa roasting procedures to ensure food safety.

P2-125 Growth Kinetics of *Salmonella enterica* during Rehydration and Subsequent Storage of Dehydrated Carrots

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Introduction: Dehydrated plant foods have low water activities and do not support growth of pathogenic bacteria such as *Salmonella enterica*. Once rehydrated, these foods could be held for later use. An assessment of these rehydrated foods during storage is required to determine the extent to which they support *S. enterica* growth.

Purpose: To determine the growth kinetics of *S. enterica* during rehydration and subsequent storage of dehydrated carrots at 5, 10, and 25°C.

Methods: Dehydrated carrots were inoculated with 4 log CFU/g of a 4-strain cocktail of *S. enterica* and dried for 24 h. Carrots were rehydrated using 4-volumes of 25°C water for 24 h. At 1 h intervals, approximately 30 g of carrots were removed and water drained for 10 min. Ninety mL of BPB was added to triplicate 10-g samples. Serial dilutions of the homogenate were plated onto TSA overlaid with XLD agar for enumeration of *S. enterica*. After 24 h rehydration, the remaining carrots were drained and 30-g portions stored in deli-style containers at 5, 10, or 25°C for 7 d. *S. enterica* was enumerated at 1, 3, 5, and 7 d. Three independent trials were conducted. Growth kinetics were determined using DMFit and data were statistically analyzed using Student's *t*-test (α=0.05).

Results: The population of *S. enterica* after 24 h rehydration was 4.38 ± 0.73 log CFU/g. During storage at 5, 10, and 25°C, the growth rates of *S. enterica* were 0.03, 0.55, and 0.44 log CFU/g per d, respectively, corresponding to a 1 log CFU/g increase in the pathogen population in 29.13, 1.82, and 2.29 d. The highest population, 8.07 ± 0.88 log CFU/g, was observed after 7 d storage at 25°C (an increase of 3.69 log CFU/g).

Significance: The data obtained in this study can be used in risk assessments to determine proper storage temperatures of carrots after rehydration.

P2-126 Impact of Air Velocity on Enterococcus faecium Inactivation during Dry Roasting of Peanuts

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Introduction: Dry roasting is used to control *Salmonella* contamination on peanuts. While previous studies have quantified impact of product temperature, process humidity, product moisture, and/or product water activity on *Salmonella* lethality, the effect of air velocity on lethality remains unclear.

Purpose: This study quantified the interaction of air velocity with temperature and moisture on *Enterococcus faecium* (a *Salmonella* surrogate) inactivation on peanuts.

Methods: Shelled peanuts inoculated with *E. faecium* were treated at various air temperatures (121.1, 148.9, and 176.7°C) and air velocities (0.65 and 1.25 m/s) at several times. Sample temperature was measured during treatment. Moisture content was measured and *E. faecium* were enumerated after treatment. Inactivation was modeled as a function of time, product temperature, and product moisture for the whole data set, and then separately for each air velocity. Parameters (D_{ref}, z_T, and z_{MC}) were compared between the model fits.

Results: Parameters estimated for the entire data set were least accurate, showing the largest relative errors and model fits with the largest RMSE (0.617 log CFU/g). Parameters estimated for high velocity conditions showed the best fit, with relative errors below 10% and RMSE of 0.327 log CFU/g. Confidence intervals for D_{ref} and z_{MC} overlapped for low and high velocities indicating no difference between conditions, but those for z_T did not overlap. Residual analysis showed some systematic bias with respect to process temperature and air velocity, so there may be effects that were not being accounted for in this model.

Significance: This study clarified the role of air velocity in microbial inactivation in dry foods, and indicated that further investigation is needed to account for minor systematic model biases in temperature and air velocity.

P2-127 Effect of Sugar Composition on Resuscitation of Salmonella and Enterococcus faecium NRRL B-2354 Survivors in Heat-treated Skim Milk Powder and Lactose-Free Skim Milk Powder

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❖ ***Developing Scientist Entrant***

Introduction: *E. faecium* is a commonly used nonpathogenic surrogate for validating *Salmonella* thermal controls. Because relative thermal resistance of these two bacteria is influenced by food composition, the impact of different sugars in powders needs to be elucidated.

Purpose: This study aimed to assess the effect of lactose and glucose-galactose on resuscitation of bacterial survivors in heated skim milk powder (SMP) and lactose-free skim milk powder (LSMP) on non-selective differential media.

Methods: Dairy powders (100 g each) were inoculated either with a *Salmonella* cocktail (Montevideo, Mbandaka, Reading, Tennessee, Agona) or *E. faecium* by directly adding 1 mL and then hand-massaged for 3 min. After pre-equilibrating to 0.25 a_w, the sample was placed in a bag, hand-massaged, and then pulverized with a pestle to break up any clumps, and then re-equilibrated to 0.25 a_w. Once inoculation homogeneity was ensured, SMP and LSMP were heat-treated at a single temperature and split into two sub-samples for analysis. In half of the samples, sugar composition of SMP and LSMP was equalized by adding glucose-galactose to SMP (SMPX), and lactose to LSMP (LSMPX), after which the survivors were enumerated on nonselective differential media at 37°C for 24 h.

Results: *E. faecium* exhibited similar thermal resistance ($P > 0.05$) in SMP and SMPX, whereas *Salmonella* was more thermally resistant in SMP ($P < 0.05$) (~14% greater) than in SMPX at 90°C. Both *E. faecium* and *Salmonella* exhibited similar thermal resistance in LSMP and LSMPX, at 70°C.

Significance: Measured thermal resistance of *Salmonella* and *E. faecium* was not influenced by sugar composition. The relative thermal resistance between these two bacteria was influenced by food composition; hence, although *E. faecium* is generally an acceptable *Salmonella* surrogate in low-moisture foods, it cannot be universally accepted as such without appropriate supporting evidence.

P2-128 The Effect of Superheated Steam on the Inactivation Kinetics of *Enterococcus faecium* Inoculated in Peanut Butter at Different Water Activities

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Introduction: Superheated steam is an emerging sanitation technology for treatment of food plant surfaces that offers minimal water and chemical utilization while providing enhanced efficacy against biofilms and penetration into environmental niches compared to other dry sanitation technologies. However, limited data are available on the mediating effects of food soil residues present on environmental surfaces as they impact microbial inactivation.

Purpose: The objective of this study was to investigate the inactivation kinetics of *Enterococcus faecium* in peanut butter as a function of superheated temperatures and peanut butter water activity (a_w).

Methods: *E. faecium* NRRL B-2354 was inoculated in peanut butter (7.96 log CFU/g ± 0.63) adjusted to different a_w (0.18, 0.40, 0.60, and 0.80) and kept at 25°C for 48 h for adaptation, then coated (31.5 mm × 20.0 mm × 0.60 mm) onto aluminum foil of 0.016 mm thickness. The steam treatment was conducted at 125, 175, 225°C, and 250°C. The coated samples were placed in a custom coupon holder inside the treatment chamber, treated for a given time interval, and then quickly removed and immediately transferred to peptone water to stop the thermal process. Survivors were recovered by plating on TSA.

Results: The temperature was stable at the steady state for each target temperature (SD < 1.12°C). As a_w increased from 0.18 and 0.80, $D_{125°C}$-values decreased from 123.46 s to 8.13 s. Similarly, $D_{250°C}$ values were 18.83 s and < 0.58 s, respectively. The z_{aw}-value and z_T-value were 0.52 ± 0.12 and 157.71°C ± 40.76, respectively (R^2 > 0.87).

Significance: While low a_w food matrices offer a protective effect and organic material often minimizes sanitizer efficacy, these results indicate that superheated steam may be an effective alternative technology achieving up to 5-log reduction on peanut butter coated surfaces in 94.16 s at 250°C.

P2-129 Understanding the Impact of Inoculation Methods on Thermal Inactivation Rates of Edible Insect Powder Using Enterococcus faecium

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✦ ***Undergraduate Student Award Entrant***

Introduction: Edible insect powder has grown in demand as a protein supplement. Despite the high risk of microbial contamination, thermal inactivation kinetics for pathogens in edible insect powders as low-moisture foods remains poorly understood.

Purpose: The purpose of this study was to evaluate the effect of inoculation methodology on the thermal inactivation kinetics of *Enterococcus faecium* in edible insect powder and roasted ground whole crickets.

Methods: Commercial cricket powder and whole roasted crickets were separately inoculated with *Enterococcus faecium*. After inoculation, the whole crickets were then milled into a powder of similar bulk density to the commercial cricket powder. Both powders were conditioned to 0.25 a_w prior to isothermal treatment at 78.6°C. Aluminum test cells (n = 10) were filled with the powders, submerged in a water bath at the set temperature, with duplicate test cells removed at six different time points (0, 10, 20, 30, 40, and 50 min). Thermal inactivation was halted by submerging the test cells in an ice-water bath immediately after treatment. The powders within each test cell were then transferred into sterile bags, appropriately diluted and plated on modified trypticase soy agar media containing esculin to quantify *E. faecium* survivors.

Results: $D_{78.6°C}$-values were 11.67 ± 6.04 and 18.32 ± 3.9 min, for *E. faecium* in the whole ground cricket powder and commercial powder, respectively. No statistical differences were seen between the inactivation rates (P > 0.05).

Significance: Understanding the impact inoculation methodologies have on the thermal inactivation kinetics of pathogens in processed products will help to establish appropriate industrial kill-step validations for the edible insect powder industry.

P2-130 Effect of UV-C Light and Hot Air on Quality and Microbiological Safety of Chia, Amaranth and Sesame Seeds

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❖ ***Developing Scientist Entrant***

Introduction: Seed consumption has increased in recent years due to the high nutrient content. Unfortunately, the number of outbreaks caused by *Salmonella* associated with consumption of low water activity foods, including seeds, has also increased. New technologies to improve microbiological safety of seeds have been used; however, the quality of the products is affected.

Purpose: The main goal in this study was to evaluate the effect of UV-C light and hot air on microbiological safety and bromatological, color and antioxidant characteristics of chia, amaranth and sesame seeds.

Methods: Chia, amaranth, and sesame seeds were inoculated with *Enterococcus faecium* ATCC 8459 (8 log CFU mL^{-1}) as a surrogate for *S. enterica*, dried for 45 min and exposed to UV-C radiation (10 or 30 g; 21.2 W m^{-2}/1 to 5 min) and hot air (10 or 50 g; 100, 120 and 140°C/1 to 10 min). Survival population was quantified and changes in bromatological content, color and antioxidant capacity were determined.

Results: Both technologies were effective in the inactivation of *E. faecium*. The best treatment of UV-C was 5 min in 10 g of seeds which reduced 5.7, 4.2 and 4.9 log CFU g^{-1} in chia, amaranth and sesame seeds, respectively. Hot air at 120°C for 10 min in 10 g of seeds reduced 4.9 log CFU g^{-1} in chia and sesame seeds, and 4.4 log CFU g^{-1} in amaranth. Quality of the seeds (content of phenolic compounds, bromatological composition and color) was minimally modified after the treatment application (P <0.05)

Significance: The proven technologies were effective to inactivate a significant population of the *Salmonella* surrogate, which in some cases meet FDA recommendation (5 log CFU g^{-1}).

P2-131 Thermal Resistance of *Salmonella* Spp. and *Enterococcus faecium* nrrl-B2354 in Whole Chia Seeds

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Introduction: The involvement of chia seeds in a 2014 multistate salmonellosis outbreak calls for the implementation of preventive controls to prevent future outbreaks. In order to design thermal pasteurization/sterilization processes, the thermal resistance of *Salmonella* spp. and an appropriate non-pathogenic surrogate in whole chia seeds must be determined.

Purpose: To determine the thermal resistance of *Salmonella* spp. and *Enterococcus faecium* NRRL-B2354 in whole chia seeds.

Methods: Whole chia seeds from three production lots were spray-inoculated in 1-kg batches with a 20 mL aliquot of either a 5-strain *Salmonella* cocktail or *E. faecium*. The inoculated samples were equilibrated to 0.53 a_w for at least two days in humidity-controlled chambers. For the thermal treatments, 2.1±0.2 g inoculated seeds were packed into pouches and then heat-treated at 80, 85, and 90 °C with a minimum of six timepoints and two replicates per timepoint. Treated samples were chilled before being plated on tryptic soy agar with yeast extract, ammonium iron (III) citrate, and either sodium thiosulfate for *Salmonella* spp. or esculin hydrate for *E. faecium*. Inoculated plates were incubated at 37°C for 24±2 h before enumeration.

Results: The D-values of *Salmonella* spp. at 80, 85, and 90°C were 15.16±3.16, 7.78±1.62, and 3.06±0.68 min, respectively. As for *E. faecium*, the D-values at corresponding temperatures were 23.06±5.65, 11.04±2.83, and 4.78±1.31 min, respectively. Interestingly, the R^2 value was more than 0.9 when only considering the first two production lots but dropped to between 0.78 to 0.87 upon including the third lot.

Significance: The thermal resistance of *E. faecium* was approximately 1.5 times that of *Salmonella* spp. in whole chia seeds, suggesting its suitability as a conservative surrogate. The differences between production lots suggest that natural variations in agricultural products should be considered when designing a thermal pasteurization/sterilization process.

P2-132 Effect of Inoculated Ingredient on the Isothermal Inactivation of *Enterococcus faecium* NRRL B-2354 in a Multicomponent Cookie Dough

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❖ ***Developing Scientist Entrant***

Introduction: In several published validation studies, only a single 'worst-case' ingredient of a multicomponent product was inoculated. However, studies have shown that product composition has a significant effect on the thermal inactivation rate of bacteria in low moisture foods. It is not known how the rates of microbial inactivation vary in a composite dough when different individual ingredients are inoculated versus when all ingredients in the dough are individually inoculated.

Purpose: To compare thermal inactivation rates of *E. faecium* NRRL B-2354 in a dough formulated with different single ingredients inoculated (other ingredients non-inoculated) and with all ingredients individually inoculated.

Methods: *E. faecium* grown on tryptic soy agar with 0.6% yeast extract (TSAYE) plates was inoculated into soy protein powder, wheat flour and peanut butter, then conditioned to their respective native water activity levels 0.30, 0.45 and 0.25. One inoculated ingredient was mixed with other non-inoculated ingredients in a ratio of 3:3:3:11 (soy protein powder:wheat flour:milk powder:peanut butter) for 30 min in a 25% relative humidity controlled glovebox. The water activity was measured and the dough was loaded into aluminum test cells for isothermal treatment at 95°C. Three subsamples at each of six time points were enumerated on TSAYE.

Results: The $D_{95°C}$-value of *E. faecium* in cookie dough formed with inoculated soy protein powder (5.34±0.25 min) was significantly higher ($P < 0.05$) than in cookie dough formed with inoculated wheat flour (4.46±0.11 min) and peanut butter (4.31±0.17 min). When the D-values reported above were compared to that of cookie dough formulated with all ingredients individually inoculated (4.67±0.19 min), there was no difference ($P > 0.05$).

Significance: Though composition has a significant effect on the thermal inactivation rate of bacteria in low moisture foods, comparable *D*-values were obtained under isothermal and iso-moisture conditions in the dough tested regardless of which ingredients were inoculated.

P2-133 Validation of Simulated Commercial Baking of Peanut Butter Cereal Bars to Control *Salmonella,* Shiga Toxin-producing *Escherichia coli* and *Listeria monocytogenes*

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Introduction: Raw flour as an ingredient of bakery products poses a foodborne pathogen risk, as demonstrated by illness outbreaks and flour recalls linked to *Salmonella* and Shiga toxin-producing *E. coli* (STEC) contamination. Processors must validate their baking process across a diverse product category to ensure the safety of the finished products.

Purpose: This study was conducted to validate a representative commercial oven baking process for the manufacture of peanut butter bars against mixed inocula of *Salmonella* (7 serovars), STEC (7 serogroups), and *Listeria monocytogenes* (5 strains).

Methods: Wheat flour was inoculated with the target pathogen cocktail (~7 log CFU/g), dried overnight to the original preinoculation weight of the flour, and used to create cereal bar dough (50-g bars). Bars were baked at 177°C for 13 minutes, followed by 15 min of ambient air cooling (B+C). Surviving populations were determined every 2 min using an agar overlay plating protocol to improve detection of injured cells. Oven relative humidity and product water activity, pH, product internal temperature, moisture content, and microbial counts were determined at each sampling point. Three replications of the study were conducted.

Results: Internal temperature of the bars increased from ~25°C to ~91°C during 13 min of baking. *Salmonella,* STEC and *L. monocytogenes* population reductions ($P \leq 0.05$) were 2.4, 3.0, and 3.9 logs CFU/g, respectively, compared to raw dough levels. Water activity of bars decreased from 0.81 to 0.70, while pH increased from 7.0 to 8.7 during baking.

Significance: Similar studies in our laboratory on a wide range of bakery products have achieved >5-log reductions in *Salmonella* and STEC populations after the commercial-simulated baking step. Intrinsic characteristics of peanut butter cereal bars (e.g., lower a_w and higher fat content) are likely contributors to the decreased process lethality observed in the current study and should be considered in food safety plans for similar products.

P2-134 Heat Resistance of *Salmonella,* Shiga Toxin-Producing *Escherichia coli* and *Listeria monocytogenes* in Peanut Butter Cereal Bar Dough with Lowered Water Activity

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Introduction: *Salmonella,* Shiga toxin-producing *E. coli* (STEC) and *Listeria monocytogenes* have been linked to foodborne outbreaks in products like wheat flour, peanuts and nut butters, which are ingredients in peanut butter cereal bars. Bakers must incorporate preventive control steps during manufacturing to eliminate these pathogens.

Purpose: This study was conducted to determine *D*- and *z*-values against mixed cocktails of *Salmonella*, STEC and *Listeria monocytogenes* during heating of peanut butter cereal bar dough adjusted to an a_w of 0.65 representative of surface drying during oven baking

Methods: Dough for peanut butter cereal bar was prepared using flour inoculated with each cocktail and dried at 37°C to an a_w of 0.65±0.2. Dried dough was placed in thermal-death-time disks or small vacuum-sealed bags and were submerged in circulating water baths at 85, 90, or 92.5°C. Upon reaching target product temperatures, disks/bags were removed (n = 7) at 7, 3 and 1-min intervals, respectively, to determine *D*- and *z*-values. Viable pathogen populations were enumerated using an agar-overlay method. Three independent replications were conducted.

Results: Respective *D*-values (min) for *Salmonella,* STEC and *L. monocytogenes* in a_w-adjusted dough were 9.4, 10.2 and 20.9 at 85°C; 3.9, 3.3 and 12.2 at 90°C; and 1.7, 1.7, and 2.8 at 92.5°C, respectively. The calculated z-values for *Salmonella,* STEC and *L. monocytogenes* were 10.1, 10.1 and 9.3 min, respectively.

Significance: During a previous baking validation of these bars, *L. monocytogenes* demonstrated a lower survival rate compared to STEC and *Salmonella.* The current study found similar heat resistance for STEC and *Salmonella*. However, *L. monocytogenes* survival in the reduced a_w dough was higher, suggesting the need for additional research on the mechanism for greater survival. Reduced a_w bakery products may present an elevated pathogen survival risk.

P2-135 Validation of a Low-moisture Viscous Cookie Baking Process

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Introduction: *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), and *Listeria monocytogenes* can be potentially introduced into bakery products pre-thermal processing through ingredients including flour, dairy, and eggs. Additionally, pathogens are known to exhibit higher thermal resistance in low-moisture foods. Hence, there is a need for food industries to validate their thermal processes.

Purpose: The purpose of this study was: (1) to provide scientific justification that the baking process during manufacturing was adequate to achieve an acceptable reduction in *Salmonella* spp., *Listeria monocytogenes* and STEC in low-moisture viscous cookies and (2) to evaluate *Enterococcus faecium* as an appropriate surrogate for use in the validation of low-moisture viscous cookies.

Methods: Four types of dough samples were evaluated. Dough samples were inoculated with a cocktail of *Salmonella* spp., *Listeria monocytogenes,* STEC, and *E. faecium* to achieve target levels of 10^7 to 10^8 CFU/g. Inoculated dough samples were deposited into parchment paper trays. Samples (n = 9) were baked at 290°F or 340°F for 21 to 22 minutes depending on the dough type. These baking parameters result in a viscous center required for product quality. Samples were analyzed for log reductions for each microorganism post-baking using scientifically valid methods.

Results: Regardless of the baking parameters used in the study, greater than 5-log reductions (P < 0.05) were found for *Salmonella* spp., STEC, *Listeria monocytogenes*. A minimum of 1.82-log reduction in *E. faecium* was achieved in samples baked for 290°F or 340°F for 21 to 22 minutes.

Significance: These data suggest that low-moisture viscous cookies when baked at 290°F or 340°F for 21 to 22 minutes, achieved at least a 5-log reduction of pathogens of concern while maintaining the desired saleable quality in the final product. Further work must be completed before utilizing *E. faecium* as a surrogate for validation in this product.

P2-136 Efficacy of Automatic Dishwashing in Reducing Microbial Load of Porous and Non-porous Surfaces Soiled with Cake Batter

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❖ ***Developing Scientist Entrant***

Introduction: Wheat flour is known to harbor shigatoxigenic *E. coli* and has been implicated in several outbreaks. Kitchen utensils used to prepare batter may harbor residual bacteria from contaminated wheat flour.

Purpose: This study evaluated the ability of an automatic dishwasher to reduce *E. coli* O157:H7 on porous (ceramic) and non-porous (stainless steel) surfaces soiled with cake batter.

Methods: Commercial cake mixes were evaluated for the presence of coliforms by plating on Violet Red Bile (VRB) agar. A cocktail of five isolates of *E. coli* O157:H7 was used to inoculate a cake-mix batter that was negative for coliform presence. The batter was applied to stainless steel and ceramic coupons (n = 3) at a rate of 0.0775g/cm^2. The coupons were dried for 1 h and then washed for 2, 10, 15, 20, and 30 min periods in the Farberware® Portable Countertop Dishwasher. An untreated control coupon was included in each trial. Wash water and drain water from the dishwasher were also sampled. Cells were quantified by plating on Sorbitol MacConkey agar and by an MPN assay (LOD=1 CFU/mL).

Results: One of the 5 commercial cake mixes was negative for coliform growth on VRB and thus was selected as the soiling medium. A five-log reduction in *E. coli* O157:H7 was observed on both the stainless steel and ceramic coupons after 10 min of washing (maximum temperature 48.5°C). No *E. coli* O157:H7 cells were recovered from the wash water at any time point, but the drain water contained 6.43 log CFU/mL after 10 min of washing.

Significance: The data suggest that automatic dishwashers with the parameters used in this study can potentially mitigate pathogenic *E. coli* from porous and non-porous surfaces that have been contaminated with wheat flour batter after 10 min of washing. Furthermore, drain water from these dishwashers may harbor bacteria.

P2-137 Modeling the Effect of Temperature and Water Activity on the Survival of *Escherichia coli* during Dehydration of Plant-based Food Products

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Introduction: Although plant products have been associated with pathogen contamination, the relative efficacy of moderate-temperature (≤ 60°C) dehydration of plant-based foods on pathogen inactivation is unknown. Air currents, temperature, and humidity within the dehydration process dynamically

change product temperature and water activity (a_w), critical factors for pathogen inactivation. Modeling in-process inactivation must account for these dynamics.

Purpose: Model the reduction of *E. coli* as a function of product, a_w, and temperature under isothermal experimental conditions, and test those models via dynamic drying in a commercial dehydration unit.

Methods: Apple, kale, and tofu were each adjusted to a_w 0.90, 0.95, or 0.99 and inoculated with an *E. coli* cocktail (~8.0 CFU/g), followed by isothermal water bath treatment at 49.0, 54.4, or 60.0°C to calculate *D*-values (in triplicate). A global log-linear/Bigelow-type model was developed for each product, accounting for temperature and a_w. Inoculated samples were placed at predetermined locations in a dehydration unit operating at 60°C for up to 24 h (in triplicate).

Results: *D*-values varied by product type and a_w level ($P < 0.05$), ranging from 3.0 to 6.7, 19.3 to 55.3, and 45.9 to 257.4 min at 60.0, 54.4, and 49°C, respectively. The relative impact of a_w was product dependent and appeared to have a non-linear impact on *D*-values. The global model was estimated with reasonable root mean squared errors (RMSE) of 1.54, 1.24, and 0.75 log (CFU/g) for apple, kale, and tofu, respectively. However, application of those models to the dynamic data from the dehydrator system had poor predictive performance (RMSE > 15 log (CFU/g)).

Significance: Isothermal data described the impact of product matrix, a_w, and temperature on *E. coli* inactivation but did not accurately approximate observations in the dehydrator. The deviations were likely due largely to differences in the a_w range. For commercial applications, improved models or dehydrator-specific data may be necessary to validate dehydration as a kill step.

P2-138 Patented Organic Peracetic Acid and Hydrogen Peroxide-based Sanitizing Solution Achieves > 4 Log CFU/g Reduction in *Salmonella*, *Listeria monocytogenes,* STEC and *Enterococcus faecium* NRRL B-2354 on Almonds While Maintaining Nutrition and Shelf Life

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Introduction: Although the FDA has approved several almond pasteurization processes, investigation into new technologies improving almond quality and throughput is a continued industry endeavor. One such technology is the Neo-Pure Continuous Food Safety System, which applies a peracetic acid and hydrogen peroxide-based sanitizing solution to almonds and then removes residual solution.

Purpose: Investigations were conducted to identify a suitable surrogate for almond treatment and optimize parameters to achieve a minimum 4 log efficacy while maintaining quality, measured by nutrient content and shelf life.

Methods: Almond kernels were inoculated (120 mL inoculum/2000 g kernels) with the following cultures: A) *Enterococcus faecium* NRRL B-2354, B) *Salmonella* spp. cocktail (serovars Newport, Senftenberg, Oranienburg, Saintpaul, Typhimurium DT104 and Cubana), C) Shiga toxin-producing *Escherichia coli* (STEC) cocktail (serotypes O26:H11, O103:H2, O111:NM, O121:H19, O145:NM, O45:H2 and O157:H7), D) *Listeria monocytogenes* cocktail (ATCC 19115 of serotype 4b, LI0530 of serotype 1/2a, LI0540 of serotype 1/2b, ATCC 7644 of serotype 1/2c, and strain 16-079213- 0001).

Inoculated samples were dried under sterile, ambient conditions overnight. Subsequently, 500 g samples were treated with 100 L/t of the sanitizing solution and dried (220°F, 7 minutes). Five 50 g samples were enumerated from both untreated and treated samples.

Un-inoculated raw almonds, treated under the same parameters, were compared to untreated ($n = 3$ for each) for peroxide value, para-anisidine value, fiber, total fat, calcium and potassium (single factor ANOVA).

Results: The minimum log reduction for *Enterococcus faecium* NRRL B-2354, *Salmonella*, STEC and *L. monocytogenes* was 4.91, 5.78, 7.03 and 6.07 log CFU/g, respectively. No significant change ($P \geq 0.05$) between treated and untreated samples was observed for all components analyzed.

Significance: *E. faecium* NRRL B-2354 is a suitable surrogate for *Salmonella*, STEC and *L. monocytogenes*. The treatment is an effective pathogen control method for almonds as it gave a >4 log minimum reduction for all bacteria populations while maintaining nutrient content and shelf life.

P2-139 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 Cashews While Maintaining Shelf Life and Nutrition

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Introduction: Recalls and outbreaks associated with raw cashews due to *Salmonella* contamination necessitate implementation of an intervention method for pathogen control in cashews. Previous studies involving treatment of cashews with a peracetic acid-based sanitizing solution had found i) *Enterococcus faecium* NRRL B-2354 to be a suitable surrogate for *Salmonella* inoculated onto cashews and ii) > 5-log CFU/g reductions for *E. faecium* were achieved using an industrial scale applicator to apply the solution at a rate of 40 mL/kg followed by drying in an industrial scale fluidized bed dryer (220°F, 6 min).

Purpose: Identification of a suitable surrogate and promising efficacy results warranted further investigation to determine impact of treatment on nutrient content and shelf life of cashews, at parameters that achieved a > 5-log reduction on *Salmonella* surrogate.

Methods: Raw whole cashews were treated in the industrial scale applicator and dryer (3 MT/h throughput), under the same parameters that achieved a > 5-log CFU/g reduction for cashews inoculated with *E. faecium* (40 mL/kg application rate, 220°F drying for 6 min). Untreated and treated cashews ($n = 3$ of each) were compared for free fatty acid, peroxide value, and para-anisidine value after 3 and 6 weeks under accelerated shelf life storage conditions (40°C, 70% RH). In addition, before commencing the accelerated shelf life study, moisture content, iron, zinc, magnesium, protein, free fatty acid, peroxide value and para-anisidine value were compared (single factor ANOVA).

Results: No significant change ($P \geq 0.05$) between treated and untreated samples was observed for all components analyzed throughout the entire 6 weeks of the study.

Significance: The industrial scale applicator and dryer is a suitable pathogen control method for cashews, achieving a > 5-log reduction in a *Salmonella* surrogate while maintaining shelf life and nutrition.

P2-140 Population Dynamics of *Listeria monocytogenes* in Nut and Seed Butters

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❖ ***Developing Scientist Entrant***

Introduction: Nut and seed butters are low water activity foods and do not support the growth of foodborne pathogens. Research has determined that some pathogens, such as *Listeria monocytogenes*, can survive for long periods of time in legume butters, such as peanut butter. However, there is a dearth of information on the persistence of *L. monocytogenes* in nut and seed butters.

Purpose: To determine the population dynamics of *L. monocytogenes* in nut and seed butters stored at 5 and 25°C.

Methods: Nut (almond, hazelnut, pecan) and seed (pumpkin, sesame, sunflower) butters were inoculated with a 4-strain cocktail of rifampicin-resistant *L. monocytogenes* at 4 log CFU/g. Butters were mixed by hand for 15 min and 100-g portions were weighed into deli-style containers with lids and stored at 5 or 25°C for 4 months (112 d). During storage, 25 g, in triplicate, was homogenized with 225 mL BPB (or BLEB for FDA BAM enrichments when necessary) and serial dilutions of the homogenate were plated onto BHI with rifampicin for enumeration of *L. monocytogenes*. Data were statistically analyzed using Student's *t*-test (α = 0.05).

Results: The initial population of *L. monocytogenes* in the butters was 3.58 ± 0.25 log CFU/g. After 4 months storage at 5°C, the population of *L. monocytogenes* decreased by 1.50, 1.32, and 1.28 log CFU/g in almond, hazelnut, and pecan butter, respectively. Significantly less population reduction was observed in pumpkin, sesame, and sunflower butters (0.68, 1.16, and 0.61 log CFU/g, respectively). After 4 months storage at 25°C, the *L. monocytogenes* population in almond butter decreased by 1.24 log CFU/g. The population in all other butters decreased to below the level of enumeration (1.67 log CFU/g) but the pathogen was still present via enrichment.

Significance: The results of this study can aid in understanding the survival of *L. monocytogenes* in nut and seed butters at different storage temperatures.

P2-141 *Listeria monocytogenes* Growth Kinetics during Rehydration and Storage of Dehydrated Potatoes

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Introduction: Dehydrated potatoes do not support the growth of pathogenic bacteria. However, once rehydrated, the high water activity and neutral pH of this food may support the growth of pathogens, such as *L. monocytogenes*, during storage.

Purpose: To examine the growth kinetics of *L. monocytogenes* during 5, 10, and 25°C storage after rehydration of dehydrated potato.

Methods: A 4-strain rifampicin-resistant *L. monocytogenes* cocktail was inoculated onto dehydrated potatoes at 4 log CFU/g and dried for 24 h. Potatoes were rehydrated in 4-volumes of 5 or 25°C water for 24 h. During rehydration, 30 g of potatoes were removed and drained for 10 min. Potatoes were homogenized 1:10 with BLEB and the homogenate was plated onto BHI- rifampicin for *L. monocytogenes* enumeration. After rehydration, potatoes were drained and portioned into deli-style containers for storage at 5, 10, and 25°C and *L. monocytogenes* was enumerated at 1, 3, 5, and 7 d. Triplicate samples were assessed at each timepoint and three independent trials were conducted. Growth rates were determined using DMFit and data were statistically analyzed using Student's *t*-test (α=0.05).

Results: The populations of *L. monocytogenes* on potatoes after 24 h rehydration in 5 or 25°C water were 1.54 ± 0.37 and 3.42 ± 0.35 log CFU/g, respectively. When rehydrated with 5°C water, the pathogen growth rates during storage at 5, 10, and 25°C were 0.71, 1.61, and 2.71 log CFU/g per d, respectively. When rehydrated with 25°C water, growth rates at 5, 10, and 25°C were 0.08, 0.43, and 1.07 log CFU/g per d, respectively. The fastest time to a 1 log population increase in *L. monocytogenes* (8.88 h) was observed on potatoes rehydrated with 5°C water and stored at 25°C.

Significance: The results of this study can aid in determining appropriate time and temperature control for safety for dehydrated potatoes during rehydration and subsequent storage.

P2-142 Quantitative Microbial Risk Assessment to Evaluate the Public Health Risk of Avian Influenza H7N9 in Chicken from Live Poultry Markets

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Introduction: Avian influenza A (H7N9) is a subtype of the influenza virus that is of emerging concern to humans, with over one thousand reported cases of human infection and several deaths between 2013 and 2017. However, due to many factors, such as H7N9's current sporadic human case pattern, rapid mutation and recombination rates, and unclear transmission dynamics, a clear quantitative microbial risk assessment for H7N9 from exposure to consumption of contaminated poultry remains lacking.

Purpose: The aim of this study was to develop a risk model and evaluate the human health risk of exposure to H7N9 from live poultry markets.

Methods: The growth, transmission, and die-off of H7N9 in the live poultry market supply chain was modeled from farm to at-home consumption. Risk factors included the environmental conditions, frequency of disinfection of the storage areas, and scale of vaccination. Relevant information was obtained from the WorldClim database (meteorological information) and from previously conducted survey data and qualitative risk assessments. While the growth and die-off models were modeled on @Risk software, the transmission dynamics and risk of human infection were modeled on R (R-3.6.2).

Results: The model was simulated using Latin Hypercube sampling for 100,000 iterations. Increased risk factors for viral spread in live poultry flocks included the introduction of new infected birds to an existing vaccinated flock (with a predicted increase of 23.3%), mean temperature of the coldest season, and average flock size (25,000~30,000 birds). Alternately, screening for, and culling of, infected poultry was found to effectively decrease the number of human H7N9 cases. Sensitivity analyses indicated the re-contamination of vaccinated poultry and exposure to live poultry markets had the largest impact on number of illnesses per year.

Significance: The developed risk model can be used to estimate the microbiological risks associated with avian influenza H7N9 and identify major risk factors to develop effective mitigation strategies.

P2-143 Assessing the Risk of Salmonellosis from Consumption of Conventionally and Alternatively Produced Broiler Meat in the United States

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* spp. have long presented a major problem for the food safety of broiler meat. As the popularity of alternatively produced (e.g., organic) broiler meat increases, an understanding of the food safety risks associated with these types of products is needed.

Purpose: The purpose of this study was to develop a retail-to-consumption quantitative microbial risk assessment model that could be used to estimate the differences in risk of salmonellosis acquired from the consumption of conventionally and alternatively produced broiler meat in the United States annually.

Methods: Data were extracted from literature surveys and studies and used to define distributions that could be used to estimate *Salmonella* growth during retail storage, transportation, and home storage, as well as concentration changes during preparation and due to cross-contamination. A Monte Carlo simulation with 100,000 iterations was performed to estimate the total risk per serving and total number of illnesses in the United States annually from both meat types. Sensitivity analyses were performed to determine the factors that were highly correlated with increased risk of salmonellosis.

Results: Conventionally produced broiler meat was estimated to have a median risk per serving of 7.62×10^{-8} and cause a median of 1,695 illnesses annually compared with a median risk per serving of 9.23×10^{-8} and 280 estimated illnesses for alternatively produced chicken. The sensitivity analysis identified cross-contamination of hands during meal preparation as the most important factor linked to risk. The 'what-if' scenario analysis estimated that control measures such as using antimicrobial soap during hand washing after handling raw chicken can reduce the risk considerably.

Significance: The developed risk assessment model provides information on the public health risk of conventionally and alternatively produced broiler meat. These results will be useful in determining the key intervention strategies to mitigate the food safety risks associated with the consumption of contaminated chicken products.

P2-144 The Public Health Outcome of the Continued Removal of Specified Risk Materials (SRMs) from Regulated Beef Products in Domestic Production

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Introduction: Bovine spongiform encephalopathy (BSE) is a progressive degenerative neurologic disease of cattle, affecting the central nervous system. As the United States is a negligible BSE risk country per the World Organization for Animal Health (OIE) standards since 2013, the US is not required to remove specified risk materials (SRMs). Yet, current Food Safety and Inspection Service (FSIS) regulations require removal of SRMs from all cattle presented for slaughter to mitigate the risk of BSE and variant Creutzfeldt–Jakob disease (vCJD) in humans.

Purpose: Assessing effectiveness of five domestic SRM removal options on the risk of vCJD.

Methods: FSIS developed a model to assess the annual risk of vCJD cases in the U.S. as a function of the prevalence of BSE-infected cattle in this country, the probability of a vCJD case occurring given slaughter of a BSE-infected cattle, and the effectiveness of SRM removal options. US APHIS Veterinary Services surveillance data by birth-year (1993–2018) cohorts and the UK BSE and vCJD epidemic data were utilized to estimate model inputs. Monte Carlo simulations were done to estimate the annual risk of vCJD cases.

Results: Risk estimates were generated for all five SRM removal options. The upper bound estimate for the odds of a single case of vCJD was 8.7 in 1 trillion with current SRM removal regulations, while the odds of a single vCJD case is 1.6 in 10 billion with no SRM removal requirement.

Significance: As the U.S. has been a negligible BSE risk country per the OIE standards since 2013, these findings should assist risk managers in their decision-making process when using data to assess their relative risk from vCJD.

P2-145 Quantitative Microbial Risk Assessment of Diarrhea *Bacillus cereus* in the Ready-to-eat Lunch Box and Dried Mango

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Introduction: The demand for ready-to-eat (RTE) lunch boxes and imported dried mango for the convenience store has recently increased in Korea due to increasing one-person households. *Bacillus cereus* has been detected in various processed foods, including lunch boxes and dried fruits, thus there is a potential of foodborne illness by *B. cereus* due to increasing consumption of these foods.

Purpose: The objective of this study was to conduct a microbial risk assessment of *B. cereus* in ready-to-eat lunch box and dried mango, which is the highest consumption among dried fruits.

Methods: The contamination levels of *B. cereus* in various RTE lunch boxes (n = 80) and dried mangos (n = 80) from convenience stores and on-off line markets were monitored, respectively. The predictive models of *B. cereus* in RTE lunch boxes and dried mango were developed as a function of temperature. The daily consumption amount and intake rate of these foods were investigated with 1,500 residents in major eight provinces in Korea, respectively. The probability of *B. cereus* outbreak with these foods was compared by simulation with @Risk.

Results: *B. cereus* was detected 23 in 80 RTE lunch boxes (28.75%) and 31 in 80 dried mangos (38.75%). The growth of *B. cereus* in RTE lunch boxes observed at the temperature of 17℃ above, while the survival model of *B. cereus* was developed as a function of temperature (4~15℃). The daily consumption amount of RTE lunch boxes and dried fruits were 417.27 g and 5.6 g per person, respectively. The risk of *B. cereus* in both foods was very low with consideration of the infectious dose of *B. cereus* (10^5) because of no dose-response model for *B. cereus.*

Significance: The contamination levels of *B. cereus* in the lunch box and dried fruits weren't changed from market to home, but prevention of contamination during processing and temperature control at the retail markets are needed.

P2-146 Quantitative Microbial Risk Assessment of *Listeria monocytogenes* in Raw Julienned Beef Purchased from On and Off Line Markets

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Introduction: Raw julienned beef is commonly eaten with seasoning in Korea and has the potential to cause listeriosis.

Purpose: This study conducted to investigate quantitative microbial risk assessment of *Listeria monocytogenes* in raw julienned beef from the retail market to home.

Methods: Raw lean beef for yukhoe (n = 330), which purchased from on-off line markets, were monitored for the contamination of *L. monocytogenes* and a predictive model of *L. monocytogenes* in raw beef was developed as a function of temperature (4 to 36℃). The daily consumption amount and intake rate of raw julienned beef were collected on 1,000 individuals in Korea. The data was calculated by the Monte Carlo simulation model using @Risk.

Results: The initial contamination level of raw julienned beef was -3.77 log CFU/g on average. The growth of *L. monocytogenes* in raw julienned beef was not observed at the temperature of 27℃ below. The daily consumption amount of raw julienned beef was 92.14 g per person. The probability of illness

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by *L. monocytogenes* of raw julienned beef per person per day was 2.51×10^{-16} for the susceptible population and 1.89×10^{-19} for the general population. The result of the sensitivity analysis shows that the intake rate was the highest correlation with the risk of listeriosis for both general and susceptible population

Significance: Since *L. monocytogenes* growth was not observed in raw julienned beef at refrigeration and ambient temperature, the risk of listeriosis due to raw julienned beef was very low in this work.

P2-147 Microbial Risk Assessment of Highly Pathogenic *Vibrio* spp. by Raw Oyster Consumption

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Introduction: Oyster is usually consumed raw in Korea. Hence, there is a possibility to be infected by highly pathogenic *Vibrio* spp. (*Vibrio vulnificus* and *Vibrio cholerae*). However, there are microbial risk assessments only for *Vibrio parahaemolyticus* and norovirus in Korea.

Purpose: The objective of this study was to estimate the probabilities of highly pathogenic *Vibrio* spp. foodborne illness caused by raw oyster consumption.

Methods: Eighty-eight raw oysters were collected from south coast, west coast, and Seoul in Korea to examine the prevalence of *V. vulnificus* and *V. cholerae*. The growth patterns of *Vibrio* spp. in raw oyster were evaluated by plating the samples on CHROM™agar Vibrio. Consumption frequency and amounts for raw oyster were surveyed. With the collected data, simulation models were prepared in @RISK to show the probabilities of *V. vulnificus* or *V. cholerae* foodborne illnesses caused by intake of raw oyster.

Results: Of 88 raw oysters, there was no *V. vulnificus*- or *V. cholerae*-positive sample (detection limit: 30 MPN/g). Thus, initial contamination levels of *V. vulnificus* and *V. cholerae* in raw oyster were estimated to be -3.6 log CFU/g by Beta distribution (1,89). Cell counts of *Vibrio* spp. were not changed in raw oyster during storage. Exponential distribution (55.792,Shift(10.968)) showed that average consumption amount was 66.76 g per day per person at 0.35% of frequency. The simulations with the data showed that the probabilities of the foodborne illness caused by raw oyster consumption were 9.08×10^{-15} for *V. vulnificus* and 8.16×10^{-13} for *V. cholerae*. In addition, the correlation coefficient showed that consumption frequency had the greatest effect on the probability of the foodborne illness.

Significance: In conclusion, the risk of highly pathogenic *Vibrio* spp. foodborne illnesses caused by raw oyster consumption is low in Korea.

P2-148 Quantitative Microbial Risk Assessment of Highly Pathogenic *Vibrio* spp. in Whiparm Octopus in Korea

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Introduction: *Vibrio* spp. are mainly found in seafood, and *Vibrio cholerae* and *Vibiro vulnificus* are known as highly pathogenic. In Korea, *V. cholerae* and *V. vulnificus* have been related to outbreaks because of raw seafood consumption, but the risk of the foodborne illness has not been evaluated.

Purpose: The objective of this study was to evaluate the risk of highly pathogenic *Vibrio* spp. (*V. cholera* and *V. vulnificus*) by whiparm octopus consumption.

Methods: One hundred eighty raw whiparm octopus samples were collected from restaurant or market to detect *V. cholerae* and *V. vulnificus*. Predictive models were developed to describe the fates of highly pathogenic *Vibrio* spp. at 4 to 20°C. Temperature and time data for storage were collected by measurement and personal communication, followed by fitting the data with @RISK program to obtain appropriate probabilistic distributions. Consumption patterns of whiparm octopus were investigated by a survey. Dose-response models for *V. cholerae* and *V. vulnificus* were investigated through the literature. Eventually, simulation models with @RISK were developed with the collected data to estimate the risk of *V. cholerae* and *V. vulnificus* foodborne illness.

Results: Of 180 whiparm octopus products, there were no *V. cholerae* and *V. vulnificus* positive samples. Thus, Beta distribution (1,181) and an equation [Initial contamination level=-ln(1-prevalence)/25] estimated an initial contamination level as -3.9 log CFU/g. The predictive models showed that the initial cell counts slightly increased. The exponential distribution for the consumption amount showed that average amount was 60.29 g per person at 0.47% of frequency. The simulation in @RISK with the data showed that the probabilities of *V. cholerae* and *V. vulnificus* foodborne illness by the intake of raw whiparm octopus were 7.08×10^{-13} and 8.71×10^{-15} per person per day, respectively.

Significance: This result indicates that the risk for the highly pathogenic *Vibrio* spp. can be considered low in Korea.

P2-149 Modeling the Invasion *of Campylobacter jejuni* into Small Intestinal Cells for the Key Events Dose-response Framework

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❖ ***Developing Scientist Entrant***

Introduction: The current approaches to dose-response modeling of low-dose exposures of pathogens rely on assumptions and extrapolations, although the models play an important role in quantitative risk assessment. The Key Events Dose-Response Framework has been proposed as an alternative approach for evaluating low-dose exposures that is a mechanistic way to predict infection/illness probability from pathogen exposures. Quantitative evaluation and modeling the bacterial invasion into intestinal cells would be essential for the estimation of the dose-response relationship.

Purpose: The objective was to investigate one critical process related to campylobacteriosis, the invasion behavior of *Campylobacter jejuni* into Caco-2 cells as a model for intestinal cells and to develop a mathematical model for the invasion counts.

Methods: Caco-2 cells were cultured at 37°C and 5% CO_2 condition in Dulbecco's minimal essential medium supplemented with 10% fetal calf serum, as a monolayer in 96-well microplate. Three isolates of *C. jejuni* were cultured microaerobically at 42°C with Bolton broth. The cultured Caco-2 cells and diluted *C. jejuni* suspensions ($10^{1\text{-}6}$ CFU/mL) were co-cultured for 12 h and invasion counts of *C. jejuni* were enumerated at every 1.5 h by gentamicin method with Preston agars. The observed behaviors of bacterial invasions were described by a mathematical model based on Bayesian modeling.

Results: The changes in the *C. jejuni* invasion counts into Caco-2 cells showed exponential convergence behaviors to the maximum invasion counts, and the rates of bacterial invasions were directly proportioned to the exposed bacterial concentrations. The estimations of parameter distributions were successfully converged. The observed values of 99% were successfully described by the 99% prediction band derived from the present developed Bayesian model.

Significance: The invasions of *C. jejuni* into intestinal cells were successfully demonstrated and described by the developed Bayesian model. The findings will be the base of an alternative approach for dose-response modeling.

P2-150 Quantitative Microbial Risk Assessment for *Salmonella* Foodborne Illness by Chicken Nugget Consumption

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Introduction: *Salmonella* shows high contamination levels in poultry products. Chicken nugget is minced chicken ball, and thus, *Salmonella* from chicken may still exist in the product and survive in the chicken nugget because of insufficient frying.

Purpose: The objective of this study was to evaluate the probability of *Salmonella* foodborne illness by chicken nugget consumption.

Methods: One hundred three chicken nugget samples were collected from conventional and traditional markets to evaluate the *Salmonella* prevalence. The *Salmonella* cell counts from chicken nuggets inoculated with *Salmonella* stored at 7°C to 30°C were fitted with the Baranyi model to calculate lag phase duration and growth rate. These kinetic parameters were fitted with a polynomial model as a function of temperature. Distribution temperature and time were surveyed, and consumption data on cooking methods were surveyed. A dose-response model was searched for in the literature. With these data, the probability of *Salmonella* foodborne illness was estimated through the simulation with @RISK.

Results: In the tested samples, *Salmonella* was not detected. Thus, initial contamination level was estimated by Beta distribution (1, 104), and the level was -3.7 log CFU/g. The developed predictive models showed that the *Salmonella* cell counts increased under the investigated distribution conditions simulated with Uniform distribution (0, 8760) for time and Pert distribution (-23.6, -18.8, -1.4) for temperature. Average consumption amount was 78.2 g, calculated by Weibull distribution [RiskWeibull (1.2696, 84.016, RiskShift (0.19973))] at 0.6% of frequency. Beta-Poisson model [$1-(1+Dose/4.4\times10^{5})^{-0.89}$] was appropriate to evaluate the dose response. The simulation showed that the probability of *Salmonella* foodborne illness by chicken nugget consumption was 3.62×10^{-12} per person per day.

Significance: The results indicate that the risk of *Salmonella* foodborne illness by chicken nugget consumption is low in Korea.

P2-151 Estimation for Probability of *Staphylococcus aureus* Foodborne Illness from Ready-to-Eat Salad Consumption

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Introduction: Consumption of ready-to-eat (RTE) salad is gradually increased every year in Korea because of the health benefit. In general, RTE salad in a market is prepared by hand in a plant during packaging. Thus, there is high possibility for *Staphylococcus aureus* cross-contamination from worker's hands to the RTE salad, which may cause the foodborne illness.

Purpose: The objective of this study was to estimate the probability *S. aureus* foodborne illness from RTE salad consumption in Korea.

Methods: Forty-three RTE salad samples collected from supermarket were analyzed to detect *S. aureus* by plating and gene identification. Predictive models (primary and secondary models) to describe fates of *S. aureus* were developed with cell count data collected at 4°C to 37°C during *S. aureus*-inoculated RTE salad storage. Distribution temperature and time, and consumption amount and frequency for the salads were surveyed. A dose-response model was searched through the literature. With these collected data, a simulation model was prepared in @RISK program, and a probability of *S. aureus* foodborne illness by RTE salad consumption was estimated.

Results: Of 43 samples, 1 sample was *S. aureus*-positive, and thus, Beta distribution (2, 43) was used to estimate the initial contamination level and the value was -2.8 log CFU/g. With this initial contamination level, the developed predictive models showed that *S. aureus* cell counts gradually increased as storage temperature increased. Lognormal distribution (RiskLognorm(142.96,157.36,Riskshift(-5.5945)) showed that average consumption amount was 137.7 g per person per day, and the frequency was 9.8%. The combination of these results with the Exponential dose-response model [$risk=1-exp(-r\times dose)$ ($r=7.64\times10^{-8}$)] showed that the probability of *S. aureus* foodborne illness by RTE salad consumption was 2.1×10^{-9} per person per day in Korea.

Significance: This result should be useful in estimating the risk of *S. aureus* foodborne illness by RTE salads consumption in Korea.

P2-152 Estimated Risk of *Bacillus cereus* Foodborne Illness by Perilla Leaf Pickle Consumption in Korea

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Introduction: Perilla leaf pickles are one of the kimchi that are salted and seasoned with spices, followed by fermentation. Thus, *Bacillus cereus* spore in spices can be introduced into perilla leaf pickles.

Purpose: The objective of this study was to assess the risk of *B. cereus* in perilla leaf pickles.

Methods: Fifty perilla leaf pickle samples were collected from supermarkets, and *B. cereus* were enumerated on CHROMagar™ B. cereus. Distribution temperature and time were surveyed. *B. cereus* inoculated perilla leaf pickles were fermented at 7°C to 35°C up to 7 days, and the cell counts were enumerated on CHROMagar™ B. cereus to develop predictive models with the Weibull (primary model) and exponential (secondary model) models. Consumption data and a dose-response model were also obtained. From the simulation with these data in @RISK, the probability of *B. cereus* foodborne illness per person per day was estimated.

Results: Of 50 perilla leaf pickle samples, 17 samples (34%) were *B. cereus* positive, and the initial contamination level was estimated to be 0.7 log CFU/g by Exponential and Uniform distributions. *Delta* values decreased 44.41 to 3.23 h as storage temperature increased from the primary model, and the developed secondary model was appropriate with 0.888 of R^2. Gamma distribution showed that average consumption amount was 79 g, and frequency was 60.7%. There was no dose-response model for *B. cereus,* and thus, the minimum infection dose (10^5 CFU/g) was used in hazard characterization by Discrete distribution. The simulation with these data showed that the probability of *B. cereus* foodborne illness was 0 per person per day through consumption of perilla leaf pickles.

Significance: These results indicate that the risk of *B. cereus* in perilla leaf pickles is very low in Korea.

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P2-153 Modeling the Risk of Salmonellosis/Listeriosis from the Consumption of Frozen Food Products under Alternative Consumer Handling Scenarios

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Introduction: Quantitative microbial risk assessment (QMRA) models are increasingly viewed as one of the important tools in helping food processors make scientifically supported decisions.

Purpose: The purpose of this study was use Battelle's PRIA™, a QMRA tool, to evaluate: (1) Salmonellosis risk in a not-ready to-eat (NRTE) frozen plant-based protein product that is consumed with minimal preparation and (2) Listeriosis risk due to *Listeria monocytogenes* contamination in frozen vegetables consumed as a ready-to-eat (RTE) food.

Methods: Ensembles of simulations using Battelle's PRIA™ modeling software were performed using data, including product formulation, processing, and prevalence of contamination in ingredients, based on a combination of published sources, internal company data, and expert opinion. Multiple ensembles were performed to explore possible scenarios, including potential temperature abuse during consumer handling.

Results: For the frozen plant-based protein product average predicted Salmonellosis cases were estimated to be 307 illnesses/million servings and <1 illness/million servings, for the NRTE scenario with improper cooking and RTE scenario with improper cooking, respectively. For frozen vegetables, the model estimated Listeriosis risk increased from 16 to 162 illnesses/million servings when *L. monocytogenes* contamination of frozen vegetables increases from a low level (1-10 CFU/serving) to a high level (100-1000 CFU/serving). When cooking instructions were followed properly, the model predicted <1 illness/million servings in all evaluated scenarios.

Significance: The results from these case studies underscore the importance of considering consumer behaviors during the design and development of frozen food products. QMRA tools provide a means for food safety professionals to assess the potential risk of Salmonellosis/Listeriosis when managing minimal contamination events in frozen food products. It is important to note that the risk of illness based on modeling must be interpreted by food safety professionals with appropriate education and experience since the model estimates do not necessarily represent true food safety risk.

P2-154 Assessing Foodborne Risk of Metal Exposure Associated with Produce Crops Irrigated with Oilfield Produced Water

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Introduction: Oilfield produced water (OPW), a byproduct of oil extraction operations, has been used as an alternative irrigation water source in some U.S. regions for decades. There is potential for safe beneficial use of OPW for irrigation to mitigate the impact of water scarcity. However, the safety of this practice for soil, crop, and human health has not been sufficiently assessed.

Purpose: This three-year integrated project funded by the USDA National Institute of Food and Agriculture (NIFA) sought to estimate potential foodborne risk associated with consuming crops irrigated with OPW, with focus on metals. Crops irrigated with OPW that were included in the study are citrus, almonds, pistachios, grapes, and root vegetables.

Methods: Water concentration data were obtained by sampling OPW, soil, and food crops and analyzing them for heavy metals and radionuclides. Additional publicly available concentration data were obtained from the California Water Boards. Irrigation needs were estimated based on evapotranspiration and precipitation data in the south San Joaquin Valley of California. Transfer, partition, and uptake coefficients were obtained from the literature. Data were incorporated into a spatially-explicit probabilistic multi-pathway risk assessment modeling framework to quantify human exposure dose of selected metals, by consumer age and crop. Doses were compared with cancer and non-cancer health endpoints.

Results: Results showed low foodborne risk for most metals, relative to U.S. EPA endpoints. We also identified that boron levels may be of concern for crop health and sustainability.

Significance: Study outputs aim to enable growers and policy makers to tailor OPW use to the appropriate application, and mitigate potential risk factors so that OPW may be safely applied to ensure continued agricultural productivity in drought-prone areas.

P2-155 Organophosphate Pesticides Exposure and Risk Assessment from the Consumption of Vegetables in Thailand

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Introduction: Thailand was ranked as one of the top five countries in terms of annual pesticide consumption. Consumers could be exposed to more than one pesticide at the same time and the risk would be increased if the pesticides which share the same mechanism of action come from different commodities. Organophosphate Pesticides (OP) are compounds widely use in agriculture and exhibit a neurotoxin via inhibition of the acetylcholinesterase enzyme in the nervous system causing poisoning symptoms if this inhibition is significant.

Purpose: Assess the acute cumulative dietary OPs exposure from vegetables consumption of Thai consumers.

Methods: Cumulative dietary OP exposure was estimated using a deterministic approach and considering residue and food consumption data. Pesticide residue data were obtained from three peer-reviewed studies published by Wanwimolruk et al. during 2013-2015. Food consumption data were obtained from a validated questionnaire-based dietary survey performed by Mahidol University. The most frequent consumed vegetables in Thailand were included in the assessment: cabbage, tomato, Chinese kale, pakchoi, and water morning glory. The relative potency factor (RPFs) approach was used to estimate cumulative dietary exposure using Disulfoton as an Index Compound (IC). Two approaches for calculating RPF were used (BMD_{10} and LD_{50} as relevant doses) to solve the OP data availability, resulting in two different assessments.

Results: The acute cumulative dietary OP exposure estimates for Thai consumers do not exceed the IC reference dose (RfD) using LD_{50} approach. Children between 3 - 6 years are the most exposed population reaching 10.6 % of IC RfD. Exposures estimates suggest insignificant dietary risk. However, OP exposure estimates using BMD_{10} approach exceed 4 times the RfD of the IC. Findings suggested RPF are crucial for the assessment's accuracy.

Significance: The results provide evidence for policymakers, health educators, and consumers to make informed decisions on pesticide use and risk in Thailand.

P2-156 Integration of Pathogen Reduction Models within Computational Fluid Dynamics Simulations of the Spray Drying Process

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❖ ***Developing Scientist Entrant***

Introduction: There is a concern of pathogenic contamination in spray-dried products due to recent outbreaks and recalls, although spray drying is popular to manufacture low-moisture food powders. Computational fluid dynamics (CFD) is a common method to optimize the spray drying process. However, there is a significant lack of integration of microbial risk assessment and pathogen-reduction models within CFD simulations of spray drying systems for a better process validation. Furthermore, such an integrated model can assist to measure critical process variables of non-conducive systems, such as spray dryers, indirectly.

Purpose: This study aimed to develop and validate a CFD model of a pilot-scale spray drying system with an integrated microbial reduction model.

Methods: Using ANSYS FLUENT, a CFD model was developed of 10% soy protein (w/w) isolate pumped through the spray dryer at an operating temperature of 180°C, feed-rate of 7 mL/min. Simulation data were collected for the powder formation, temperature, and residence time within the spray dryer. Thereafter, previously obtained experimental data of *Enterococcus faecium* inactivation and temperature distribution within a pilot-scale spray dryer was utilized for the validation of the CFD model.

Results: Temperature distribution within the simulated spray dryer system was confirmed to match the experimental data points with a variance of 2.3°C. The simulated droplet residence time within the system resulted in a bimodal distribution of low and high modes of 0.208 ± 0.062 and 2.269 ± 0.087 s, respectively. The average water evaporation time for each droplet was 0.200 ± 0.009 s. CFD simulation results estimated average *E. faecium* reduction of 0.133 (0.003-0.639) log compared to experimental end point log reduction of 2.83 ± 0.38 log.

Significance: Integrating predictive microbiological models within CFD simulations of industrial-scale food-processing systems can significantly improve process validation model accuracy where direct measurement of controls is challenging.

P2-157 Shelf-life Estimation of Pacific White-leg Shrimps Using Micro Isothermal Calorimetric (MIC) Data at Accelerated Storage Temperature - A Machine Learning Approach

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Introduction: The basis of traditional shelf life estimation models is Arrhenius equations which determines loss of quality over time using rate laws. However, this modelling approach is not always valid as it strictly depends on boundary conditions resulting in the lack of robustness. Since sensors produce a large set of data, the industry is in need of more robust models with higher generalization ability. A more accurate shelf-life estimation is possible using MIC data, which represents non-specific heat flow (µW) in a food system.

Purpose: The application of machine learning theory as an alternative method to interpret MIC data using a radial basis function network (RBFN) and compare it against a first-order Arrhenius model.

Methods: Five grams of fresh pacific white leg shrimp were stored at 3, 6, 9, 12°C for 4 days (N = 30, triplicated). MIC data was collected by incubating at 21°C for 25 h and 30°C for 48 h for psychrotrophic and mesophilic bacteria in pre-sterilized vials, respectively. Two models; rate of reaction, $k = k_0 \exp(-Ea/RT)$ and, the RBFN were fitted with data, where the µW corresponds to N (CFU/mL).

Results: The initial load of 3.73 log CFU/ml was increased to 2 to 4 log cycles with the passage of time and increase in temperature. Remaining shelf life (days) was calculated in terms of k (h^{-1}) and the specific heat flow per gram per sec ($\mu W.g^{-1}s^{-1}$) for TVC and MIC data, respectively. Both models were compared after a cross-validation procedure (N = 10) which yielded high R^2 values (>0.9) with low avg RMSE (0.0202) for both models, however, RBFN demonstrated higher accuracy, lower cost of calibration, fewer iterations than the kinetic model.

Significance: The MIC data and RBFN has the great potential to predict microbial quality of pacific white-leg shrimp distributed as fresh at refrigerated temperature.

P2-158 Pork "Gyros": Assessment of Microbial Safety Under Commercially Occurring Roasting Scenarios

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Introduction: Pork "gyros" (fresh or frozen) is composed of marinated non-cured pieces of meat and fat, subjected to roasting (5-8 h), and served as slices from outside to the core. Composition, thickness, and roasting practices applied (e.g., duration, intermittent heating) may compromise "gyros" quality and/or safety, due to growth of spoilage and/or pathogens, respectively, at the coldest spots.

Purpose: i) To assess *Salmonella*, *Escherichia coli* O157:H7 (EHEC), and *Staphylococcus aureus* growth in pork "gyros," in the presence of indigenous microbiota, at temperatures occurring inside meat during commercial roasting. ii) To compare growth data with existing broth or meat-based predictive models, and update the latter with product-specific data.

Methods: Pork "gyros" pieces (5 x 5 cm) were surface inoculated (3 log CFU/cm^2; 3-strains composites) of *Salmonella* Enteritidis and *S.* Typhimurium, EHEC, or *S. aureus*. Three inoculated pieces were piled up, vacuum packaged to mimic the microaerobic conditions in the interior of "gyros," and stored at 10-45°C or non-isothermal profiles. Total Viable Counts, pseudomonads, lactic acid bacteria (LAB), *Brochothrix thermosphacta* were also monitored. Growth curves were compared to simulation curves on pork meat or ground pork retrieved by existing predictive models from GroPin and ComBase.

Results: *Salmonella* reached 6-7 log CFU/cm^2 close to the optimum temperature (30°C and 40°C), while EHEC and *S. aureus* attained 4-5 log CFU/cm^2 or no growth, respectively. Growth was restricted <20°C, while, at 45°C, growth was restricted (*Salmonella* and EHEC), or even inactivation occurred (*S. aureus*). Indigenous microbiota, dominated by LAB played significant role on pathogen inhibition. Existing models showed good agreement with growth data at 30 - 40°C, especially for S*almonella*, but overestimated pathogens' growth in the other temperatures, since they do not take into account the competitive effect of indigenous microbiota.

Significance: Current data enable the update of existing predictive models for pathogen growth in pork "gyros" and relevant products, when temperatures within the dangerous zone occur internally during heating.

Poster

P2-159 Simulating Shelled Corn Sampling to Improve Sampling Plans for Mycotoxin Detection

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❖ ***Developing Scientist Entrant***

Introduction: One common method to sample bulk corn for mycotoxin detection is probe sampling. Approaches to improving probe sampling performance remain unclear due to spatial clustering of contaminated kernels.

Purpose: This study aims to improve grain sampling effectiveness by elucidating the impact of clustering on probe sampling performance given different number of probes and sampling strategies.

Methods: A novel simulation model was built to mimic the process of taking 5 to 100 probe samples from a contaminated grain lot, grinding, testing for aflatoxin by ELISA, and return the probability of acceptance. A combination of four parameters were investigated, including bulk aflatoxin concentration (5, 10, 20, 40, 80, 100 ppb), number of probes (5, 7, 10, 100), sampling strategy (SRS, STRS, SS), and the number of kernels in each cluster (1 – no clustering, 10, 100, 1000 - highly clustered). Each combination was iterated for 100 times by the Monte-Carlo technique. A sensitivity analysis using partial rank correlation coefficients (PRCC) was conducted to quantify the influence of the four parameters on probability of acceptance. A one-way ANOVA was utilized to compare the effect of 3 sampling strategies and number of probes on probability of acceptance given different levels of clustering.

Results: The probability of acceptance is negatively impacted by increasing aflatoxin concentration (PRCC = -0.85) and increasing number of probes (PRCC = -0.10). Meanwhile, the probability of acceptance is positively influenced by higher degree of clustering (PRCC = 0.33) and choosing STRS/SS over SRS (PRCC = 0.03). Only when the degree of clustering is at the highest does the sampling performances among the 3 sampling strategies and among different number of probes differ significantly (*P*-value = $1.38\times10^{-5,}$ 7.20×10^{-6}, respectively).

Significance: Sampling plans should prioritize (i) taking more probes or auto-sampling and (ii) choosing SRS over STRS and SS to facilitate rejection of a contaminated lot.

P2-160 Hazard Identification and Characterization for the Development of a Share Table Quantitative Microbial Risk Assessment.

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Introduction: Food recovery through school share tables could be viable mechanism to reduce food insecurity and food waste in the US, but food safety concerns from stakeholders are limiting the implementation of this recovery method. The extra steps added by the implementation of share tables increase the opportunities for (i) cross-contamination and (ii) hazard amplification. The development of a quantitative microbial risk assessment (QMRA) will address stakeholder's food safety concerns and identify the best food safety practices.

Purpose: Identify hazards that could cause illness due to the consumption of foods that have been exposed to the share table process.

Methods: Hazard identification consisted of the development of a representative flow chart of the share table process through the observation of an operation share table system at an elementary school in Danville, Illinois. Hazard characterization consisted of finding relevant literature to determine characteristics, transmission routes and significance of hazards that are present or that could be added to a school lunch program by the incorporation of share tables.

Results: The share table flowchart identifies *n* = 8 fundamental steps and their interactions within the school lunch program. The incorporation of share tables enables the recurrence of steps such as food selection, consumption, and storage as well as increasing the number of interactions between food, consumers, and food workers. Norovirus*,* hepatitis A, *Salmonella* spp., *Campylobacter, Clostridium perfringens,* Shiga toxin-producing *E.coli, Listeria monocytogenes, Shigella*, *Staphylococcus aureus,* and allergens are significant hazards in a school lunch program. Risk factors for these hazards include (i) cross-contamination between hands, utensils, surfaces, foods and any combination of these, and (ii) hazard amplification due to holding temperatures, re-storage and transportation.

Significance: This hazard identification & characterization process will serve as a foundation for the development of a share table QMRA.

P2-161 Effect of Type of Staphylococcal Enterotoxins on the Risk of Ready-to-eat (RTE) Triangle-Sushi at Retail Market

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Introduction: Staphylococcal food poisoning is an intoxication resulting from the ingestion of food containing the enterotoxins (SEs) produced by various types of Staphylococcal enterotoxins (SEs). The most common toxin implicated in staphylococcal food poisoning is SEA. Triangle-shaped sushi is a popular RTE food in Korea, which is made with most of the rice and other ingredients, such as pork, beef, etc.

Purpose: This study investigated the effect of various type (SEA, SEB, SEC, SED, and SEE) of staphylococcal enterotoxins on the risk of food poisoning outbreak of RTE Triangle-sushi at a retail market.

Methods: Various triangle-sushi (*n* = 170) were purchased from convenience stores in Korea and monitored for the contamination of *S. aureus*. To estimate the level of change of contamination from markets to home, the growth models of *S. aureus* in triangle-sushi were developed as a function of temperature (10 to 37℃). The daily consumption amount and intake rate of triangle-sushi were investigated with 1,000 residents in Korea. Finally, Monte Carlo simulation analysis was run for the worst-case scenarios using @RISK.

Results: *S. aureus* was not detected in triangle-sushi among 170 samples. Triangle-sushi inoculated with SEA grew at the temperature of 10℃ below, while a five-toxin cocktail of Staphylococcal (SEA, SEB, SEC, SED, SEE) grew at the temperature of 11℃. The probability of *S. aureus* foodborne illness from triangle-sushi per person per day is $2.06\text{x}10^{-9}$ with the *S. aureus* toxin cocktail. The most influential factor for the risk of *S. aureus* foodborne illness was the consumption frequency.

Significance: The result indicates that only SEA can grow in triangle-sushi at the refrigeration temperature of the retail market (10℃) in Korea, which proves that it causes the most common staphylococcal food poison.

P2-162 A Meta-analysis of Worldwide Mycotoxin Prevalence in Beers

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Introduction: Beer is the second most popular alcoholic beverage consumed in the world. Mycotoxins have been reported to occur in beers around the world.

Purpose: The purpose of this research was to estimate the worldwide prevalence of mycotoxins in beers through a systematic review and meta-analytic approach for incorporation into quantitative risk assessment.

Methods: Citations related to mycotoxin occurrence in beer from 1986 to 2018 were retrieved from Web of Science, Scopus and PubMed. The prevalence of mycotoxins in beers by WHO region and country was subjected to meta-analysis. Data on total aflatoxins (AF), AFB_1, AFB_2, AFG_1 and AFG_2; deoxynivalenol, 3- and 5A deoxynivalenol (DON); total fumonisins, FB_1, FB_2, FB_3, (FBs); ochratoxin A (OTA); T-2 and HT-2 toxin (T2t); zearalenone (ZEA) and "other mycotoxins" was extracted from collected citations. Regression was performed to assess correlation between mycotoxin prevalence and year of publication. Logit transformation was applied to normalize the data and random-effects were estimated by maximum likelihood. Heterogeneity was calculated via the I^2 statistics test. Data analysis was performed using Metafor and Meta packages in R Statistical Software using $P < 0.05$.

Results: A total of 73 articles with 4970 samples from among 5219 potential articles were included in the meta-analysis and meta-regression. The global pooled prevalence of mycotoxins in beers was 17% (95%, C=14-22%), with substantial heterogeneity (I^2=96.55%, $P < 0.0001$). The overall rank order of prevalence was OTA>FBs>DON>other mycotoxins>ZEA>AFs>T2t. The regions with highest and lowest reported beer mycotoxin prevalence were Africa (24%) and Eastern Mediterranean (4%), respectively. Countries with highest and lowest prevalence were Iran (99%) and China (1%), respectively. Meta-regression showed a significant decrease in the prevalence of mycotoxins in beers over time (C=-0.0094; $P < 0.05$).

Significance: Data showed substantial heterogeneity in the occurrence of mycotoxins in beer and suggest further actions to reduce the risk of exposure may be needed.

P2-163 A Meta-Regression Model Describing the Effects of Essential Oils on *Escherichia coli* Inactivation in Cheese

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❖ ***Developing Scientist Entrant***

Introduction: The antimicrobial properties of plant essential oils have been described in the literature and proposed as hurdles to increase the microbiological safety of many food products, including cheese.

Purpose: The objective of this study was to integrate data on *Escherichia coli* (EC) inactivation in cheese containing essential oils (EOs) by means of an overarching meta-regression model.

Methods: Suitable primary studies were identified through exhaustive literature search. Sixteen studies were considered appropriate for inclusion (N = 362), and the following study characteristics were extracted: antimicrobial, mean log reduction, storage temperature, exposure time, antimicrobial application (i.e., cheese mixture, cheese surface, milk, or film), and antimicrobial and pathogen's inoculum concentrations. Studies were assigned weights according to the sample size (n) used along the experiment while the different antimicrobials were assumed to cause shifts as random effects. The significance of moderators was evaluated by analysis of variance (α=0.05).

Results: The meta-regression revealed the significant impact of application type ($P < .0001$) and antimicrobial concentration ($P = 0.002$) on EC log reduction. Exposure time was also found to affect EC inactivation, although such effect is dependent on the type of application ($P < .0001$). This means that, for a specific antimicrobial, the same inhibitory effect is achieved with distinct exposure times, depending on the application type selected. Milk was found to be the application mode leading to highest microbial reduction, while incorporation in cheese mixture, cheese surface and films presented lower inhibitory effects. Among the types of EOs meta-analyzed, shallot, sage and lemon balm produced the greatest mean bactericidal effects. Heterogeneity analysis revealed that the moderators explain >95% of the between-antimicrobial variability.

Significance: This meta-regression model offers insight on the main causes of variability in microbial reduction, which can be useful for the experimental design of challenge studies and for the optimization of the use of essential oils as a biopreservation technology for pathogen control in foods.

P2-164 Inactivation of Antimicrobial Resistant Bacteria during Manure Storage as Static Stockpiles

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Introduction: Humans may be exposed to antimicrobial resistant bacteria (ARB) with an origin of food animal husbandry through environmental routes due to the contamination from livestock manure. Appropriate treatment and management of manure is critical to mitigating ARB spreading to the environment.

Purpose: Quantitatively evaluate the survival of tetracycline resistant (TETr) *E. coli* during manure storage as static stockpiles and estimate storage time sufficient to inactivate TETr *E. coli* below the limit of detection (LOD) or quantification (LOQ).

Methods: Manure samples were collected from various depths (core, mid, and surface) of three manure stockpiles at 9 sampling occasions over a three-month period. The presence and load of TETr *E. coli* along with moisture content for each sample were measured. The survival curve of ARB was described by fitting several generalized linear mixed models (Gamma, Exponential, and Weibull) with and without the covariate effect, i.e., moisture content. The upper bound of 95% confidence interval was used to conservatively estimate the time required to inactivate TETr *E. coli* below LOD or LOQ.

Results: The response denoting loads of TETr *E. coli* in log CFU/g showed a positively skewed distribution. Weibull distribution had the smallest mean square prediction error (MSPE) and was the best fit for modeling the response variable with random effects due to pens, depths, and sampling days. Overall, 48 days were required for TETr *E. coli* to reach the LOQ (2.30 log CFU/g) and 170 days to reach the LOD (-1.00 log CFU/g).

Significance: These findings provide a science-based evidence informing appropriate manure management practice and may contribute to combating antimicrobial resistance.

P2-165 Survival of *Listeria monocytogenes* in Cow Milk through a Dynamic Human Stomach Model

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Introduction: *Listeria monocytogenes (Lm)* is an opportunistic foodborne pathogen that causes a rare but serious disease, listeriosis, mainly in high-risk groups including pregnant women, neonates, the immunosuppressed and the elderly. Ready-to-eat foods, such as deli meat, produce and dairy products have been causally linked to outbreaks of listeriosis. Current research on acid tolerance of *Lm* in human gastrointestinal tract primarily focuses on the behaviors of the pathogens in the absence of food using static and dynamic *in vitro* models. Understanding how *Lm* behaves in the stomach in the presence of food is important since the stomach is the first and major barrier against pathogens.

Purpose: This research aims to evaluate survival of *Lm* strains in artificially-inoculated commercial cow milk in an *in vitro* dynamic human gut model, Simulator of the Human Intestinal Microbial Ecosystem (SHIME), which can mimic the physiological conditions of the human stomach.

Methods: Three major human listeriosis-causing strains, 1/2a, 1/2b and 4b that were inoculated in cow milk were exposed to simulated gastric fluid of healthy adults (pH 1.5, 2.0, 3.0) with gastric emptying time of 2 hours. Samples were collected every hour and viable count of *Lm* were determined based on culture-based enumeration.

Results: All serotypes display various degrees of susceptibility < 1 log reduction during 1 h and 2 h of emptying time across all acidity levels except for pH 1.5, which resulted in 3- to 5-log reduction after 2 hours of exposure ($P < 0.05$). Survival of *Lm* in simulated human stomach is strain-dependent at certain timepoints. There is a greater log reduction of *Lm* found in higher gastric pH compared to lower gastric pH from t=1 to t=1.5 h, which might be an indication of acid tolerance development in *Lm.*

Significance: This study will provide a better understanding on the behaviors of *Lm* strains as they transit through the human stomach with food and can potentially enable the development of novel treatments or prevention strategies for listeriosis.

P2-166 Effect of Relative Humidity on Survival of *Salmonella enterica* in Raw Cut Peppers Stored at Distinct Temperatures

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Introduction: Raw cut peppers are used in sandwich fillings and as finger foods. Outbreaks involving *Salmonella* contaminated peppers have been widely reported. Little is known about the effects of relative humidity (RH) and temperature on survival of *S. enterica* in cut peppers.

Purpose: We assessed the survival of *S. enterica* strains linked to disease outbreaks on cut peppers (*Capsicum annuum* L.) at different temperature and relative humidity (RH) conditions.

Methods: Peppers were cut into pieces (3 cm^3) and spot inoculated with *S.* Enteritidis resistant to nalidixic acid (NA; 15 µg/mL) and *S.* Typhimurium resistant to tetracycline (TET; 15 µg/mL) at final counts of ~4.5 log CFU/g pepper/strain. Peppers were placed in desiccators containing saturated solutions of potassium sulfate and potassium carbonate (100% and 50% RH, respectively) and stored at 7, 14, and 21°C. *Salmonella* were enumerated on BHI agar containing NA or TET, at time intervals from 0 to 144 h, with a detection limit of 1.5 log CFU/g.

Results: Counts of *S.* Typhimurium and *S.* Enteritidis increased over time in cut peppers stored at 14°C and 21°C at 100% RH (up to 1.5 log CFU/g after 144 h). Counts of *S.* Typhimurium decreased ~1.5 log CFU/g after 72 h at 7ºC at 100% RH, while counts of *S.* Enteritidis did not change. When cut pepper samples were stored at ~50% RH, counts of both *Salmonella* strains decreased after 48 h storage, regardless of temperature. After 96 h of storage at 7°C at 50% RH, no viable counts of S. Typhimurium were recovered, while counts of S. Enteritidis were ~3.0 log CFU/g.

Significance: These findings show the effects of RH on survival of *Salmonella* in cut peppers and highlight the ability of different *Salmonella* strains to survive in cut peppers at different conditions of temperature and RH.

P2-167 Validation of Existing Combase Models for Suitability in Ten Different Types of Whole Uncut Fresh Produce

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Introduction: While *L. monocytogenes* causes relatively few cases per year in the US, it causes more deaths than most other bacterial pathogens. Risk management tools are increasingly needed when products experience out of temperature conditions.

Purpose: We assessed the ability of ComBase Predictor to model *L. monocytogenes* growth on ten different whole fresh produce items stored between 2 to 35°C.

Methods: Ten different produce types (blueberry, broccoli, carrot, cauliflower, cherry, mandarin orange, lemon, raspberry, tomato) were investigated. Five *L. monocytogenes* outbreak strains were made resistant to rifampicin to facilitate recovery. A cocktail of the 5 strain in 0.1% peptone water was used to inoculate samples (~3.5 log CFU/sample), incubated at 2, 12, 22 and 35°C and enumerated over 28 days. Experiments were replicated six times for each temperature/produce combination. Growth rates were estimated with DMFit and "growth" was defined as an increase of >1 log CFU for at least two time points. Growth rates were compared with ComBase modeling predictions for *Listeria* at different pH values and temperatures at high water activities.

Results: DMFit provided generally good fits to initial growth curves. Growth was not observed for any produce items stored at 2°C. Apparent growth was almost always observed at 12, 22 and 35°C for all produce types, with the exception of whole carrots, where no growth was observed under any conditions. In many cases growth of *L. monocytogenes* up to a maximum of >6.0 log CFU/sample was followed by a decline for the remainder of the storage period. The ComBase model (assuming pH 5) was generally fail safe for all produce items except for tomatoes stored at 35°C.

Significance: Our results demonstrate that the ComBase model is failsafe for *Listeria*. More research is needed to investigate factors causing apparent growth, especially for items not typically refrigerated.

P2-168 Predictive Model for Growth of *Clostridium perfringens* during Cooling of Cooked Pork Supplemented with Sodium Chloride and Sodium Pyrophosphate

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Introduction: The thermal cooking processes can activate the heat-resistant spores causing them to germinate, especially if the meat products stay between 40 and 50°C for too long during cooling. Intrinsic factors, such as NaCl concentration, pH, and water activity, can influence *C. perfringens* growth and may be modified through product formulation.

Purpose: The objective of this study was to develop a dynamic model to predict growth of *C. perfringens* in cooked ground pork supplemented with salt (0, 1, 2, and 3% wt/wt) and sodium pyrophosphate (0, 0.1, 0.2, and 0.3% wt/wt) under continually varying temperature.

Methods: Inoculated 5 g meat samples supplemented with various levels of salt and/or sodium pyrophosphate in sterile bags were completely immersed in a circulating water bath stabilized at temperatures ranging from 15 - 51°C. Periodically, samples were analyzed for *C. perfringens* count on tryptose–sulfite–cycloserine agar and the plates were incubated for 48 h at 37°C. The primary Baranyi model was fitted to the various *C. perfringens* growth data. A secondary model in the form of a quadratic polynomial was developed and subsequently, a dynamic model was developed and validated using growth data retrieved from six published studies.

Results: All primary models gave a satisfactory fit ($R^2 \geq 0.85$). Secondary model predicted the maximum specific growth rate as a function of temperature, salt, and phosphate concentrations ($R^2 = 0.92$). During validation of our developed model, 28 out of 38 predictions by the model were within acceptable prediction zone ($-0.5 \leq$ prediction error ≤ 1.0).

Significance: This predictive model can be used to develop and follow safe cooling rates and design pork products containing ingredients (salt or pyrophosphates) that can control *C. perfringens* spore germination and out growth.

P2-169 Growth Kinetics of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on the Surface of Whole Cantaloupes and Watermelons during Storage

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Introduction: Outbreaks linked to whole cantaloupes highlight the importance of understanding the growth potential of pathogens on melon surfaces at various storage temperatures.

Purpose: Here we analyze the growth kinetics of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on the surface of whole cantaloupes and watermelons.

Methods: Experiments were conducted in triplicate to determine the growth curves of the pathogens. Athena cantaloupe and mini seedless watermelon were spot inoculated (10^3 CFU/3.1 cm^2) on the sun-side with a multi-strain cocktail of *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes*, and allowed to dry for 1 h ($n = 6$). To simulate postharvest handling conditions, melons were stored at 4, 10, 15, 20, or 25°C, and sampled up to 21 days. At each sampling, the inoculated area was excised, stomached, and plated onto selective and non-selective media to enumerate pathogen concentration. To describe pathogen growth as a function of time, primary modeling was performed using ComBase. The specific growth rate values were used to develop secondary models to explain the relationship between the growth parameters and the temperature.

Results: The surface of cantaloupe is more susceptible to pathogen growth compared to watermelon. On cantaloupe, populations of *L. monocytogenes* increased linearly with temperature; calculated growth rates were ~0.0006, 0.001, 0.008, 0.0118, and 0.0119 log CFU/h at 4, 10, 15, 20 and 25°C, respectively. Growth rates at 15, 20, and 25°C were significantly higher than at 4 or 10°C ($P < 0.05$). Populations of *L. monocytogenes* declined on watermelon when stored at 4, 10, and 15°C. Growth rates of *E. coli* O157:H7 on cantaloupes stored above 10°C increased linearly, with maximum growth at 25°C for 3 days (4.9 log CFU/3.1 cm^2); populations of *E. coli* O157:H7 declined regardless of temperature on watermelons. *Salmonella* populations declined on both melon surfaces at all temperatures with higher reduction rates at lower temperatures.

Significance: Refrigerated storage of whole melons reduces the risk of foodborne pathogen growth on whole cantaloupes and watermelons.

P2-173 Models for Growth of *Listeria monocytogenes* on Whole Intact Fresh Produce from Literature Data

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Introduction: *Listeria monocytogenes* cells can multiply on whole intact fresh produce, however the factors that influence growth are not well understood.

Purpose: We developed models for factors that influence the growth of *Listeria monocytogenes* on whole intact fresh produce using data extracted from the published literature.

Methods: Published or unpublished datasets ($n = 29$) characterizing the behavior of *Listeria monocytogenes* on 21 different types of whole intact fresh produce were found by searching seven databases. Growth models were fit to each data set to estimate growth rates using DMfit. Multiple linear stepwise regression models were developed using R software. Model factors included: incubation temperature, inoculation buffer, initial and final cell concentrations, inoculation method, container characteristics and produce surface characteristics. Subset regression modeling was used to further refine the models. The olsrr package was used to create best subset models of the significant parameters.

Results: Parameters used in the reduced model were incubation temperature, inoculation buffer type, initial and final cell concentrations, container characteristics and produce surface characteristics. The reduced multiple linear regression model for growth rate had an adjusted R^2 value of 0.51 with a *P*-value of <4.00e-15. ANOVA analysis showed that incubation temperature, initial cell concentration, final cell concentration, and produce surface characteristics all had significant ($P < 0.05$) effects on growth rate. The best regression model for growth rate had 3 parameters: incubation temperature, and initial and final cell concentrations. The model created using these parameters had an adjusted R^2 of 0.37 and a *P*-value of 3.33e-13. Our findings show the importance of inoculum concentration and produce microbial carrying capacity on the estimated growth rate and highlight the overall importance that temperature has on growth rate.

Significance: These models can be used to guide future experimental design and in quantitative microbial risk assessments for *L. monocytogenes* on whole produce.

P2-174 Quantification of the Influence of Strain Type and Inoculum Preparation on the Survival of *Salmonella* in Whole Milk Powder

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Introduction: Dry foods have been increasingly shown to be a potential vehicle for transmission of foodborne pathogens. Factors that affect the survival of *Salmonella* in dry foods are not well understood.

Purpose: This research evaluates and models the survival of *Salmonella* in dry foods as influenced by serovar and inoculation method.

Methods: Six strains of *Salmonella* (of three serotypes: Enteritidis, Montevideo and Typhimurium), and a cocktail of those strains, were inoculated into 5 g of whole milk powder. Powder was dried for 24 h under conditions to restore the water activity of the uninoculated powder (~0.30). Inocula were prepared using an overnight broth culture and a lawn grown culture. Survival was measured over 56 days, and each experiment was repeated 3 times. Linear regression and Weibull models were fit to each dataset and summary statistics created using R.

Results: Lawn culture inocula showed greater survival after 58 days, with generally <1 log CFU reduction, where broth cultures showed at least 2 log CFU. The cocktail of broth grown strains had a rate of reduction faster than the rate of reduction of 5/6 individual broth grown strains, while the cocktail of lawn grown strains had a rate of reduction faster than only 1/6 lawn grown strains. Linear models showed fairly high variability for both methods with R^2 values from 0.42 to 0.82 for broth culture inocula and 0.01 to 0.77 for lawn culture. Lawn culture inocula showed slower rates of reduction ranging from 0.001 to -0.0159 log CFU/day while broth inocula showed rates ranging from -0.007 to -0.033 log CFU/day. Modified Weibull models generally provided better fits than linear models.

Significance: The rate of survival of *Salmonella* is highly influenced by strain type and inoculum preparation method. More research is needed to determine the biological reasons behind these differences.

P2-176 Source Attribution at the Fish Sub-Product Level for 11 Foodborne Pathogens of Importance in Fish for the Development of a Risk Assessment Model

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed a systematic risk-based approach to better prioritize its inspection resources. This includes the development of an Establishment-based Risk Assessment (ERA) model that categorizes food establishments according to the risk level their products represent to Canadians, which considers the volume of each sub-product they manufacture, as well as other food safety risk factors. As no studies were available on the source attribution for fish sub-products, an expert elicitation was conducted to address this need.

Purpose: The study aimed at estimating the relative contribution of individual fish sub-product categories to the overall foodborne illness burden associated with the consumption of fish for 11 pathogens.

Methods: The elicitation was conducted over a 2-month period, and targeted norovirus, non-typhoidal *Salmonella* spp., verotoxigenic *E. coli* (O157, non-O157), rotavirus, *Campylobacter* spp., *Yersinia enterocolitica*, hepatitis A virus, *Toxoplasma gondii*, *Listeria monocytogenes*, *Clostridium botulinum*, and *Vibrio parahaemolyticus*. Seven fish sub-product categories created based on similar food safety risks were evaluated and 40 experts were invited to complete a web-based survey (available in English and French). Experts provided estimates at the fish sub-product level for each pathogen and evaluated their certainty level using a scale from 1 (low) to 10 (high).

Results: Seventeen experts (43%) with over 21 years experience (average) in government, academia, and industry participated in the study. Their background disciplines included mostly microbiology (35%), biology (24%), and food science (18%). Data analysis showed that shellfish was predominantly associated with norovirus (75%), *V. parahaemolyticus* (77%), and hepatitis A virus (59%); while ready-to-eat hot and cold smoked fish products were mainly associated with *L. monocytogenes* (36%) and *C. botulinum* (32%). Expert profiles did not influence the source attribution estimates.

Significance: These results will be included in the CFIA ERA model and used to enhance its applicability for risk prioritization and effective inspection resource allocation.

P2-177 Assessing the Relative Risk of Feed Safety Criteria Included in the Canadian Food Inspection Agency Risk Assessment Model for Feed Mills through an Expert Elicitation

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Introduction: The Canadian Food Inspection Agency (CFIA) is developing an Establishment-based Risk Assessment model for feed mills (ERA-Feed model) to allocate inspection resources according to the feed safety risks associated with each feed establishment. A previous study was conducted to select the risk factors (n = 13) and their assessment criteria (n = 206) to be included in this model based on a literature review and expert advice.

Purpose: The objective of this study was to quantify the relative risk (RR) of the assessment criteria based on their impact on feed safety to design the ERA-Feed model algorithm.

Methods: A two-round, face-to-face expert elicitation was conducted on November 7, 2019. During the first round, experts were asked to individually estimate the RR of each assessment criteria through an online questionnaire. In the second round, results from all experts were shared and discussions occurred. Experts were then invited to adjust their RR estimates, if needed. Statistical analyses were performed using Excel 2016 for descriptive analysis and R version 3.4.0 for the non-parametric Kruskal-Wallis and Shapiro-Wilk tests.

Results: A total of 28 experts from the feed industry (54%), academia (29%) and government (18%) across Canada participated. Results showed no statistically significant association between respondent profiles and assigned criterion weight. Uniformity of answers between experts improved between rounds. The criteria having the highest increase in risk (median RR ≥ 4.5) were the use of prohibited materials combined with the production of ruminant feed and non-compliances related to the feed mill's process control programs. The risk mitigation criteria having the highest impact were the presence of feed safety certifications and the use of dedicated manufacturing lines.

Significance: The median results assigned to each criterion will be used to build the algorithm of the CFIA's ERA-Feed model. This model will help in allocating inspection resources based on risk for the protection of animal and public health.

P2-178 Predicting the Growth of *Listeria monocytogenes* in a Deli Style, Uncured Turkey Meat Product Formulated with Vinegar Powder as a Function of Product pH, Moisture, and Salt

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Introduction: The growth of *Listeria monocytogenes* in refrigerated ready-to-eat meat products is primarily affected by the presence or absence of a bacterial inhibitor and intrinsic product properties (pH, moisture and salt content). Predictive modeling can assist in the development and rapid commercialization of microbiologically-safe formulations.

Purpose: Develop a mathematical model to evaluate the growth and inhibition of *L. monocytogenes* in deli style, uncured turkey meat formulated with Verdad® Powder N6 (vinegar powder) at various physicochemical product compositions.

Methods: Box-Behnken design was used to evaluate the growth and inhibition of *L. monocytogenes* in turkey meat formulated with 0.6, 0.75, and 0.9% vinegar powder at product pH 6.2, 6.4, and 6.6; moisture content 70, 73, and 76%; and salt content 1.0, 1.5, and 2.0%. Twenty-seven treatment combinations including three center points were evaluated in a three-block design by surface inoculating with ca. 3.0 log CFU/g of a 5-strain cocktail of *L. monocytogenes*, vacuum packaged, and stored at 3.3°C. Populations of *L. monocytogenes* were enumerated at prescribed intervals to develop growth curves. These curves were subjected to linear fitting, and growth rates and lag times were obtained. These data were used to calculate time to 2-log outgrowth of *Listeria* (TTG2), which was used for Box-Behnken analysis in SAS JMP.

Results: Box-Behnken analysis was performed, and effects (e.g., pH, antimicrobial level, and the interaction of pH and antimicrobial) deemed significant ($P \leq 0.01$) were used to generate a predictive model equation to predict *Listeria* outgrowth.

Significance: A mathematical model was developed to predict *Listeria monocytogenes* growth impacted by the presence of antimicrobial and product proximate characteristics. This model will assist in faster product commercialization while maintaining food safety.

P2-179 Progress Toward Standardizing Metagenomics: Application of Metagenomic Reference Materials to Develop a Reproducible Microbial Lysis Methodology with Minimum Bias

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Introduction: Metagenomics research has grown rapidly in the previous decade, but often of reproducibility between different methods and laboratories, which risks limiting our ability to compare between studies and decreases confidence in previous conclusions.

Purpose: We sought to compare the performance of many commercially- and academically-sourced lysis protocols using both typical sample types and known input mock microbial communities.

Methods: We developed a statistical method attached to a computational package called the Measurement Integrity Quotient (MIQ). This measurement is based on the root mean square of errors (RMSE) measurement modified to consider manufacturing tolerances in a standard and transformed for ease of understanding when comparing observed compositions relative to expected values from a manufactured standard. As a computational package, this system incorporates automated bioinformatic analysis to start with raw sequencing reads and deliver the score with little bioinformatic knowledge required of the user. We compared the effects of several different chemical, enzymatic, thermal, and mechanical lysis methods, including over 40 different bead size/material combinations using the MIQ scoring package. Additionally we looked at the performance of several commercial kits and laboratory-derived protocols in the hands of different groups.

Results: We were able to determine that a lysis bias against gram-positive microbes and fungi was present in many different methods. We also determined that the use of hard, dense ceramic beads (such as zirconium oxide) of mixed size provide reliable, unbiased lysis of microbes, often scoring MIQ values of 90 and above when paired with an appropriate bead beating system.

Significance: This study systematically evaluates biases in microbiome preparation techniques and suggests some best practices to minimize these biases. This study also introduces the MIQ score, which can be used for evaluation of microbiome preparation techniques as well as quality control for individual preparations.

P2-180 Development of a Bacterial Metabarcoding Analysis Pipeline

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Introduction: Metabarcoding technology brings tremendous value to understanding how microbes affect food spoilage, fermentation, changes in flavor and shelf life. However, various analysis methods/algorithms perform differently, and the difficulty of data analysis limits routine application of metabarcoding by the food industry.

Purpose: This study evaluated several data clustering and classification methods/algorithms, with the goal of developing an easily executable data analysis pipeline that characterizes bacterial communities at the genus, and in some cases, the species level.

Methods: The bacterial communities in food (cheese, lettuce, chicken deli meat) and environmental samples, plus a bacterial mock community sample as a positive control, were profiled by targeting the V3-V4 region of the 16S ribosomal RNA and using the Illumina MiSeq. DADA2, Deblur, VSEARCH, and MED were evaluated for data clustering. BLAST, ScikitLearn, and VSEARCH were evaluated for operational taxonomic unit (OTU) classification. A total of 11 pipelines (various combinations of the data clustering and classification approaches, plus mothur) were hosted in Snakemake, a platform that executes commands automatically. The pipeline performance score was calculated based on computing efficiency and OTU classification results (precision, recall, F-measure, specificity, taxonomic ranking). The score was normalized on a scale of 0-100, with 100 representing the best performance. The OTU tables were visualized using EasyBioM, a web-based and in-house developed tool.

Results: MED+BLAST proved to be the best pipeline, demonstrating the highest efficiency and OTU accuracy, with a performance score > 98 for all datasets, compared to the other pipelines (average score of 91, ranging from 70 to 98).

Significance: This study evaluated several metabarcoding data analysis methods/algorithms and identified a pipeline that performed the best. The MED+BLAST pipeline, implemented in the Snakemake environment, enables an easy to operate data analysis workflow to survey bacterial communities, with little or no bioinformatics expertise needed.

P2-181 Serotype Characterization of Salmonella Isolates from Traditional Wet Markets from Cambodia through Whole-genome Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Traditional wet (informal) vegetable markets are an important part of the Cambodian culture and economy. However, vegetables can potentially be contaminated by pathogens due to lack of hygiene and sanitary practices, food safety regulations, and basic infrastructure.

Purpose: The overall objectives of this study were to i) isolate *Salmonella* from food contact and non-food contact surfaces, ii) perform whole-genome sequencing (WGS), and iii) assess in-silico serotype abundance.

Methods: Samples collected from two informal markets in Battambang, Cambodia, were screened for *Salmonella enterica* according to USDA-FSIS guidelines. Isolates were confirmed by PCR. Genomic DNA was extracted from 81 isolates with a commercial kit (Qiagen). Libraries were prepared from genomic DNA with the Nextera XT DNA Library Preparation Kit, and WGS was carried out on either the MiSeq or NextSeq sequencer, using a MiSeq Reagent Kit V2 (500-cycles) or a NextSeq 500/550 High-Output Kit V2 (300-cycles), respectively (Illumina). De novo assemblies were obtained with Shovill 0.9 (https://github.com/tseemann/shovill). Serotype for each isolate was determined *in silico* using SeqSero 1.0 on draft genomes (http://denglab.info/SeqSero). The NCBI Pathogen Detection database (https://ncbi.nlm.nih.gov/pathogens), was used to determine the antimicrobial resistance (AMR) profiles and the SNP clusters.

Results: Sixteen *Salmonella enterica* serotypes were detected across multiple surfaces. The most common serotypes were Rissen (*n* = 18), Hvittingfoss (*n* = 11), Corvallis (*n* = 10), Krefeld (*n* = 8), Weltevreden (*n* = 6), and Altona (*n* = 6). *Salmonella* I 4,[5],12:i:- was recovered from a single sample. Sixteen SNP clusters grouped 75 of the isolates. A total of 23 AMR genes were detected overall. At least one AMR gene was carried in 53% of the strains. $bla_{\text{CTX-M-14}}$ was observed in 2 isolates.

Significance: The analyzed isolates have the potential to be pathogenic, posing a public health concern. Given our data, informal markets can be an important source of pathogen contamination for the vegetable value-chain in Cambodia.

P2-183 Genometrakr Database and Network: Lessons on What Can be Done with a Quarter Million *Salmonella* Genomes

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Introduction: A national database of federal, state, academic and international laboratories has been using WGS data to rapidly characterize pathogens. This GenomeTrakr network is part of NCBI Pathogen Detection web site.

Purpose: Public health agencies (FDA, CDC and USDA-FSIS) collect and share data in real time. This high-resolution, rapidly growing database is actively being used in outbreak investigations at state, national, and international levels.

Methods: GenomeTrakr database has demonstrated how a distributed network of desktop WGS sequencers can be used in concert with traditional epidemiology and investigation for source tracking of foodborne pathogens. This "open data" model allows greater transparency between federal/state agencies, industry partners, academia, and international collaborators.

Results: This database has continued to grow and diversify the foodborne pathogen database doubling in the last year to ~500,000 draft genomes with a projected growth of over 1,000,000 draft genomes by the end of 2020. Two new international surveillance efforts were added to collect food, animal and environmental isolates including *Campylobacter* and *Listeria* in Africa. NCBI has release new data analysis tools that improve rapid interpretation. NCBI, currently is producing daily clustering results for 30 bacterial pathogens including: *Salmonella*, *Listeria*, *E. coli, Shigella* and *Campylobacter*.

Significance: The high-resolution WGS signal in concert with epidemiological and inspection evidence has drastically enhanced our ability to identify the food sources of current outbreaks for foodborne pathogens with ~200 regulatory clusters examined in 2019. Results demonstrate global benefits of having an open data model. Understanding root causes of foodborne contamination assists our academic, public health and industry partners to develop preventative controls to make food safer globally.

P2-184 *Campylobacter jejuni* Clonal Complex 45 Isolates Harboring Multiple Resistance Determinants are Largely Restricted to Sequence Type 2109

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Introduction: *Campylobacter jejuni* is a zoonotic pathogen transmitted through contaminated food and water or contact with animals. *C. jejuni* Clonal Complex 45 (CC45) is a major clone associated with multiple animal hosts including chicken and cattle. To date, no large studies have examined antimicrobial resistance among CC45.

Purpose: This study's aim was to examine the distribution of antimicrobial resistance determinants in a collection of sequenced CC45 isolates.

Methods: Sequenced CC45 isolates (*n* = 1,636) were identified from the PulseNet National and PubMLST databases. *De novo* assemblies were generated using Shovill with SPAdes. Reported sequence types (STs) were confirmed using *mlst*. Resistance determinants were detected from assemblies using starAMR. Mutations in the 23S rRNA gene were identified from reads using ARIBA. Mashtree was used to assess isolate similarity.

Results: Isolates were classified into 39 STs using *mlst. Mlst* confirmed reported STs except for 3 isolates that could not be classified. The most common STs observed were ST45 (*n* = 909; 56%), ST137 (*n* = 217; 13%), ST2109 (*n* = 181; 11%), ST583 (*n* = 110; 7%), and ST538 (*n* = 40; 2%). Remaining STs had fewer than 20 representatives. Variants of the β-lactamase encoding bla_{OXA} gene were detected in most isolates (*n* = 1557; 95%) with $bla_{\text{OXA-61}}$ being the most common. Genes encoding tetracycline resistance were also commonly observed (*n* = 524; 32%). Isolates harboring determinants encoding resistance to clinically

relevant drugs such as azithromycin (*n* = 151; 9%) and ciprofloxacin (*n* = 234; 14%) were less frequently observed. The majority of isolates with ≥3 resistance determinants formed a distinct cluster in Mashtree and were classified as ST2109 (159/192; 83%).

Significance: ST2109, previously linked to outbreaks associated with exposure to pet store puppies, often harbors multiple resistance determinants including those encoding resistance to clinically important antimicrobials, a characteristic that does not frequently extend to other CC45 STs including the more common ST45 and ST137.

P2-185 Genomic Characterization and Growth Rates of *B. cereus* Group Isolates from Diverse Sources

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Introduction: The *B. cereus* group can be separated into seven phylogenetic clades, which contain foodborne pathogens as well as agriculturally-beneficial strains that are challenging to differentiate among each other.

Purpose: The purpose of this study was to carry out genomic virulotyping and investigate potential relationships between virulence gene content, phylogenetic grouping, and the growth rate of *B. cereus* group isolates from diverse sources.

Methods: A collection of 122 *B. cereus* group isolates sourced from food, environment, and clinical samples were whole-genome sequenced (WGS). WGS were assembled with SPAdes and analyzed using BTyper to detect virulence genes and classify isolates into phylogenetic clades. Isolates were inoculated into BHI at 10^6 CFU/mL and their growth was monitored for 24 h at 37°C via optical density. Logistic curves were fitted to the optical density data to determine times of entry into the stationary phase.

Results: Of 122 isolates, 119 were classified into one of the seven phylogenetic clades (I=6, II=60, III=4, IV=28, V=4, VI=16, VII=1). All isolates carried at least one of the major diarrheal virulence toxin genes (*hblABC, nheABC, cytK*), and 41 isolates carried at least one of the cereulide synthetase-encoding genes (*ces*). The mean time to stationary phase was 10 ± 3 h. Overall, sixteen (13%; all from clade II) isolates were classified as fast growers (<7 h). Eighty-five (70%) isolates were classified as intermediate growers (7 – 13 h), and 21 (17%) as slow growers (>13 h). There was a significant association between the phylogenetic clade and the growth rate (P = 0.019).

Significance: The findings of this study demonstrate considerable differences in growth rate among *B. cereus* group isolates from different phylogenetic clades. Given that the expression of major diarrheal toxins is regulated by quorum sensing, times at which individual isolates are harvested for cytotoxicity assessment need to be considered.

P2-187 Genomic Characterization of Prophage Encoding Regions in *Cronobacter sakazakii*

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✦ ***Undergraduate Student Award Entrant***

Introduction: The genus *Cronobacter* causes life-threatening infections in individuals of all age groups, and among *Cronobacter* species, *C. sakazakii* is epidemiologically the most prominent pathogen. Prophages are thought to drive bacterial adaptation, ecology, and evolution. Numerous *Enterobacteriaceae* genomes have been sequenced and the role of prophage acquisition, frequency, and prevalence in the genomic plasticity of *Cronobacter* genomes *is* currently not well understood.

Purpose: The purpose of this study was to understand the prevalence and characterization of prophages using whole genome sequence assemblies in *C. sakazakii* strains of food safety interest.

Methods: Sixty-three *C. sakazakii* isolates, obtained from dried plant-associated foods, spices, and filth flies were sequenced using Nextera libraries on an Illumina Miseq platform. *C. sakazakii* assembly data sets were analyzed using webservers like PHASTER (https://phaster.ca), custom bioinformatics tools, and scripts.

Results: Two hundred and twenty-six incomplete and intact prophage encoding regions were found representing 45 different prophages. Among these *C. sakazakii* assemblies, PHASTER analysis identified (i) multiple prophages within single assemblies, (ii) 106 intact prophage encoding regions representing 20 different *Enterobacteriaceae* prophages, and (iii) only three intact *Cronobacter* prophages; phiES15, ENT39118, and ENT47670. To date there have been 25 *Cronobacter* phages identified; PHASTER contains 17 of these. Some of the strains possessed two of the three *Cronobacter* prophages; no strain possessed all three. Inverted repeat sequences (IRS), known prophage insertion sites, showed widespread sequence variations and some were shared among the strains suggesting common re-combinatorial mechanisms. These results have been corroborated by multiple analytical methods. The prevalence and distribution of IRS suggest the occurrence of robust micro-evolution events which may be driving strain-specific adaptation and evolution.

Significance: The prophage diversity observed in this foodborne pathogen correlates with its adaption to a variety of food and environmental niches and together fills gaps in our understanding of the ecology of this organism.

P2-188 Evaluation and Comparison of *Salmonella* Genome-based Serotyping Methods with Bead-based Molecular Serotyping and Traditional Methods for *Salmonella* Isolated from Food and Environmental Samples

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Introduction: *Salmonella* serotyping is essential to surveillance and outbreak investigations, with over 2,600 *Salmonella* serovars identified to date. Since the antisera required for traditional serotyping are difficult to produce and expensive, methods have been developed that target genes encoding the antigens recognized by traditional serotyping following the White-Kauffmann-Le Minor scheme. These include bead-based *Salmonella* molecular serotyping (SMS) methods and genome-based serotyping using publicly available internet tools and whole genome sequencing (WGS) data.

Purpose: To evaluate and compare the genome-based *Salmonella* serotyping tools SeqSero v1 (SS1), SeqSero v2 (SS2) and Salmonella in silico typing resource (SISTR) with SMS and traditional serotyping methods.

Methods: Illumina MiSeq-generated WGS data was downloaded from instruments in real-time or obtained from the public GenomeTrakr depository maintained at the National Center for Biotechnology Information (NCBI). Data was analyzed by SS1, SS2 and SISTR, and results were compared to SMS and traditional serotyping. The WGS data for 475 (83%) isolates from a previous evaluation of SMS on archival isolates from FDA regulatory samples or reference strains were available in GenomeTrakr. The results of 206 additional real-time regulatory isolates, research strains, and serovar reference sequences were also analyzed.

Results: A total of 681 isolates were analyzed, SISTR identified 97.4 % (663) isolates correctly, with 97.6% to a single serovar, SS1 identified 97.2% (662) correctly, with 74.8% to a single serovar, and SMS identified 97.3% (463/476) as expected, with 46.9% to a single serovar. Five isolates from each serovar

and all isolates from serovars that could not be narrowed to a single serovar by SS1 were reanalyzed by SS2 once available. SS2 identified 98.3% (467/475) correctly, with 98.1% to a single serovar. Combined SS1 and SS2 results identified 99.3% (676/681) correctly with 98.5% to a single serovar.

Significance: Genome-based methods provided improved results over SMS and were comparable to traditional serotyping.

P2-189 Identification of Mobile Genetic Elements and Evolutionary Analysis Based on Long-read Sequencing of Listeria monocytogenes in the Food Processing Environment

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Introduction: Whole-genome sequencing (WGS) has been increasingly used for public health surveillance on pathogens in the food industry. Long-read sequencing offers a unique opportunity to identify large mobile genetic elements. Recent advancement in WGS has enabled closing genomes with speed and accuracy.

Purpose: Combining long-read and short-read sequencing technologies, we closed and characterized genomes of *L. monocytogenes* epidemic clone II (ECII) isolates from geographically dispersed food processing facilities and investigated the genetic relationship among isolates from different facilities.

Methods: Seventeen ECII *L. monocytogenes* isolates were obtained from seven meat processing facilities and sequenced using Illumina MiSeq and Oxford Nanopore MinION platforms. Whole genomes were compared to determine single nucleotide polymorphisms (SNPs) and to construct a maximum likelihood phylogeny. Putative prophages were predicted by PHASTER from complete genomes. The presence of putative prophages and plasmids in shotgun genomes was identified. In addition, genes associated with virulence and persistence were also identified in these isolates.

Results: Isolates discovered in the same meat processing facility were clustered together and differed by less than 20 SNPs; those from different facilities differed by 56 to 240 SNPs. A *comK* prophage (~41K bp) was found in 11 isolates from four facilities, and its sequence was conserved among isolates in the same facility, which represented short-term evolution scenarios. *comK* prophages among isolates from different facilities had significant differences, which could be caused by recombination events. Isolates from three facilities had an identical plasmid. A single isolate from another facility had a different plasmid, which was the only plasmid containing benzalkonium chloride resistance genes.

Significance: This study suggests that Nanopore sequencing when combined with MiSeq sequencing, could accurately produce complete genomes and identify mobile genetic elements. Prophages and plasmids were important markers to study *L. monocytogenes* persistence and evolution.

P2-191 Whole Genome Sequence Analysis of *Campylobacter jejuni* and *coli* from Ovine Carcasses in New Zealand

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Introduction: Source attribution analysis of campylobacterisosis in New Zealand (NZ) has been ongoing since 2004. However, data from NZ ovine sources are scarce and therefore it is still unclear to what degree, if any, ovine sources may contribute to the significant health burden observed for the disease.

Purpose: To determine the genotype of *Campylobacter jejuni* and *C. coli* isolated from NZ ovine carcasses.

Methods: N60 excision samples were collected from ovine carcasses from processing plants across NZ. All samples were enriched and screened using polymerase chain reaction for the presence of *C. jejuni* and *C. coli* and isolation was attempted for all screen-positive samples. Whole genome sequencing was performed on a selection of *C. jejuni* and *C. coli* isolates and the data were used for sub-typing using multi-locus sequence typing (MLST) and whole-genome MLST. Antimicrobial resistance genes were also inferred using the genome sequence data.

Results: Twenty-five MLST sequence types (STs) were identified among 44 isolates, including STs (ST42, ST50, ST3222 and ST3072) which have been previously reported to be associated with ruminant sources. Four novel STs were also identified. Whole-genome MLST further discriminated isolates within a single ST type and demonstrated a genetic diversity among the ovine isolates collected. Genes associated with the oxacillinase class of β-lactamase enzymes were identified in 41 out of 44 isolates analyzed.

Significance: This study improves our understanding of *Campylobacter* genotypes that will aid refinement of existing source attribution models to assist in determining any potential contribution from ovine sources to the burden of campylobacteriosis in NZ.

P2-192 Whole Genome Sequencing-based Analyses of *Campylobacter* Isolates from Clinical Samples and Retail Poultry Meats

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Introduction: *Campylobacter* causes approximately1.5 million illnesses annually in the U.S. and majority of cases are caused by unrecognized outbreaks. Typically, human infections are acquired from food of animal origin, including poultry. When treatment is indicated, macrolides and fluoroquinolones are primarily used.

Purpose: Our aim was to characterize genomic relatedness and genetic antimicrobial resistance determinants (gAMRd) in *Campylobacter*isolated from humans and poultry meat sources in Pennsylvania.

Methods: Clinical isolates (N=123) were obtained from patients treated in facilities associated with an academic hospital in southeast Pennsylvania during 2017-2018. *Campylobacter* was isolated from retail meats purchased in the same catchment area and sequenced by National Antimicrobial Resistance Monitoring in collaboration with FDA. Isolates from humans were sequenced on Illumina MiSeq platform. Sequences were assembled using SPAdes 3.13.1 and assemblies examined for quality using Quast 4.6.1. The gAMRd were identified based on the presence of AMR genes and mutations using ResFinder 3.2 and PointFinder 3.1.0, respectively. Isolates' phylogenetic relatedness was characterized using CFSAN SNP pipeline and core genome multi-locus sequencing typing via PubMLST.

Results: Overall, 89% of human isolates and 79% poultry isolates carried gAMRd. No macrolide resistance-conferring mutations in 23S rRNA was detected, and the *erm(B)* gene was present only in human isolates (1.6%). The occurrence of fluoroquinolone resistance-conferring mutations in *gryA* was significantly higher in isolates from human than from poultry (28% vs. 9%, p=0.0049). The occurrence of *tet(O)* gene associated with tetracycline resistance was similar in isolates from human and poultry sources (46% vs. 36%, p=0.22). We identified three clades of closely related *C. jejuni* isolates from both human and poultry sources with ≤72 SNPs difference per clade. One of the clades (MLST ST 50) contained isolates separated by ≤17 SNPs.

Significance: Close genomic relatedness of a clade of isolates suggests a potential epidemiological link that warrants further investigation.

P2-193 Whole Genome Sequencing Analysis of Non-top 7 STEC Serogroups Suggests Novel Serotypes and Relatedness to Human Clinical Isolates

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❖ ***Developing Scientist Entrant***

Introduction: Shiga toxin-producing *E. coli* (STEC), serogroups O157:H7, O26, O45, O103, O111, O121, and O145 are often referred to as the "top seven" STEC and have caused severe illness in humans. Other STEC serogroups, although not frequently causing large-scale outbreaks, still have the potential to cause human illnesses.

Purpose: This study was to apply whole-genome sequencing (WGS) to determine serotype, antimicrobial resistance (AMR), and virulence potential of non-top 7 STEC isolates from cattle.

Methods: A total of 284 isolates of non-top seven STEC from cattle feces were sequenced using MiSeq paired-end sequencing. Trimmed raw reads were mapped to reference sequences from the Center for Genomic Epidemiology database to determine the serotypes, and virulence genes of these isolates. Assemblies were fed into AMRFinder-plus to determine AMR genes and point mutations. When an isolate was untypable, Nanopore long-read sequencing was applied to generate a hybrid assembly using reads from both sequencing platforms to determine corresponding novel O-antigen gene clusters (OAGC). A SNP-based phylogenetic tree was built to examine the relatedness between these isolates and human clinical STEC isolates obtained from NCBI.

Results: Twenty-two serotypes were identified. Two major serotypes, O168:H8 (*n* = 86) and O109:H10 (*n* = 46) constituted more than 40% of the isolates. Three novel serotypes were identified and their OAGCs were characterized. About 80% (*n* = 232) of the isolates carried *stx2* only whereas 14% (*n* = 40) contained *stx1* only. Approximately 4% (*n* = 12) of the isolates carried both. Twenty-seven AMR genes and point mutations were found among these isolates, with *acrF* (*n* = 284), *blaEC* (*n* = 284), *mdtM* (*n* = 276) being present in more than 95% of all isolates. Phylogenetic analysis indicated that some isolates were closely related to clinical isolates.

Significance: WGS is a powerful tool to study microbial pathogens. The results suggest non-top 7 SETC have the potential to cause human illness.

P2-194 Evolutionary Relationship, Virulence and Stress Response Genes in a Persister *S.* Typhimurium PT4 Strain Involved in Foodborne Outbreaks in Brazil

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Introduction: *Salmonella enterica* subsp. *enterica* serovar Typhimurium is one of the major etiological agents of salmonellosis in outbreaks worldwide and has the highest human incidence in the US. Genomic investigation of high-priority foodborne pathogens is a fundamental component of epidemiological surveillance, particularly in major food producing and exporting countries such as Brazil.

Purpose: To investigate the evolutionary relationship, virulence, stress responses and antibiotic resistance genes in a *S.* Typhimurium PT4 (118/01) strain isolated from chicken meat involved in outbreaks in the south of Brazil.

Methods: *S.* Typhimurium PT4 was subjected to whole genome sequencing (WGS) after cultivation by 16 h at 37°C in Trypticase Soy Broth. Libraries were prepared according to Nextera XT protocols and sequenced on the Illumina NextSeq 500 using NextSeq 500/550 High Output Kit v2 (300 cycles). Raw reads were trimmed and assembled *de novo* using SPAdes v3.8.2. Pathogenicity Islands (SPIs) were identified by using SPIFinder 1.0. ResFinder and blastn were used to identify antibiotic and stress response genes, respectively. GARLI v2.01 was used to construct the maximum-likelihood phylogenetic trees using publicly available *S.* Typhimurium isolates recovered from poultry, chicken and human origin in Brazil in the last decades.

Results: The analyses of the WGS showed that *S.* Typhimurium PT4 118/01 has eight SPIs, including SPIs 1-5, SPIs 13-14 and C63PI, the aminoglycoside resistance gene aac(6')-laa and genes *spvABCD, fimA, fimC, fimD, fimH, avrA, csgABDF, csgG, dps, flhD, fliA, invA, motB, ogt, ompR, ompS1* and *hilA* associated with virulence and stress response. Phylogenetic analyses using publicly available *S.* Typhimurium strains indicated the persistence of this strain among sources associated with poultry spanning for more than 16 years and its virulence for humans.

Significance: Findings indicate that survival and virulence abilities of *S.* Typhimurium PT4 118/01 contribute to its persistence in poultry production chain, which impacts public health.

P2-195 AMR Determinants and Virulence Factors in *Salmonella* Typhimurium Isolated from Outbreak Patients and Implicated Foods

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Introduction: *Salmonella* Typhimurium has become one of the major serovars causing foodborne gastroenteritis worldwide. The one-health approach based on genomic diversity is important for understanding the evolutionary relationship of high-priority foodborne pathogens isolated from clinical and distinct environmental sources.

Purpose: To investigate the antimicrobial resistance (AMR) determinants, virulence factors and evolutionary relationship in seven *S.* Typhimurium strains linked to outbreaks in Brazil.

Methods: *S.* Typhimurium strains were subjected to whole genome sequencing on the Illumina NextSeq 500. The resistome, plasmid replicons, multilocus sequence type, serotype, and pathogenicity islands were identified using ResFinder 3.2, PlasmidFinder 2.1, MLST 2.0, SeqSero 1.2, and SPIFinder 1.0 databases. Virulence genes were identified using IslandViewer 4. The GARLI v2.01 was used to construct the maximum-likelihood phylogenetic tree. SNP differences among isolates were calculated using MEGA7 (1,000 bootstrap iterations).

Results: The average genome size of the strains was 4.8-5.1 Mb. The assemblies have between 55 and 71 contigs. All *S.* Typhimurium strains carried aminoglycoside resistance gene aac(6')-laa. One strain carried two extra aminoglycoside resistance genes aph(3")-lb and aph(6)-ld, and *tetB* gene (tetracycline resistance). The plasmids IncHI2 and IncHI2A were identified in one strain and plasmids IncFIB(S) and IncFII(S) in six strains. All *S.* Typhimurium strains have eight SPIs, including SPIs 1-5, SPIs 13-14 and C63PI. Ninety genes associated with virulence were identified in these strains, including *phoP, phoQ, fimA, fimC, fimD, fimF, fimH, fimL, avrA, sipA, sipB, sipC, sopB/sigD, mgtB, mgtC, gogB, pipB2, pipB, sifA, sifB, sseF, sseI/srfH, sseK1, sseK2, invA, invB, invC, invE, invF, invG, invH, hilA, hil, hilD, gtrA, sodCI, ssaC, ssaD, ssaE, ssaG.* The phylogenetic tree indicated persistence of these strains in the poultry production chain in Brazil for more than 18 years.

Poster

Significance: *S.* Typhimurium strains carrying AMR and virulence features can survive and persist in the environment for decades and cause foodborne outbreaks.

P2-196 Application of Metagenomics to Define Microbiomes and Resistomes in Food Manufacturing Facilities and Seafood

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Introduction: Microbial communities consist of diverse bacteria containing various drug resistant mechanisms. Metagenomics possesses the ability to determine the microbiome composition and its resistance potential.

Purpose: This work investigated the microbiomes and resistomes of environmental swab culture enrichments from food manufacturing facilities and imported seafood culture enrichments to assess the dissemination of antibiotic resistance genes in these environments.

Methods: A total of 67 culture enrichments were analyzed using shotgun metagenomic sequencing: 57 environmental swabs from 7 facilities and 10 seafood from 8 commodities originating from 7 countries.

Results: Microbiomes in food manufacturing facilities harbored spoilage-associated taxa such *Carnobacterium* and *Enterococcus* and seafood samples included taxa associated with freshwater and marine environments. Shotgun metagenomic analyses revealed more than 110 antibiotic resistance (AR) genes representing 13 classes across all samples. Tetracycline AR genes were observed in six food manufacturing facilities and all imported seafood, with at least one gene in 85% (6/7) of food manufacturing facilities and 90% (9/10) of seafood enrichments. The most highly abundant genes were *tetM*, *tetS* and *tetW*. Quinolone resistance genes (*qnr* and *oqx* families) were present in 90% (9/10) of seafood culture enrichments and absent in 100% (23/23) of environmental swab culture enrichments from three of the seven manufacturing facilities. Fosfomycin AR genes were ubiquitous in 100% (7/7) of food manufacturing facilities, specifically *fosX,* in facilities that harbor *Listeria monocytogenes*. Environmental swab culture enrichments harboring vancomycin resistance genes, namely *vanXYC*, *vanT*, *vanS*, *vanR,* also harbored *Enterococcus* (56%; 29/51).

Significance: This study provides important information about microbiome and resistome composition and diversity within food manufacturing facilities and imported seafood. Metagenomics holds promise as a tool for facilitating public health management decisions aimed at reducing foodborne exposure to pathogens and AR genes.

P2-197 Microbiome-informed Food Safety and Quality: A Longitudinal and Cross-sectional Survey of Retail Chicken Microbiomes

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❖ ***Developing Scientist Entrant***

Introduction: Microbiological safety and quality of livestock products are of great concern to public health. Chicken microbiomes promise rich information regarding the occurrence and ecology of foodborne pathogens, antibiotic-resistant bacteria, and spoilage organisms associated with broiler production. However, a systematic metagenomics survey of retail chicken products is still missing.

Purpose: The objectives were to 1) characterize longitudinal (three months) and cross-sectional (five brands) variations among retail chicken microbiomes; 2) compare the diversities and abundances of antibiotic resistance genes (ARG) between antibiotic-free and conventional chicken breasts; and 3) compare the performance of *Salmonella* detection between shotgun metagenomics sequencing and culture enrichment.

Methods: Seven retail chicken breast products from five brands were purchased from local groceries every other week for three months. The chicken meats were stored at 4°C until the expiration dates. Each aliquot of 250-g meats was rinsed using buffered peptone water. The rinsates were used for culture-based detection of *Salmonella*. Total DNA was extracted using pellets centrifuged from the rinsates and was subjected to shotgun metagenomics sequencing on a MiSeq platform. Microbial compositions and ARG abundances were analyzed.

Results: Microbiome patterns specific to particular brands and processing plants were identifiable and consistent over time. Vacuum packaging significantly influenced the microbial community ($P < 0.01$, PERMANOVA). Antibiotic-free and conventional products shared similar microbial compositions ($P > 0.05$, PERMANOVA) and ARG abundances ($P > 0.05$, PERMANOVA). Co-occurrence patterns revealed by network analysis suggested important contribution of certain microbial taxa to ARG content ($\rho > 0.8$, $P < 0.01$, Spearman test). *Salmonella* detection results were inconsistent between shotgun metagenomic sequencing and culturing enrichment, with more positive results (19/33 *vs.* 4/33) obtained by sequencing.

Significance: Metagenomics microbiome may indicate the microbial safety and quality of chicken products and can help risk assessment for pathogen contamination and ARG accumulation in poultry production systems.

P2-198 Changes in a Closed Feedlot *Escherchia coli* O157:H7 Population Over 22 Years

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Introduction: Shiga toxin-containing *Escherichia coli* O157:H7 (STEC O157:H7) is a naturally occurring pathogen in beef feedlots. Many epidemiology studies have tracked STEC O157:H7 populations in feedlots but typically for only a short period. To better understand how STEC O157:H7 populations changes over time, we studied the genomes from strains isolated over 22 years.

Purpose: The objective of this study was to characterize genetic variation of STEC O157:H7 in cattle from a closed feedlot over a 22-year period.

Methods: From 1997 to 2019, STEC O157:H7 isolates were collected annually from the same feedlot and stored at -80°C. Up to 10 isolates per year were inoculated in TSB and grown overnight. DNA was extracted, libraries constructed, and sequenced on an Illumina NextSeq sequencing platform. Sequencing reads were assembled with SPAdes. Contigs from each isolate were typed for Shiga toxin subtype and *tir* (translocated intimin receptor) A/T variant and used to construct a phylogenetic tree with Parsnp along with previously sequenced strains for a total of 191 strains.

Results: Phylogenetic analysis classified the strains into four clades that associated with *tir* A/T alleles and Shiga toxin subtypes. One clade had multiple Shiga toxin subtypes when compared to the three other more homologous clades. All four of the clades were present in strains isolated from 1997 through 2010 although not necessarily every year. Outside of one strain from a different clade, only strains from one of the four clades were isolated from 2011 to 2019.

Significance: These data provide a first glimpse into the ecology of STEC O157:H7 in a natural environment and the evolutionary changes that have occurred over 22 years as it has adapted to this environment. Additionally, these data provide valuable information about strain relatedness that will help improve foodborne outbreak tracking.

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P2-199 High Prevalence and Genomic Characteristics of Multi-drug Resistant Extended-spectrum β-Lactamase-producing *Escherichia coli* in Feral Swine

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Introduction: Due to the potential role of wildlife as reservoirs and sources of antibiotic resistant pathogens, an increasing number of studies are focusing on antimicrobial resistance in wildlife. However, the genomic characteristics of antimicrobial resistant bacteria (ARB) in wildlife is largely unknown.

Purpose: The purpose of this study was using whole genome sequencing and comparative genomics to investigate the genomic characteristics of ARB in wildlife.

Methods: We collected 224 fecal sample from feral swine and cefotaxime-resistant bacteria (CRB) were isolated by plating on MacConkey agar containing cefotaxime (4 µg/mL). By using a polymerase chain reaction (PCR) method, isolates carrying either CTX-M or CMY-2 gene were selected for whole genome sequencing to characterize extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase-producing *E. coli*. Antibiotic susceptibility test (AST) against 13 different antibiotics were conducted to determine the multi-drug resistance of these isolates.

Results: Of 224 feral swine fecal samples, 84 samples contained CRB corresponding to the prevalence as 37.5%. Ninety isolates carrying either CTX-M or CMY-2 genes were conducted for whole genome sequencing. Phylogenetic analysis revealed that these strains clustered into 15 clades that coincided with their sequencing types (STs). Twenty-four representative strains were selected based on whole genome architecture for further comparative genomics analysis and antimicrobial susceptibility test. All the representative isolates were multi-drug resistant and carried a variety of virulence genes. Out of 24 strains, 16 isolates contained the same conjugative IncR plasmid. In addition, the same insertion element, *IS5* and *IS1380,* were found near CTX-M-1 and CMY-2 genes, respectively, which indicated that the plasmids were transferred among these multi-drug resistant *E. coli* isolates.

Significance: Our results provide critical knowledge to better understand the prevalence and genomic characteristics of these multi-drug resistant *E. coli* in feral swine that may serve as reservoir.

P2-200 Gut Microbiota in Beef Cattle and Its Association with Antimicrobial Resistance

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Introduction: Antimicrobial resistance is a global threat to public health. Our previous study found that gut microbiota of beef cattle carries antimicrobial resistant microorganisms (ARMs), even though cattle were raised without antibiotics.

Purpose: The purpose of this study was to understand the dynamics of ARMs in the lifespan of cattle and its relationship with the development of gut microbiota.

Methods: We collected fecal samples from one generation of beef cattle (n = 278) belonging to the Angus-Brahman multibreed herd every three months throughout their lifespan, and analyzed the associations between changes in gut microbiota structure and the prevalence and concentration of cefotaxime (a third-generation cephalosporin antibiotic) resistant bacteria (CRB) in the gut.

Results: The CRB prevalence fluctuated from 10% to 40% when calves were kept on pasture, while the CRB concentration was decreased after birth (P < 0.05). However, when calves were relocated to the feedlot, both the CRB prevalence and concentration were dramatically increased. The major CRB isolated in the herd were *Pseudomonas*, *Acinetobacter* and *Escherichia-Shigella*, which all belong to Proteobacteria. Notably, the bacterial diversity of gut microbiota was gradually increased across lifespan when calves were kept on pasture, but significantly decreased when calves were moved to the feedlot (P < 0.05). The Proteobacteria accounted for 50% of total meconium bacteria after birth, and decreased to 6% after three months of birth, and maintained at 2% afterwards when calves were on pasture, but increased to 6% in feedlot. Moreover, we identified several genera that belong to Firmicutes with their proportions were negatively associated with the relative abundance of *Pseudomonas*, *Acinetobacter* and *Escherichia-Shigella* in the gut microbial community (P < 0.05).

Significance: Our study indicates a role of gut microbiota in regulation of ARMs, shedding light in controlling ARMs by shaping the structure of gut microbiota.

P2-201 Genomic Characterization of a Subset of *Listeria monocytogenes* Isolates from Fresh Produce Packing Facilities in California

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Introduction: Whole Genome Sequencing (WGS) is a powerful typing tool that is transforming the way foodborne bacteria are identified and characterized. WGS has allowed precise subtyping of *L. monocytogenes* opening up novel insights to diversity, routes of dispersal, and regional distribution in produce environments.

Purpose: Apply two approaches of WGS analysis, core genome MLST (cgMLST) and single nucleotide polymorphism (SNP), to increase understanding of *L. monocytogenes* diversity recovered from fresh packing environments.

Methods: A subset of 174 *L. monocytogenes* isolates, from a library of >700 collected during a five-year survey, representing geographically diverse tree fruit packing operations were selected for WGS. Samples were prepared for a 2x150-bp paired-end sequencing aiming for minimum 80X coverage. *De novo* assembled genomes were obtained using SPAdes assembler. cgMLST profiles were extracted using the BIGSdb-*Lm* platform and minimum span trees (MST) were generated. *In silico* MLST was performed and clonal complexes (CCs) were assigned on the basis of the definition of the Pasteur MLST database. Filtered contigs were used for SNP calling using *Geneious Prime 2020.0.* Maximum likelihood phylogenetic trees were inferred with RAxML v8.2.11.

Results: Analyzed genomes represented lineages I, II and III, including 37 clonal complexes identified by *in silico* MLST. Among the 174 isolates cgMLST identified 142 cgMLST types (CT). Despite the high diversity observed, several isolates from different facilities belong to same CT with only ≤10 different alleles. Some of the cgMLST complexes identified contained strains recovered in different years (persistence) and from different facilities (predominant). Isolates from *in silico* MLST complex cluster1 (CC1) differed between 6 to 640 SNPs. The choice of reference genome for SNP analysis was restricted by cooperator requirements and significantly influenced the outcome.

Significance: WGS analysis provides information to understand the ecology and distribution of *L. monocytogenes* in fresh food-associated environments. WGS can provide useful for source-tracking and accurate assessment of persistent strains.

P2-203 Phylogenomic Characterization of *Cronobacter* Species Isolated from Fresh Produce, Frozen Vegetables, and Farm Environments in the Czech Republic

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Introduction: *Cronobacter* species are opportunistic pathogens associated with life-threatening infections. Surveillance studies have shown that *Cronobacter* continues to be isolated from various types of ready-to-eat vegetables and fruits, sprouts, herbs, flours, and environments, which poses a significant risk to susceptible consumers. However, relatively little information of genomic characteristics and virulence factors of *Cronobacter* obtained from plant-origin foods is available.

Purpose: This study aimed to understand antimicrobial resistance, presence of plasmids and prophage sequences, and phylogeny of plant-associated *Cronobacter* strains using molecular and whole genome sequencing analyses.

Methods: Thirty-two *Cronobacter* isolates obtained from fresh produce (lettuce, cucumber, carrot, sprouts, herbs), frozen vegetables, and farm environments (irrigation water, gloves) in the Czech Republic (CR) were characterized using PCR and whole genome sequencing (WGS) analyses. WGS was conducted using Illumina's MiSeq platform with Nextera XT library kit. Multi-locus sequence typing (MLST), antimicrobial resistance (AMR) genes, and phage genomic regions were identified using CFSAN's GalaxyTrakr and PHASTER bioinformatic tools.

Results: PCR analysis showed that the strains possessed both the virulence and the heavy metal resistance plasmids; 94% positive for pESA3/pCTU1 (IncF1B) and 47% for pCTU3 (IncH1). Phylogenetic analysis showed that the strains clustered according to four different species of *Cronobacter* (*C. sakazakii, C. turicensis, C. malonaticus, and C. dublinensis*) and according to sequence type (ST) including clinically-relevant ST1, ST8, ST13, and malonate-positive ST64 strains. Antimicrobial susceptibilities of the strains were analyzed using the AMRFinder tool, which showed potential resistance to cephalothin, fosfomycin, and colistin. Additionally, PHASTER bioinformatic analysis of the strains identified 61 intact phage genomic regions including 15 different phages of *Aeromonas*, *Cronobacter*, *Edwardsiella*, *Enterobacter*, *Escherichia*, *Haemophilus*, and *Salmonella* among the 32 plant-associated strains.

Significance: This study provides in-depth genomic features of plant-associated *Cronobacter*, which is helpful for understanding how the genetic characteristics can link to molecular interaction with plant, a potential host for this pathogen.

P2-204 *Salmonella* Survival and Transcriptomic Response on Cantaloupe Flesh With and Without Organic Acid Pretreatment

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks in recent years have been associated with *Salmonella enterica* contamination of melons, including cantaloupe. It is known that *Salmonella* can survive on non-treated cut cantaloupe flesh, however, less information is available on how the pathogen survives.

Purpose: To examine the survival and gene regulation of *Salmonella* on fresh-cut cantaloupes with and without malic acid pretreatment.

Methods: Cut cantaloupe flesh was pretreated by submersion into 2% w/v malic acid for 1 min or left untreated. Flesh was aliquoted into 100-g portions in deli-style containers and spot-inoculated with *Salmonella* Typhimurium at 7 log CFU/g. Samples were air dried for 1 h then stored at 4°C for 7 d. Enumeration of *Salmonella* was conducted by homogenization of the flesh with BPB and cultivation on TSA with XLD overlay. Three independent trials were conducted. Data were analyzed by Student's *t*-test, $P \leq 0.05$. Samples for sequencing were prepared using the TruSeq Stranded mRNA kit and run on a MiSeq.

Results: The initial population of *Salmonella* on the cantaloupe flesh was 6.50 ± 0.31 log CFU/g. After 7 d, the pathogen population on the untreated cantaloupe was 6.85 ± 0.15 log CFU/g, whereas it was significantly lower on the treated cantaloupe (6.11 ± 0.24 log CFU/g). After 1 d the most upregulated genes in *Salmonella* on the treated cantaloupe compared to untreated were those that encoded starvation-inducible proteins including *phoH* and *csiD* (upregulated 207 and 167-fold, respectively). Other notable upregulated genes (>100-fold) included those encoding ABC transporters and associated proteins (*sitB*, *sitC*) and metabolic functions (*ygjG*, *zntA*, *acs*, *melA*, and *nagA*).

Significance: The results of this study can aid in understanding how *Salmonella* survives on cantaloupe flesh with a malic acid pretreatment.

P2-205 The Effect of Sequential Antimicrobial Treatments on *Listeria* Biofilm-forming Ability and Survival

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Introduction: *Listeria* has the ability to adapt and proliferate in a variety of environments. This pathogen has shown niche adaptation and its ability to form biofilm is a hurdle for food safety. After the biofilm formed, it becomes difficult to disrupt. Chemical sanitizers combined with non-thermal processing technologies might represent an effective way to control *Listeria* biofilm formation.

Purpose: The objective of this research was to understand the effect of several chemical sanitizers alone or in combination with UV light on the transcriptional and phenotypic response of *Listeria* biofilm forming ability and survival.

Methods: A CDC Reactor was used to grow 4-day-old, multi-strain *Listeria* biofilms on stainless steel coupons. Biofilm survivability was investigated after 10 min exposure to lactic acid (4%), peracid (200 ppm), quaternary-ammonium (400 ppm) alone or in combination with UV light (254 nm) for 15 or 30 min. Sequential treatments effect was also evaluated. Biofilm RNA was extracted and submitted to short-read sequencing. Comparison among gene expression and treatments were performed using CLC Genomic workbench v.12. Experiments were run in triplicate.

Results: *Listeria* biofilms reached 6 log CFU/cm^2 after 4 days. When *Listeria* biofilms were exposed to peracid, a reduction of 4.2 log CFU/cm^2 ($P < 0.05$) was observed. A maximum reduction of 1.8 log CFU/cm^2 ($P < 0.05$) was recorded after UV treatment for 30 min. The sequence of antimicrobial treatments was significant only when UV was preceded by lactic acid ($P < 0.05$), while no difference was observed for peracid and quaternary ammonium. A total of 430 genes were differentially expressed ($P < 0.05$) after treatments. Among those, 115 were upregulated in categories responsible for protein fate, cellular processes and energy metabolism.

Significance: The results obtained in this research offer a preliminary understanding of *Listeria* biofilm response to chemical sanitizers and support the development of effective intervention strategies to control this pathogen in food processing environment.

P2-206 Metagenomic Analysis of Refrigerated Products Treated with High-pressure Process and Natural Antimicrobials

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Introduction: Two refrigerated ready-to-eat model meals (chipotle black bean dip and grilled corn esquites) were treated using High Pressure Process (HPP) for extended refrigerated shelf life. Since HPP is ineffective against spores, both cultured celery juice powder (CCJP) and Nisaplin (NIS) were added to control Gram positive spores, specifically *Clostridium* and *Bacillus*, respectively.

Purpose: The purpose of this study was to understand the shifts in microbial populations and investigate the combined effect of different hurdles (pH, antimicrobials, HPP and refrigeration) during shelf life.

Methods: Microbial populations were monitored using APC, psychrotrophic APC and lactic acid bacteria, collecting six samples from duplicate meals from each variable. 16S rRNA metagenomic analysis was performed on the microbial communities extracted from one meal from each variable to identify specific species of bacteria.

Results: Control (no HPP and no antimicrobials) bean dip (pH 4.8) spoiled at 28 days and *Leuconostoc* was the prevailing spoilage organism. HPP treatment in presence or absence of natural antimicrobials (CCJP and NIS) extended the shelf life of the bean dip to 70 days and showed that the composition of the microbial community stayed constant and *Leuconostoc* remained low. Control samples of grilled corn esquites (pH 6.4) had mixed populations of bacteria when spoiled (day 28), including *Leuconostoc*, *Serratia* and *Pseudomonas*. HPP treatment alone was not enough to retard spoilage. HPP plus natural antimicrobials extended the shelf life, changing the time to spoilage to 70 days and changed the relative percentages of different spoilage bacteria at time of spoilage.

Significance: This study showed the benefits of using natural antimicrobials in HPP refrigerated RTE model meals influencing microbial populations and spoilage. DNA sequences of *Bacillus* and *Clostridium* were either not detected or were detected at very low levels; thus, neither showed signs of growth during this study in either matrix.

P2-207 Organic Amendments Alter Soil Microbiome: Implications for Produce Microbial Safety

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Introduction: Organic soil amendments are applied to soil to provide crops with nutrients and improve water infiltration and water-holding capacities in the soil.

Purpose: This study aimed to assess the effect of applying various organic soil amendments on soil microbiome.

Methods: Three organic fertilizers (chicken manure, cow manure, or milogranite) were applied at four levels (control: 0 kg/ha, low: 168 kg/ha, medium: 336 kg/ha, high: 672 kg/ha) in soil. Collard greens were grown in the treated soils in open farm 1.5×3.0 m plots. Six soil samples were collected from each plot. DNA was then extracted and V4 region of bacterial 16S rRNA gene was amplified and sequenced.

Results: At family level, chicken manure and milogranite were associated with significant enrichments in *Bacillaceae*, *Chitinophagaceae*, *Rhizobiaceae*, whereas cow manure resulted in significantly higher abundance of *Anaerolinaceae* and *Hyphomicrobiaceae* and a depletion in *Bacillaceae* compared to untreated control soil ($P \leq 0.05$). *Burkholderiaceae* was enriched in milogranite treated soils, and *Enterobacteriaceae* abundance was higher in both milogranite and chicken manure treated soils ($P \leq 0.05$). *Bulkholderiaceae* and *Enterobacteriaceae* were significantly depleted with cow manure ($P \leq 0.05$). At genus level, *Escherichia/Shigella* abundance was significantly higher in soils treated with cow manure and milogranite ($P \leq 0.05$). *Burkholderia_Caballeronia_Paraburkholderia* was enriched with milogranite but depleted in cow manure treated soil ($P \leq 0.05$).

Significance: While organic amendments improve soil microbial health, some potential bacterial pathogens may enrich in the amended soils. This might increase the risk of transfer of bacterial pathogens to produce.

P2-208 Biomarker Identification from Next-generation Sequencing Data Using Bioinformatics Analysis

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Introduction: The technology of next-generation sequencing (NGS) is advancing rapidly and it is being widely applied in pathogen detection and surveillance. With the increasing of the size of the data from clinical and public health laboratory, data analysis and interpretation are becoming challenging.

Purpose: The purpose was to develop an analytical pipeline for specific gene analysis and biomarker discovery from next generation sequencing (NGS) data for pathogen characterization and surveillance.

Methods: An NGS data set of 323 *Salmonella* isolates was retrieved from National Center for Biotechnology Information (NCBI) database, as well as a dataset of *fliC* gene reference sequences of 24 *Salmonella enterica* strains of 13 serotypes. Random Forest (RF) and Supporting Vector Machine (SVM) algorithms were applied on pre-processed NGS data. Topic modeling was used to reduce data dimension and feature selection.

Results: An analytical pipeline was developed, and SNPs were generated using our previously developed framework. RF and SVM algorithms were then applied on both the SNPs dataset the SNPs corpus to predict the serotypes of *Salmonella*. High prediction (more than 98%) accuracy and specificity were obtained in both datasets, with even better performance in the SNPs corpus.

Significance: The pipeline developed provides an effective bioinformatics tool for genetic diversity clarification and marker sequences discovery for pathogen characterization and surveillance. The implementation of topic modeling and other machine learning algorithms provides a new way in food safety data analysis.

P2-209 Utilization of Metagenomics for Evaluation of Three Enrichment Procedures for Detection and Isolation of *E. coli* O157:H7 in Mung Bean Sprout Irrigation Water

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Introduction: Spent sprout irrigation water (SSIW) has been tested in compliance programs for detection of *E. coli* O157:H7 in sprouts. However, detection and isolation by standard cultural methods can be difficult due to the high background microflora associated with this matrix.

Purpose: This study used enriched SSIW microbiomes to evaluate three procedures for rapid detection and isolation of *E. coli* O157:H7 from artificially contaminated mung bean SSIW samples.

Methods: Mung bean SSIW inoculated with *E. coli* O157:H7 at low (0.075 cfu/mL) and high (0.75 cfu/mL) levels, was stored at 4°C for 72 h. Three enrichment procedures were used: (1) FDA BAM procedure using static incubation in mBPWp media for 5 h at 37°C, followed by addition of acriflavine (A), cefsu-

Iodin (C), and vancomycin (V) and then further incubation with no shaking at 42ºC to enhance selectivity; (2) mBPWp with CV at 42°C with shaking, and (3) mBPWp with CV at 42°C without shaking. SSIW prior to enrichment along with three biological replicate samples at each inoculum level for the three methods were collected for shotgun metagenomic analysis after enrichment. Sequencing was accomplished using Illumina technology and a k-mer based method was used for bacterial taxa identification.

Results: The relative abundance of *E. coli* O157:H7 at the 24 h enrichment time point for both the low and high inoculum was found to be between 15 and 30 % for test conditions (2) and (3) but less than 3 % for test condition (1). Both modifications to the BAM procedure resulted in lower levels of *Enterobacter* and *Pseudomonas* in the SSIW microbiomes, along with lesser relative abundance differences in other taxa among the enrichment procedures.

Significance: This study demonstrates how metagenomics provides an additional tool for enrichment method development studies that can be used to improve current cultural methods used for pathogen detection in difficult matrices.

P2-210 Current Processing Practices are Ineffective for Removing Residual Silver Nanoparticles from Contaminated Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Concentrations of silver nanoparticles (AgNPs) in agroecosystems are likely to increase, primarily because of their use in nanopesticides and land application of sewage sludge. Hence, AgNP exposure to fresh produce may increase their residual levels, thus posing a food safety concern. The effectiveness of produce processing practices in removing AgNPs from fresh produce is currently unknown.

Purpose: This study aimed to evaluate the effectiveness of removing AgNPs from contaminated romaine lettuce by commercial sanitizers commonly used in produce industry.

Methods: Romaine lettuce was immersed in a 40 mg/L AgNP suspension and shaken for 1 h to contaminate the leaves. Four-liter glass carboy jars with spigots were used to assess decontamination effectiveness. A chlorine-based sanitizer adjusted to pH 6.5 – 7.0 containing 100 mg/L free chlorine (XY-12, Ecolab) was used as the wash water treatment with deionized water serving as the control. After inserting 25 g of AgNP-contaminated romaine lettuce into the carboy, 10-ml water samples were collected through the opened spigot at 30-sec intervals for 5 min. AgNPs concentrations were then determined in the water and leaf samples using inductively coupled plasma mass spectrometry (ICP-MS).

Results: Ag concentrations during washing in water alone increased from 18.5 mg/L at 30 sec to 51 mg/L after 5 min but remained at 10 – 15 mg/L in water containing 100 mg/L free chlorine.

Significance: This chlorine-based sanitizer is commonly used in commercial processing of fresh-cut produce removed AgNPs from contaminated romaine lettuce to some extent. However, water alone was more efficient in removing AgNPs from Romaine lettuce leaves compared to chlorine. Finding optimal wash water conditions to decrease AgNP levels in contaminated fresh produce is important for improving end-product safety.

P2-211 The Molecular Mechanisms of Nonthermal Plasma (NTP) Induced Viable but Nonculturable (VBNC) *Staphylococcus aureus*

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Introduction: In recent years, nonthermal plasma (NTP) technology has received a lot of attention as a promising alternative to thermal pasteurization in the food industry, while little is known about the microbial stress response (e.g., viable but nonculturable-VBNC) towards NTP, which could threaten food safety and impede the development of NTP.

Purpose: The aim of this study is to investigate the molecular mechanisms of VBNC *Staphylococcus aureus* induced by NTP.

Methods: *S. aureus* was exposed to NTP treatment with various applied energies and subsequently stored at 4°C for various times. The propidium monoazide quantitative polymerase chain reaction (PMA-qPCR) combined plate count method was applied to monitor the total viable and culturable *S. aureus* to obtain the amount of VBNC cells induced by NTP. Transcriptomics (RNA-seq) was further used to reveal the profile of differentially expression genes (DEGs) in NTP-induced VBNC *S. aureus* compared with the culturable counterparts.

Results: After NTP treatment with 8.1-24.3 KJ, all viable *S. aureus* was observed to form a VBNC state at a level of 7.4 to 7.6 log CFU/mL when stored at 4ºC for 8-72 h. Transcriptomic analysis revealed a change in the energy allocation of VBNC *S. aureus*, with most energy-dependent physiological activities (e.g., metabolism) being arrested but the oxidative stress response-related genes (*katA*, *dps*, *msrB*, *msrA*, *trxA*) being up-regulated. Additionally, ATP depletion by carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) accelerated the formation of VBNC *S. aureus*. The oxidative stress produced by NTP triggered staphylococcal antioxidative response that consumed a certain amount of cellular energy (ATP) to reduce other energy-dependent activities (cell growth and division), subsequently causing the formation of VBNC cells.

Significance: This study provides better knowledge for controlling the occurrence of VBNC *S. aureus* during NTP treatment, which leads to more rational design of NTP processing and assures food safety.

P2-212 Deactivation of *Aspergillus flavus* Spores and the Fungal Toxin Deoxynivalenol Using High Voltage Atmospheric Cold Plasma

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Introduction: Fungal spores and mycotoxins are major drivers of food spoilage and safety concerns in processing, storage, and distribution. Current methods to eliminate contamination require harsh treatments that alter flavor, nutrients, and texture. High voltage atmospheric cold plasma (HVACP) benefits from short treatments, ambient temperatures, and little to no alteration of food substrates.

Purpose: The purpose of this study was to evaluate HVACP as a treatment to deactivate *Aspergillus flavus* spores and the mycotoxin deoxynivalenol.

Methods: Sporulated *Aspergillus flavus* cultures (n = 3/group) were treated with Dielectric Barrier Discharge HVACP in high-barrier film sealed boxes flushed with dry air at 85 kV for 0, 1, 2, 5, and 10 minutes at room temperature and stored for 24 hours at 4°C for maximum treatment effect. Spores were isolated and viability was assayed using the Live/Dead viability assay. Spores were enumerated using an epifluorescence microscope. Deoxynivalenol was treated at 0, 5, 10, 15, 20, and 100 µM (n = 3/group) as solid powder or aqueous solution. Treated and non-treated toxins in PBS were assayed for cytotoxicity to Caco-2 cells using MTT cell viability assay. All assays were repeated in triplicate. Significance was determined by one-way ANOVA analysis using GraphPad Prism version 6.

Results: HVACP treatments significantly reduced spore viability after 1-minute treatments compared to no treatment groups (46.85% vs. Control, $P < 0.005$), with greater reductions in viability following extended treatments: 23.86% (2-min), 13.47% (5-min) and 4.54% (10-min). Aqueous toxin groups no longer demonstrated significant cytotoxicity in cell culture after 20 minutes of HVACP treatment compared to control groups.

Significance: This study demonstrates the use of HVACP in the deactivation of a significant food spoilage fungi in culture, as well as one of the most common fungal toxins. This treatment confers a benefit over existing technologies in food protection that rely on destructive heat treatments.

P2-213 Microbial Load Reduction in Chia (*Salvia hispanica L.*) Seeds with High Intensity Light Pulses and Lemongrass (*Cymbopogon citratus)* Essential Oil in Vapor Phase

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Introduction: Chia seeds (*Salvia hispanica* L) provide an excellent environment for microbial growth; pH, aerobic conditions, and temperatures contribute to a rapid expansion as high as 10^9 CFU/g have been reported in chia seeds obtained from retail stores, with the probability to find pathogens.

Purpose: To evaluate the microbial load reduction in Chia seeds (*Salvia hispanica L.*) with the combination of high intensity light pulses (HILP) and *Cymbopogon citratus* essential oil in vapor phase (VP).

Methods: A total of 30 samples of chia were obtained from local stores in Puebla, Mexico and aerobic mesophilic bacteria (AMB), molds and yeasts (MY) were evaluated. Chia samples were processed with HILP treatment at 12 s with 16.20 J/cm^2 of energy, the AMB and MY were determined after the process. HILP samples were treated with lemongrass essential oil in vapor phase at concentrations of 227, 454, 681 and 909 mL/L(air) for 24 h.

Results: Chia AMB load was 5.6 log (CFU/g) and 4 log (CFU/g) for MY. HILP was effective to reduce AMB up to 2 log cycles; however, MY only reach 1 log cycle reduction. When lemongrass essential oil was applied in vapor phase for 24 h a remarkable reduction was determined for both parameters (2 to 4 log cycle reduction for AMB and MY, respectively)

Significance: These results suggest HILP and vapor phase combination are suitable for the reduction of chia seed microbial load.

P3-02 Reduction of *Escherichia coli* O157:H7 Contamination of Romaine Lettuce by Switchgrass Extractives

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7 outbreaks involving leafy greens have been on the rise. Extractives of switchgrass, *Panicum virgatum,* have antimicrobial activity correlated to high phenolic concentration.

Purpose: The objective of this study was to determine the antimicrobial effects of switchgrass extractives (SE) *in vitro* and *in planta* against *E. coli* O157:H7 on romaine lettuce.

Methods: For *in vitro* experiments, washed lettuce leaves were treated under ultraviolet light for 10 min, inoculated with rifampicin-resistant *E. coli* O157:H7 and treated with sterile deionized water, 0.6% sodium hypochlorite, or SE for 1 to 30 min. Bacteria were recovered, ten-fold serially diluted in phosphate buffered saline, surface spread plated on Tryptic Soy Agar (TSA) plates in duplicate and incubated at 37°C for 24 to 48 h. Each experiment was replicated thrice. For the *in planta* study, lettuce leaves on plants in the greenhouse were inoculated with rif-resistant *E. coli* O157 by swabbing demarcated leaves either prior to (pre-treatment) or after application (post-treatment) of SE (at 0, 24 and 48 h). Bacteria from inoculated control and treated leaves were recovered and enumerated as described above. Data were statistically analyzed using mixed model analysis of variance and Tukey's adjustment to determine differences between treatments and effect of treatment times.

Results: Lettuce treated with SE *in vitro* showed time-dependent reduction of *E. coli* O157:H7 after 1, 3, 10, and 30 min, with reduction ranging from 3.24 to 6.16 log CFU ($P < 0.05$), compared to ~6-log reduction with 0.6% sodium hypochlorite. Lettuce *in planta* showed 1.88 log reduction after 24 h SE pre-treatment, with significant 3.88-log CFU/g reduction ($P < 0.05$) after 0 h post-treatment.

Significance: These results indicate that SE has potential as a sustainable solution for washing lettuce prior to consumption, at pre-harvest and post-harvest. However, further research is needed to develop and optimize this technology.

P3-03 Hemp Extractives to Control *Escherichia coli* O157:H7 and *Salmonella* Typhimurium Populations on Formica Coupons

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Introduction: The extractives from industrial hemp (*Cannabis sativa* L.) stems have unexplored potential for value-added applications in food and agricultural systems, including use as natural antimicrobials. Ethanol extracts of these stems were determined to be rich in mono-phenols and cannabidiolic acid that exhibit antibacterial properties. As *Escherichia coli* O157:H7 and *Salmonella enterica* outbreaks continue to occur, methods to prevent cross-contamination from food-contact surfaces are being researched.

Purpose: The goal of this research was to determine the ability of hemp ethanol extractives (HE) to decrease rifampicin-resistant *Escherichia coli* O157:H7 and *Salmonella* Typhimurium LT2 populations on Formica coupons.

Methods: Sterile Formica coupons were aseptically inoculated with overnight cultures of *E. coli* O157:H7 or *S.* Typhimurium LT2, air-dried, and treated with HE containing 37% ethanol, 37% ethanol alone, or phosphate buffered saline (PBS) for 15, 30 and 60 min. The treatments were stopped using Dey-Engley's Neutralizing Broth, ten-fold serially diluted in PBS and surface-spread plated on Tryptic Soy Agar plates. Bacteria were enumerated after incubation at 37°C for 24 to 48 h. The experiments were replicated thrice and data were statistically analyzed ($P < 0.05$). Scanning electron microscopy (SEM) was used to determine cell-structure conformation.

Results: HE decreased *E. coli* O157:H7 by 0.91±0.44 to 1.04±0.48 log CFU/mL after 30 to 60 min, while 37% ethanol caused 0.11±0.42 log CFU/mL reduction after 60 min. HE also decreased *S.* Typhimurium LT2 by 0.85±0.60 to 0.94±0.38 log CFU/mL after 15 to 30 min, with increased reduction of 2.1±1.30 log CFU/mL after 60 min, while 37% ethanol showed no reduction even after 60 min. SEM images of HE-treated bacteria showed morphological changes compared to intact structures of control and 37% ethanol-treated cells.

Significance: HE show modest potential to decrease bacterial populations on Formica coupons by altering cell structure. Further studies are warranted using combinations of HE and other antimicrobials for improved activity.

P3-04 Reduction of Aichi Virus in Ozonated Water

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✦ ***Undergraduate Student Award Entrant***

Introduction: Aichi virus is a non-enveloped, single-stranded RNA virus that causes human gastroenteritis worldwide. Improved decontamination methods and prevention of cross-contamination during shell-fish harvesting and handling are needed. Ozonation is being widely researched as an alternate to chlorine for microbial inactivation.

Purpose: The objective of this research was to determine the ability of a small ozone-generating device to inactivate Aichi virus in water for potential use in small to mid-size farms and during shellfish-depuration.

Methods: Aichi virus at 5 log plaque forming units (PFU)/ml in sterile de-ionized water was treated with the ozone generating device for 0 to 20 min at room temperature. Viruses were recovered, ten-fold serially diluted and plaque assayed in duplicate using confluent Vero host-cells in 6-well plates. Similarly, 5 log PFU/mL Aichi virus was added to artificially contaminated water containing organic load and treated for up to 20 min, and assayed. Each experiment was replicated thrice and data were statistically analyzed using mixed model analysis of variance and Tukey's adjustment ($P < 0.05$).

Results: Aichi virus at ~5 log PFU/ml showed time-dependent decreases of 1.16, 1.50, 1.94, 2.94, 3.53 and 4.37 log PFU/mL after treatments for 1, 2, 5, 10, 15 and 20 min, respectively, with the ozone-generating device (average ~1.7 ppm ozone, pH ~6.5). Water artificially contaminated with organic load to mimic real world scenarios showed only 1.18, 1.68 and 2.03 log PFU/mL reduction after 10, 15 and 20 min, respectively. Thus, prior filtration of contaminated water may be needed before treatment with this ozone-generating system for optimal viral reduction.

Significance: The small ozone-generating system shows promise for application to decrease Aichi virus in contaminated water, albeit after initial purification that decreases organic load. The device also shows potential for use in small to mid-size farms to potentially decrease the risk of Aichi virus outbreaks.

P3-06 Substantial Thermal Stability of *Escherichia* phage OSYSP and Potential Use in Combined Phage-thermal Treatment Against Shiga Toxin-producing *Escherichia coli*

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Introduction: Lytic bacteriophages are promising biocontrol agents against pathogenic bacteria for food safety and therapeutic applications; however, their potential benefits have not been fully explored.

Purpose: This study was conducted to compare the thermal inactivation kinetics of a bacteriophage and its host in a systematic manner using the first order inactivation model.

Methods: Sterile glass vials were filled with 4 mL of pure bacteriophage OSYSP or *Escherichia coli* O157:H7 EDL933 suspensions in buffered peptone water. For each treatment, a sample holder including 5 vials of phage or bacterial suspensions was positioned to the center of temperature-controlled circulating water bath. Heat-treated sample vials were removed from the water bath at specified time intervals up to 8 h, and immediately submerged in an ice water bath for rapid cooling. Following treatments, bacteriophage OSYSP and *E. coli* O157:H7 EDL933 viable populations were determined using the double layer plaque assay and plate count method, respectively.

Results: Bacteriophage OSYSP had a remarkable heat resistance ($P < 0.05$), compared to host *E. coli* O157:H7 EDL933. The time required to reduce the 90% (D-value) of phage OSYSP PFU/mL and *E. coli* O157:H7 EDL933 CFU/mL were 166.7 and 7.3 min at 55°C, 44.4 and 0.33 min at 60°C, respectively. *E. coli* O157:H7 EDL933 (z-value 3.7°C) was significantly ($P < 0.05$) more sensitive to temperature changes compared to phage OSYSP (z-value 7.7°C) under the same experimental conditions.

Significance: Demonstration of a substantial thermal stability of bacteriophage OSYSP over *E. coli* O157:H7 EDL933 suggests that heat treatments could be used for preferential selection of phage OSYSP from *E. coli* O157:H7 EDL933 culture. These data also suggest that phage OSYSP is a potentially useful hurdle in treatment combinations for food processing using mild heat (<55°C), and as a biological marker to validate the thermal treatments targeting *E. coli* O157:H7 EDL933.

P3-07 Molecular Mechanism of Metabolites Produced by *Lactobacillus casei* on Lysis of Enterohemorrhagic *E. coli*

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❖ ***Developing Scientist Entrant***

Introduction: Enhancing the bactericidal activity of metabolites produced by probiotics is one of the promising strategies to improve overall gut health as well as control enteric infections with foodborne pathogens such as *Salmonella, Listeria,* enterohemorrhagic *Escherichia coli* (EHEC), etc. However, the underlying mechanism of action and the effective concentration of various metabolites produced by different probiotic strains including *Lactobacillus casei* (LC) are still under investigation.

Purpose: To understand the bactericidal mode of action of metabolites, including conjugated linoleic acid (CLA) produced by LC and CLA over-producing *L. casei* (LC-CLA) against the pathogenesis of EHEC EDL-933.

Methods: Peanut white kernel was used to stimulate the growth and improve the metabolites of LC. Cell-free cultural supernatant (CFCS) was collected from LC and LC-CLA at 24 h and 48 h time periods to evaluate their efficacy in inhibiting EHEC growth and pathogenesis including the expression of membrane heat shock (*htpX, cpxP, pspA,B,C,D,E*), DNA damage repair (*lexA, recA, tus, yebG*), cell division (*ftsZ*) and chaperon (*clpB, dnaK,J, grpE, ibpA,B*) genes of EHEC by qRT-PCR. ANOVA was used to determine statistical significance.

Results: The growth of LC was increased by about 0.89 log CFU/mL ($P < 0.05$) in the presence of peanut. The CFCS obtained at 48 h from LC with peanut showed better potential in inhibiting EHEC entirely in 24 h, whereas CFCS collected from LC-CLA after 24 h were highly bacteriostatic to the pathogen ($P < 0.05$). Significant upregulation of the stress genes related to membrane and DNA integrity indicated a possible effect of the metabolites produced by LC-CLA on EHEC membrane and genomic DNA. This finding was further confirmed by bacterial cell membrane integrity assay and agarose gel electrophoresis.

Significance: Metabolites produced from bio-active probiotic, LC-CLA, might have significant disruptive effects on EHEC cell membrane and genomic DNA and potentiality in controlling EHEC infections.

P3-08 *Salmonella* Control in Raw Minced Meat by Vinegar-derived Clean Label Ingredients

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Introduction: *Salmonella* is the most frequently reported bacterial cause of foodborne illness in the U.S., causing approximately 26,000 hospitalizations and 400 deaths each year, resulting in enormous economic loss by medical costs and loss of productivity. Organic acid derivatives like acetates and vinegar are known as generic microbial growth inhibitors and can be applied to reduce food safety risks.

Purpose: To demonstrate the efficacy of a vinegar-derived acetate in inhibiting *Salmonella* in order to increase safety of fresh minced meat products.

Methods: Portions of ground raw beef (80% lean, 20% fat, 62% moisture, pH 5.9) were inoculated separately with 4.5 log CFU/g *Salmonella* (including strains *S. Enteritidis* 6424, *S. Enteritidis* E40, *S. Heidelberg* S13, *S. Typhimurium* S9, and *S. Typhimurium* M-09-0001-A1), by applying a 1% inoculum (v/w). Inoculated products were treated with 0, 0.25, or 0.75% acetate-based ingredients (dry vinegar or potassium acetate-potassium diacetate blend), and mixed for 3 minutes. The product was divided into polyethylene bags (25 g/bag), and stored aerobically at 10°C and 15°C. Triplicate samples were assayed at 0, 1, 7, 10, and 14 days for both temperatures and *Salmonella* populations enumerated by surface plating on selective agar (XLD). The results were statistically analyzed using ANOVA.

Results: Populations of *Salmonella* increased ~1.5 log in Control samples stored at 15°C for 1 day, and ~1 log after 10 days storage at 10°C. In contrast, no growth (<0.3 log increase) was detected in any acetate-based treatments stored at 10 or 15°C for 14 days. The data from this study revealed that vinegar derivatives prevented *Salmonella* growth significantly ($P < 0.05$) at abuse temperatures (15 and 10°C) during prolonged storage (14 days), compared to the control without antimicrobials.

Significance: This research demonstrated the possibility to increase safety of fresh minced beef, by controlling the growth of *Salmonella,* using vinegar-derived preservatives.

P3-09 Disinfectant Wipes Transfer *Clostridioides difficile* Spores across Hard, Non-porous Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: *C. difficile* has been detected in foods and on environmental surfaces, with transmission from fomites known to cause community-acquired *C. difficile* infections.

Purpose: In this study, we evaluated the cross-contamination risk associated with the real-world use of disinfectant wipes on fomites contaminated with *C. difficile* spores.

Methods: A Formica sheet was marked into 0.5 m lengths; inoculation-zone (i-zone), 0.5 m^2, 1 m^2, 1.5 m^2, and 2 m^2. The i-zone was inoculated with 50 µL of 5.0 x 10^8 CFU *C. difficile* ATTC 43598 spores. Three quaternary ammonium (QA) and three hydrogen peroxide (HP) disinfectant towelettes with non-sporicidal claims were used to wipe the entire sheet (i-zone to 2 m^2). A sodium hypochlorite (SH) product with sporicidal claims was also evaluated. After the wiping process and after label-defined contact times, surface swab samples were collected from 10 cm x 10 cm (0.1 m^2) areas of all marked portions, and used wipes were placed in neutralizing buffer. Swab samples and used wipes were processed to recover *C. difficile* spores using BHIY-HT agar following EPA MLB SOP-MB-31.

Results: All the tested products had some sporicidal efficacy with SH being significantly more sporicidal than the QAs ($P < 0.05$) but not statistically different from the HPs ($P > 0.05$). Regardless of product type, significantly lower mean log CFU/0.1 m^2 were detected from the 0.5 m^2 and 1.0 m^2 areas compared to the i-zone ($P < 0.05$). However, there were no significant differences among the log CFU/0.1 m^2 transferred to the 1.5 m^2 and 2 m^2 and the residual spores detected from the i-zone ($P > 0.05$). Used wipes retained high levels of *C. difficile* spores with the QAs having significantly higher levels than the HPs and SH ($P < 0.05$).

Significance: Disinfectant towelettes may contribute to cross-contamination risks in environments contaminated with *C. difficile* spores.

P3-10 Strain-specific Response of *Escherichia coli* Biofilms to Chlorine Dioxide

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Introduction: Chlorine dioxide (ClO_2) is a standard sanitizer for foods and food contact surfaces. Aqueous ClO_2 has proven efficacy against *E. coli* biofilms, which are a continuous source of contamination in the processing environment.

Purpose: We will investigate *E. coli*'s behavior in biofilms and if generic serotypes are as robust as other serotypes when treated with ClO_2.

Methods: This study investigated four STECs and one generic *E. coli* (*E. coli* O45 RM:13752; *E. coli* O157:H7 RM:18959; *E. coli* O157:H7 ATCC:43888 *stx-*; *E. coli* K-12 ATCC:13706; *E. coli* O145 RM:10808). The strains were inoculated individually or combined in a cocktail in a 24-well plate. After a 72-hour incubation at 25°C, planktonic cells were aspirated off, and the well was washed twice with peptone water (PW). The remaining biofilm was exposed to either distilled water (control) or 10 ppm ClO_2 for 10 minutes. After treatment, cells were enumerated on MacConkey's Sorbitol Agar.

Results: The control biofilm indicated a 7.3-log CFU/cm^2 +/- 0.4 recovery for each strain and cocktail. Biofilm treated by ClO_2 indicated a significant ($P < 0.5$) reduction compared to the control. The recovery for ClO_2 ranged from 0-4.3 log CFU/cm^2 with an average of 2.5 +/- 1.4 log CFU/cm^2. Strain-specific differences were observed with RM:10808 having the lowest log reduction followed by RM:13752, ATCC:43888, cocktail, K-12, and RM:18959 (2.8, 3.3, 4.6, 4.8, 6.2, and 6.6 log CFU/cm^2, respectively). In post hoc analysis (Tukey's test), RM:10808 and RM 13752 were significantly more resistant to treatment than the other strains.

Significance: This study demonstrates the efficacy of ClO_2 against *E. coli* biofilms, however, the conventional indicator species K-12 nor the cocktail was as robust as the pathogenic *E. coli* O45 RM:13752 and *E. coli* O145 RM:10808. Further studies will investigate the mechanism for differential inhibition of *E.coli* serovars.

P3-11 Modeling the Efficacy of Gaseous Chlorine Dioxide against *Listeria* on Apple Surfaces

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Introduction: Cross-contamination of pathogenic bacteria like *Listeria monocytogenes* on apples may occur during the industrial apple packing operations. Gaseous chlorine dioxide (ClO_2) can be utilized during the storage of fresh produce to decontaminate the products.

Purpose: This study investigated the efficacy of gaseous ClO_2 against *Listeria innocua*, as a surrogate for *L. monocytogenes,* on apple surfaces to support future prediction and development of commercial gaseous ClO_2 decontamination in the apple storage conditions.

Methods: A cocktail of five *L. innocua* strains (ATCC 33090, 33091, 43547, 51742, and BAA-680) was inoculated onto the calyx of three Fuji apples (250 g each). An amber vacuum desiccator (45 L) was modified as a treatment chamber. Gaseous ClO_2 was generated inside the chamber, by mixing equal amounts of dry media (sodium chlorite and activating acids) in a sachet. A total of 24 apples (~6 kg) including 21 uninoculated and three inoculated apples were treated for 1 h. The initial dry media amount (inputs) were 10, 15, and 20 g of each precursor. Temperature (19 ± 1°C) and relative humidity (60 to 88%) were measured inside the chamber. After the treatment, the inoculated apples were washed with 30 mL 0.1% peptone water. The rinse was enumerated on the PALCOM agar overlaid with TSA. The first-order model was used to fit the reduction data at different initial inputs.

Results: After 1 h treatment, reductions of 0.84, 1.60 and 2.90 log CFU/mL were observed at ClO_2 initial inputs of 10, 15, and 20 g, respectively. The coefficient of determination (R^2) was 0.911 for the first-order model. Log reductions were significantly ($P < 0.05$) different between 10 and 20 g initial inputs ($N = 3$).

Significance: This is the first study that models the efficacy of gaseous ClO_2 inactivation against *L. innocua* on apples. The first-order model can be used to predict the inputs if target log reductions are known, which will provide insight into the development of industrial-scale treatments.

P3-12 Characterization of Three Lytic Bacteriophages as an Antimicrobial Agent for Biocontrol of Shiga Toxin-producing *Escherichia coli* O145

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Introduction: Shiga toxin-producing *E. coli* (STEC) O145 is one of the most prevalent non-O157 serogroups associated with numerous produce outbreaks around the world, causing bloody diarrhea, urinary tract infections, kidney failure, and high mortality among immunocompromised population. Lytic phages are alternative antibacterial agents to minimize the emergence of antimicrobial-resistant strains. However, the information regarding the characterization of lytic phages for the control of STEC O145 is scarce.

Purpose: Thus, the objective of this study was to characterize three bacteriophages for the biocontrol potential of STEC O145 strains.

Methods: Three lytic phages specific to STEC O145 strains were isolated from fecal samples (phages UDF145_2 and UDF145_3) and water sediment (phage Pr145T13lw). After purification, three phages were subjected to morphology characterization, host range tests against STEC O157 and top 6 non-O157, *stx* genes screening, and temperature susceptibility tests (50°C) for 24 hours. The antimicrobial activities of these phages were evaluated at MOI = 1 using a spectrophotometer.

Results: Phages UDF145_2 and Pr145T13lw had a similar morphology belonging to *Siphoviridae* family whereas phage UDF145_3 belonged to *Myoviridae* family. The host range test revealed that phage Pr145T13lw had a wider host range, lytic against STEC O145, O111, O26, and generic *E. coli* strains (DH5a), than phages UDF145_2 and UDF145_3, which were lytic against STEC O145 and generic *E. coli* (ATCC13706) strains. Additionally, at MOI=1, phage Pr145T13lw rendered significantly higher antimicrobial effects against both outbreak and environmental STEC O145 strains than the other two phages. All three phages were stable at temperature ranging from 4°C to 50°C and did not harbor *stx* genes.

Significance: The three phages, with different host ranges, are able to render strong lytic effects against STEC O145 strains, and their temperature stability could aid in the development of alternative biocontrol strategies for STEC O145 in the pre-harvest produce environment.

P3-13 Efficacy of Bacteriophage and Its Depolymerase Enzyme against *Escherichia coli* O45 Biofilms on Food Contact Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks caused by non-O157 Shiga-toxigenic *Escherichia coli* have increased over the years. One such serotype is *E. coli* O45 that could persist in processing environments by forming lipopolysaccharide-biofilms, posing significant challenge to the food industry. Conventional sanitizers could be ineffective, warranting the need for development of alternatives. Bacteriophage depolymerase enzyme, known to degrade lipopolysaccharides, could be used effectively against biofilms.

Purpose: Evaluate the efficacy of depolymerase and *E. coli* O45-specific bacteriophage against biofilms on food contact surfaces.

Methods: Two-day-old biofilms of *E. coli* O45 (7 logs CFU/cm^2) were formed on stainless steel (SS) and high-density polyethylene (HDPE) coupons. Following treatments were applied before (preventive) or after (corrective) biofilm formation: a) depolymerase; b) O45-bacteriophage (P9; 9 logs PFU/mL); c) depolymerase followed by bacteriophage. Surviving pathogen populations were determined by sonicating coupons (40 KHz; 5 mins), and plating on TSA and MAC. Biofilm disruption was visualized using scanning electron microscope (SEM). Data were analyzed using one-way-ANOVA ($P < 0.05$).

Results: All treatments were effective in reducing *E. coli* O45 populations within biofilms, compared to the control. For both, preventive and corrective treatments, enzyme plus phage treatment was the most effective. It reduced pathogen populations by 1.6 and 1.8 logs as preventive, and 3.1 and 1.5 logs as corrective treatment on SS and HDPE, respectively. When used alone as preventive treatment, enzyme reduced *E. coli* O45 populations by 1.3 and 1.5 logs, and phage by 1.0 and 0.7 logs on SS and HDPE, respectively. As corrective treatment, depolymerase was more effective on SS (1.9 logs reduction) while phage was more effective on HDPE (1.2 log reductions). The SEM images confirmed enumeration results, showing disrupted biofilms and reduced bacterial populations with enzyme and phage treatments, compared to the control.

Significance: Phage depolymerase enzyme could be used effectively to reduce *E. coli* O45 and its biofilms on food contact surfaces.

P3-14 Formation of Filamentous Morphotypes of Shiga-toxigenic *Escherichia coli* in Response to Antimicrobial Stressors

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❖ ***Developing Scientist Entrant***

Introduction: Shiga-toxigenic *Escherichia coli* (STEC) have been responsible for numerous recalls and outbreaks. To mitigate STEC contamination risk, antimicrobials are commonly used at the processing level. Despite several interventions in place, recalls and outbreaks have continued. Environmental stressors may trigger adaptation in bacteria such as formation of biofilms and filamentous cells, serving as their repository during unfavorable conditions. This could lead to pathogen persistence in the processing environment, increasing contamination risks. Understanding STEC adaptations to antimicrobials could help develop effective intervention strategies.

Purpose: Examine STEC morphology in response to antimicrobial stressors.

Methods: Chlorine (200 ppm), per-acetic acid (200 ppm), sodium acid sulfate (0.1, 1 and 3%), bacteriophages, and phage-enzyme, were tested against STEC serotypes (O157, O26, O111, O121, O103, O145, O45). Bacteria were exposed to the antimicrobials before or after growth and biofilm formation (8 h or 7 d) on stainless-steel (SS), high-density-polyethylene (HDPE) or polyvinyl-chloride (PVC) surfaces. Sterile distilled water was used as the positive control. Survival of STEC was determined using standard culture methods. Biofilm disruption and STEC morphology on different surfaces were observed under scanning electron microscope (SEM) at 1,000 - 50,000x magnification.

Results: Chemical sanitizers reduced pathogens to undetectable levels on SS, HDPE and PVC, compared to the control. Bacteriophages and phage-enzyme reduced populations by 0.4-3.1 log CFU/cm^2. SEM images revealed reduced or disrupted biofilms, along with dead cells, on surfaces treated with antimicrobials. However, viable, regular-sized (1 µm), rod-shaped cells, similar to positive control, were also observed. Additionally, antimicrobial-exposed populations contained filamentous cells that were multiple times longer (3 - 35 µm) than regular cells. These cells appeared to have formed due to blocked septa formation between dividing cells. STEC exposed to bacteriophages formed capsular sheaths surrounding multiple cells, giving the appearance of extremely elongated (~35 µm) single cells.

Significance: Filamentous morphotypes of STEC could be indicative of "adaptation loopholes" that result in resistance to antimicrobial challenges.

P3-15 Application of Chemical and Biological Methods to Prevent Formation of Shiga-toxigenic *Escherichia coli* Biofilms on Poly-vinyl Chloride Surfaces

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✦ ***Undergraduate Student Award Entrant***

Introduction: Shiga-toxigenic *Escherichia coli* (STEC) have been implicated in numerous foodborne outbreaks linked to beef. Cattle are common reservoirs of STEC, known to persist in the farm environment through cross-infection or cross-contamination. Water troughs and incoming water supplies (made of PVC) have been identified as potential sources of STEC contamination on cattle farms. They can harbor STEC for extended periods of time due to formation of strong biofilms by these bacteria. It is therefore important to remove these biofilms to control STEC in livestock operations.

Purpose: Evaluate the efficacy of chemical and biological methods to prevent STEC biofilm formation on PVC.

Methods: Chlorine (200 ppm), per-acetic acid (PAA; 200 ppm), sodium acid sulfate (SAS; 0.1, 1, and 3%) and bacteriophage cocktails (9 logs PFU/mL) were evaluated against STEC biofilms. A 3-strain cocktail of *E. coli* O157:H7 (ATCC 43895, 0122, wild-type KF10) and 14-strain cocktail (7 clinical, 7 wild-type) of non-O157 serotypes (O26, O45, O103, O111, O121, O145) was used in separate experiments. Bacteria (~6 logs CFU/cm^2) were allowed to form biofilms for 7 days (25°C) on PVC coupons (2x5 cm^2) placed in water containing one of the treatments. Coupons were then removed and sonicated (40 KHz, 5 min) to remove any biofilms. Surviving STEC populations in the biofilms and residual water were determined on appropriate medium. Biofilms were visually observed using scanning electron microscope (SEM). Data were analyzed using one-way ANOVA ($P < 0.05$).

Results: All chemical treatments reduced pathogen population to undetectable levels compared to control (7.4 logs CFU/cm^2). Phage-cocktail specific to *E. coli* O157:H7 was more effective at preventing biofilms than non-O157 phages, reducing pathogen population by 0.7 logs CFU/cm^2. The SEM images were consistent with the results showing reductions in pathogen populations and biofilms on PVC treated with antimicrobials.

Significance: Formation of STEC biofilms on PVC could be prevented using effective antimicrobials in water.

P3-17 The Effect of a *Carnobacterium maltaromaticum* Strain on Quality of and Inhibition of Pathogenic Bacteria on Vacuum-packaged Beef

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Introduction: Previous studies have shown that a *Carnobacterium maltaromaticum* strain, A5, can inhibit both spoilage and pathogenic bacteria *in vitro* and hence has the potential to improve beef safety and quality.

Purpose: To explore the effect of *C. maltaromaticum* A5 on the safety and shelf life of vacuum-packaged (VP) beef.

Methods: Beef steaks (8 groups) were inoculated with 0 (groups 1 and 5), 0.5 (2 and 6), 1 (3 and 7) and 2 (4 and 8) log CFU/cm^2 of *C. maltaromaticum* A5, and 0 (groups 1-4) and 2 (5-8) log CFU/cm^2 of *Salmonella* Typhimurium ATCC 14028 and *Escherichia coli* O157:H7 1934. The steaks were vacuum-packaged and stored at 2°C for 12 weeks, and three were randomly withdrawn from each group at bi-weekly intervals. Organoleptic properties of group 1-4 steaks were evaluated by a five-person panel. The population of total aerobic bacteria (TAB) and carnobacteria on all steaks, and *S.* Typhimurium and *E. coli* O157:H7 on group 5-8 steaks was determined on agar plates.

Results: No significant differences in organoleptic characteristics were observed between *C. maltaromaticum*-inoculated (1) and uninoculated (2-4) groups most of time throughout storage. Group 2-4 and 6-8 steaks showed higher numbers of TAB from week 2 to 8 and no significant difference for other weeks compared with group 1 or 5; all groups had 8.1 to 8.7 log CFU/cm^2 TAB at week 12. Compared with TAB, the proportion of carnobacteria in inoculated groups was between 2-70% in the initial microbiota, increased to about 90% at week 2 and then gradually decreased with <20% in the final microbiota. The population of both pathogenic bacterial species on group 5-8 steaks decreased during storage and no inhibition by *C. maltaromaticum* was observed.

Significance: *C. maltaromaticum* A5 does not show effect on VP beef spoilage and does not inhibit *S.* Typhimurium or *E. coli* O157:H7 on VP beef either.

P3-18 Combinations of Phenolic Compounds and Cold Shock Alters *Escherichia coli* Pathotypes Survival and Genetic Expression of Virulence Factors

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Introduction: Several phenolic compounds exhibit antimicrobial activity against *E. coli*; however, there is little information on the effectiveness of these compounds in combination with low temperatures for the control of pathogenic *Escherichia coli*.

Purpose: To evaluate the effect of phenolic compounds [tannic acid, gallic acid, methyl gallate, and epigallocatechin gallate] in combination with cold shock (CS, 10°C) on growth, swarming motility, biofilm formation, and expression of selected virulence genes of three *E. coli* pathotypes (EPEC, EHEC, and ETEC).

Methods: Inhibitory concentrations (MICs) of the phenolic compounds were determined at 37°C by the microdilution method. *E. coli* strains were grown at 37°C until mild-log phase and then subjected to 10°C for 4 h. Then, concentrations lower than MICs (sub-MICs) were added to the media and analyzed

for: viability (determined by flow cytometry), swarming motility (on LB agar), biofilm formation (by crystal violet staining), and expression of CS genes (*csp* A and B, by qPCR).

Results: The sub-MICs (2.6, 3.2, 0.8, 1mg/mL of tannic acid, gallic acid, methyl gallate, and epigallocatechin gallate, respectively) did not affect the viability of the strains. The CS plus tannic acid provoked the highest mortality (50-90%) in all pathotypes. CS plus gallic acid inhibited completely the mobility of EHEC and ETEC, whereas CS plus methyl gallate reduced the biofilm formation (79.4 and 65.8 % reduction) for ETEC and EPEC, respectively, and CS plus epigallocatechin gallate reduced 72.6 % in EHEC. In most cases, repression of the CS-related *csp* A and B genes were observed.

Significance: The combination of phenolic compounds and cold shock affected growth, biofilm formation, and swarming motility of pathogenic *E. coli* and are alternatives to be studied for its control.

P3-19 Antibiotic Resistance Influences the Growth and Biofilm Formation in *E. coli* O157:H7

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7, has been implicated in severe foodborne outbreaks. As with several foodborne pathogens, antibiotic resistance strains have emerged.

Purpose: Phenotypic changes in *E. coli* O157:H7 due to acquisition of antibiotic resistance were evaluated.

Methods: *E. coli* O157:H7 with no resistance to antibiotics was conferred resistance to 100 µg/mL streptomycin through incremental exposure (*E. coli* O157:H7 strep*). The strain was conferred resistance to 100 µg/mL ampicillin by transformation with a pGFP plasmid (*E. coli* O157:H7 amp*). A strain with dual resistance, 100 µg/mL ampicillin and 100 µg/mL streptomycin and was also developed (*E. coli* O157:H7 strep*amp*). These strains were comparatively evaluated to the parent strain for growth rates, maximum cell density, log-phase duration by comparison of growth curves and for biofilm formation using the crystal violet assay.

Results: *E. coli* O157:H7 amp* strep* had the longest lag phase duration (6.87 h), followed by *E. coli* O157:H7 strep* (6.77 h). *E. coli* O157:H7 amp* had the shortest lag phase duration (2.29 h), which was also faster than the non-resistant parent strain of *E. coli* O157:H7 (2.97 h). The short lag-phase durations among *E. coli* O157:H7 amp* and the parent strain of *E. coli* O157:H7 were also supported by the high maximum population density values of 4.09 and 3.81 OD_{600}, respectively. Acquisition of streptomycin resistance resulted in lower maximum population density values 3.27 OD_{600}. A combination of ampicillin and streptomycin resistance in *E. coli* resulted in the lowest maximum population density of 3.22 OD_{600}. *E. coli* O157:H7 amp* strep* followed *E. coli* O157:H7 strep* formed significantly higher amounts of biofilm than the parent and *E. coli* O157:H7 amp* strain ($P < 0.05$).

Significance: The results indicate that the acquisition of streptomycin resistance might slow growth rates and increase biofilm formation in *E. coli* O157:H7.

P3-20 Emerging and Multidrug Resistance of *Listeria* spp. Recovered from Produce Processing Environments

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❖ ***Developing Scientist Entrant***

Introduction: The ubiquitous nature of *Listeria monocytogenes* (Lm) and its presence in soil and agricultural environments, make it a challenging pathogen to control in the produce industry. Considering the high mortality rates associated with listeriosis and increasing reports of reduced sensitivity of *Listeria* spp. to clinically relevant antibiotics, it is imperative we understand the extent of antimicrobial resistance (AMR) of these microorganisms, particularly in strains associated with produce.

Purpose: To identify the potential emerging and multidrug resistance (MDR) of *Listeria* spp. recovered from Pacific Northwest (PNW) produce processing environments by evaluating their AMR profiles.

Methods: *Listeria* spp. (n = 165) were isolated from environmental swab samples from five produce processing facilities in the PNW. AMR profiles were determined using disk diffusion assay (CLSI) for 17 antibiotics (amikacin, AMK; ampicillin; cefoxitin; chloramphenicol, CHL; clindamycin, CLI; erythromycin; gentamicin; imipenem; kanamycin, KAN; rifampin; co-trimoxazole; tetracycline; nalidixic acid; novobiocin, NOV; penicillin, PEN; streptomycin, STR; vancomycin). Isolates were considered sensitive, intermediate, or resistant to antibiotics based on the diameter of inhibition zones and considered to be MDR when resistant to ≥ 3 antibiotics.

Results: The majority of isolates were sensitive to AMK (93%), CHL (89%), KAN (93%), and STR (52%); however, remaining tested isolates demonstrated intermediate resistance to these antibiotics, suggesting emerging resistance. Isolates with reduced sensitivity to AMK or KAN were only sensitive to one or two of the other antibiotics tested. Three Lm isolates demonstrated MDR, including resistance to CLI, NOV and PEN.

Significance: Characterization of AMR in *Listeria* strains from produce processing environments in the PNW identified emerging resistance to STR, and the presence of MDR. While the majority of *Listeria* spp. remain sensitive to clinically relevant antibiotics, the presence of MDR in strains recovered from produce processing is concerning and prompts further studies to understand the pressures driving the AMR changes in these microorganisms.

P3-21 Decontamination of *Escherichia coli* O157:H7 from Watermelon Seeds by Combined Treatments of Gaseous Chlorine Dioxide and Mild-Wet Heat

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❖ ***Developing Scientist Entrant***

Introduction: To prevent *Escherichia coli* O157:H7 infection from watermelon intake, it is important to inactivate *E. coli* O157:H7 from watermelon seeds, however, an effective seed decontamination method has not been developed yet.

Purpose: The study was done to develop a seed decontamination method using combined treatments of gaseous ClO_2 and mild-wet heat without reducing seed's viability.

Methods: Firstly, watermelon seeds were treated with gaseous ClO_2 (*ca.* 1,000 ppm) and mild-wet heat (85% RH and 60℃) for 60, 90, and 120 min and the germination rates were determined. Seed's germination rates were measured by the guidelines of the International Seed Testing Association. Next, watermelon seeds were inoculated with *E. coli* O157:H7 (*ca*. 7 log CFU/g) and treated with gaseous ClO_2 (*ca.* 1,000 ppm) at 85% RH and 60℃ for up to 120 min. After treatment, the numbers of survived *E. coli* O157:H7 on watermelon seeds were determined by direct plating method.

Results: The germination rate (96.7%) of watermelon seeds after treatment with gaseous ClO_2 and mild-wet heat for 120 min was not significantly reduced ($P > 0.05$) compared to that (98.0%) of control seeds. The initial population of *E. coli* O157:H7 inoculated in watermelon seeds was 6.6 log CFU/g. When seeds were treated with mild-wet heat for 120 min without gaseous ClO_2, the population was reduced to 2.0 log CFU/g. However, when seeds were treated with both gaseous ClO_2 and mild-wet heat, the population of *E. coli* O157:H7 was reduced below the detection limit (1.0 log CFU/g) for plating within 90 min, and completely inactivated within 120 min.

Significance: This is the first study to confirm that combined treatments of gaseous ClO_2 and mild-wet heat can inactivate *E. coli* O157:H7 in watermelon seeds without reducing its germination rate. These results may provide useful information in developing seed decontamination method.

P3-22 Inactivation Kinetics and Metabolic Responses of *Escherichia coli* in Organic Broccoli Sprouts by the Combination Treatment of Lactic Acid and Mild Heat

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❖ ***Developing Scientist Entrant***

Introduction: Organic broccoli sprouts (*Brassica oleracea* var. *italica*) could be contaminated by pathogenic *Escherichia coli* but only mild treatment could be applied for maintaining the freshness of the sprouts.

Purpose: The aim of this research was to elucidate the inactivation kinetics and metabolic responses of pathogenic and non-pathogenic *Escherichia coli* organic broccoli sprout by mild heat (MH) and lactic acid (LA).

Methods: The *in vitro* inactivation kinetics of *E. coli* ATCC 25922 and O157:H7 ATCC43895 were investigated by LA (2%, v/v), MH (45°C), or their combination. The *in vivo* inactivation and growth recovery on the bacteria and the sprout quality including color, firmness, and total phenolic content during storage were determined. The bacterial reductions were fitted using five models. The metabolic analysis of *E. coli* under different treatments was tested by nuclear magnetic resonance and the results were analyzed by multivariable (principal component analysis, orthogonal partial least squares discriminant analysis) and pathway analysis. Differences with $P < 0.05$ were considered statistically significant.

Results: *In vitro* survival kinetics revealed that the cells decreased dramatically and were not detectable after 120-135 s under combined treatment. Non-pathogenic strain was more sensitive to the treatment (around 0.5 log CFU/mL difference). The Weibull model with higher R^2 (0.66–0.99) best described the *in vitro* antibacterial effects and *in vivo* growth dynamics. Tricarboxylic acid (TCA) cycle was the most affected pathway under the combined treatment. Furthermore, lysine was concentrated in O157:H7, indicating that lysine decarboxylase system might contribute to the survival of O157:H7. Interestingly, the treatments did not affect the sprout quality significantly.

Significance: The combined treatment is promising to reduce the safety risk of organic sprouts. Elucidating the metabolomics of the bacteria under the treatment provides promising approach to enhance the sanitization treatment in the future.

P3-23 Metabolic Characterization of Eight *Escherichia coli* Strains Including "Big Six" and Acidic Responses of Selected Strains Revealed by NMR Spectroscopy

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❖ ***Developing Scientist Entrant***

Introduction: Infection caused by the "Big Six" *Escherichia coli* is an emerging safety issue for fresh organic sprouts but the effects of mild sanitizer on the pathogen and its sanitizing response remained largely unclear.

Purpose: The aim of this study was to elucidate the inactivation effect and metabolic response of the "Big Six" on organic broccoli sprouts under lactic acid (LA) treatment.

Methods: Intracellular metabolites of eight *E. coli* strains (including the "Big Six") cultured in broth were extracted and analyzed by nuclear magnetic resonance (NMR). The obtained spectra were processed by multivariable analysis including hierarchical cluster analysis, principal component analysis (PCA), and orthogonal partial least squares discriminant analysis to discriminate different variables. Under lactic acid, the *in vivo* inactivation kinetics, morphologic changes, protein leakage, and metabolic adaptation of the two strains were characterized by model fitting, atomic force microscopy, protein content determination, and NMR, respectively. The signaling pathway for the metabolic changes was analyzed and the involved biochemical pathways were proposed and verified by real-time PCR test. Data were analyzed by analysis of variance, and differences with a P value < 0.05 were considered significant.

Results: The metabolic profiles of eight *E.coli* strains showed metabolic diversity in different serotypes. For example, metabolites including lysine, arginine, α-ketoglutaric acid, adenosine, and fumaric acid were responsible for the separation of *E. coli* ATCC 25922 in PCA. Relatively large metabolic differences between ATCC 25922 and O26:H11 (ATCC BAA-2196) existed, indicating the metabolic variation between pathogenic and non-pathogenic strains. The *in vitro* and *in vivo* inactivation kinetics, morphological changes, and protein leakage showed higher acid tolerance of *E. coli* O26:H11 compared with ATCC 25922. Moreover, the metabolic analysis of selected two strains under acid stress showed different amino acid metabolisms and higher requirement for energy production in ATCC BAA-2196. Real-time PCR tests confirmed that glutamic acid dependent decarboxylase/antiporter system was the major acid resistance mechanism.

Significance: Pathogenic *E. coli* showed different metabolic response from non-pathogenic strain under acid stress. The "Big Six" contamination on organic sprouts could be controlled by LA via affecting the metabolomics.

P3-25 Identification of Antimicrobial-resistance Genes in Whole-Genome Sequences of Canadian *Campylobacter* spp. Isolates Recovered from Poultry or Clinical Sources

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Introduction: *Campylobacter* is the leading cause of foodborne bacterial illness in Canada. The increasing presence of antimicrobial resistance (AMR) in *Campylobacter* spp. recovered from clinical and livestock sources worldwide is an important threat to human health, of which the most concerning is the increasing rate of fluoroquinolone resistance.

Purpose: This study was to use whole-genome sequences (WGS) to identify genes conferring AMR (ARG) in Canadian *Campylobacter* isolates recovered from poultry and to compare ARGs in human clinical and local retail poultry isolates from Nova Scotia.

Methods: The study used WGS of *Campylobacter jejuni* and *C. coli* isolates collected during a 2012 national study of abattoir and retail poultry (*n* = 1,712), a 2012 study of Nova Scotia retail poultry (*n* = 302), and Nova Scotia clinical isolates from 2012-2014 (*n* =118). AMR profiles were predicted using ResFinder to detect acquired genes and in-house programs to detect point mutations in the 23S rRNA gene and *gyrA* gene.

Results: Overall, prevalence of AMR was similar between abattoirs and retail poultry but did differ between species and across provinces for some antimicrobials. Overall, the prevalence of tetracycline resistance was 47% and beta-lactamases were 82% across Canada. Nationally, the prevalence of fluoroquinolone resistance in isolates ranged from 3% in some provinces to 21% in isolates from British Columbia (*n* = 108/525), with *C. coli* (*n* = 198) isolates having a 20% higher prevalence of resistance then *C. jejuni*. Within Nova Scotia, however, the prevalence of fluoroquinolone resistance in clinical isolates was 35%, considerably higher than in local retail poultry (2%).

Significance: These findings demonstrate carriage of ARGs differ across the country and among *Campylobacter* species. The high prevalence of fluoroquinolone resistance in clinical *Campylobacter* compared to poultry shows the need to identify the source of these isolates to develop strategies for mitigating impacts of AMR in Canada.

P3-26 Population Dynamics and Resistance of *Salmonella* Enteritidis to a Lytic Phage

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Introduction: *Salmonella* Enteritidis is the most common *Salmonella* serovar. While phages are a promising control strategy to reduce *Salmonella* colonization on food matrices; phages are in constant interaction with their hosts in the environment, therefore, phage are considered evolving antimicrobials.

Purpose: To determine the population dynamics of *Salmonella* and a lytic phage by serial transfers in rich and minimal culture media.

Methods: Experimental evolution assays were conducted in rich (TSB) and minimal media (MM), in which phages and bacteria were inoculated in 50 mL liquid cultures, 1/100 of the mixture was transferred to fresh medium daily for 21 days. Every 24 h, the optical density and phage titer were measured. Four replicates were conducted. Twenty colonies were selected on days 1, 12 and 21 to evaluate the evolution of resistance to past, present and future phage populations. The behavior of the dynamics was modeled and simulated with mathematical mass-action models.

Results:

In the assays performed in TSB, the *Salmonella* culture increased on the first day to reach OD 1.5 and in MM increased to OD 0.8. In both cases, the *Salmonella* population was maintained for the 21 days of the experiment. The phage titer in TSB increased on the first day by 4 logs (log 10 PFU/mL), however, it gradually decreased 3 logs, but it was maintained. Resistance assays showed co-evolution in MM, where *Salmonella* isolates from day 21 (future) are susceptible to past phage, but they are resistant to present (day 12) and future phages (day 21). Despite evolution of resistant *Salmonella* mutants, the stability of the lytic phage population in this system is consistent with the leaky resistance model.

Significance: This study shows the co-evolution and resistance to phage in *Salmonella*, which is a fundamental knowledge for the design of phage-based biocontrol strategies for food applications.

P3-27 The Effect of Natural Antimicrobials on *Clostridium perfringens* Endospores and Vegetative Cells

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❖ ***Developing Scientist Entrant***

Introduction: *Clostridium perfringens* is the third most prevalent foodborne bacterial pathogen in the United States, and it is a major concern to the food industry due to its ubiquitous and resilient nature. Current consumer preference for natural ingredients offers a unique opportunity for novel combinations of food ingredients that could inhibit *C. perfringens*.

Purpose: The goal of this study was to evaluate the efficacy of select natural antimicrobial ingredients (AI) against *C. perfringens* endospores and vegetative cells in pure culture.

Methods: Anaerobic suspensions of endospores and vegetative cells of three *C. perfringens* strains were mixed with different AI combinations and concentrations using 96-well plates. AIs included commercial products based on vinegar alone or in combination with lemon juice, citrus extract, or cinnamon extract. Optical density (OD_{600}) readings were taken to determine minimum inhibitory concentrations (MIC). Treated cells or endospores were subcultured to prepared fluid thioglycolate (FTG) agar plates to determine viable counts to assess bactericidal and sporicidal activities. Sporulation efficiency was determined using modified Duncan-Strong (DS) media with sublethal antimicrobial concentrations. Statistical significance of mean value differences of OD_{600} and counts between treatments and controls were determined with ANOVA at a value of $P < 0.05$.

Results: Two vinegar treatments (dry, liquid vinegar) had little effect inhibiting growth up to 3.0% (positive controls had final OD_{600} approx. 0.95). Another concentrated vinegar preparation stopped growth at 3.0% ($P < 0.05$). Vinegar with citrus extract and vinegar with dried lemon juice treatments kept OD_{600} at <0.09 for all strains when used at ≥1.0% and ≥1.5%, respectively. Those formulations also reduced vegetative cells by approximately ≥5.0-log CFU/mL ($P <$ 0.05), endospores by ≥3.5-log CFU/mL ($P < 0.05$), and sporulation efficiency by ≥ 53.7% ($P < 0.05$) of all three strains.

Significance: These findings suggest that two of the natural antimicrobial formulations are effective bactericidal and sporicidal AIs against *C. perfringens*.

P3-29 Differential Inhibitory Potential of Prebiotics Alone and in Combination with Antibiotics on Strains of *Salmonella*

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Introduction: Antimicrobial resistance (AMR) is a major public health problem that requires serious attention. Extensive use of antibiotics has resulted in their reduced effectiveness for therapeutic and non-therapeutic purposes. Thus, alternative therapies for treating infections using naturally available resources such as prebiotics are needed.

Purpose: The aim of this study was to evaluate the effects of blueberry pomace extract (BPE) on susceptibilities of *Salmonella* strains to antimicrobial agents commonly used for therapeutic purposes against *Salmonella* infection.

Methods: Antimicrobial susceptibility tests on the *Salmonella* strains were performed on BHI or XLD agar amended with 25 mg/mL or without (control) BPE using disk diffusion method. Standard antimicrobial discs (azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone and tetracycline) were used. Effects of BPE on physicochemical properties in *Salmonella* strains were tested. qRT-PCR was used to validate effects of BPE on the expression of genes involved in physicochemical properties.

Results: Our results showed that BPE increased susceptibilities of *Salmonella* strains towards tetracycline as evident in *S.* Enteritidis and *S*. Typhimurium. Although *S*. Newport and *S*. Kentucky were still resistant to tetracycline, their zones of inhibition were nearing intermediate range indicating BPE positive effect. Also, BPE altered the physicochemical properties of *S*. Typhimurium, *S*. Kentucky, *S*. Enteritidis, and *S*. Newport strains. Swimming motility rates (%) were significantly reduced in *S*. Typhimurium (32%), *S*. Kentucky (35%), *S*. Enteritidis (29%), and *S*. Newport (32%) as compared to the control (100%) due to exposure to BPE. These findings were validated using qPCR and it was observed that *fliC*, *hilA* and *hilD* genes were down regulated in the presence of BPE.

Significance: This study suggests that BPE could be used as an adjuvant in poultry antimicrobial therapy as feed additives to reduce *Salmonella* load. This could reduce the emergence of antibiotic resistant *Salmonella* strains from poultry meat products.

P3-30 Effects of Phenolic Acids on Outer Membrane Integrity and Functionality of *Salmonella* Typhimurium

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❖ ***Developing Scientist Entrant***

Introduction: Phenolic acids, specifically Gallic acid (GA) and Protocatechuic acid (PA) are bioactive plant derivatives that have potential as alternative antimicrobials against bacterial pathogens including, *Salmonella enterica* serovar Typhimurium (ST). However, the interactions between these phenolic acids with important bacterial structures, like outer membrane and proteins, as well as the effects that they will have on physiological functions are not yet fully elucidated. This becomes especially important when developing new approaches of targeting pathogens and understanding their mechanism of action.

Purpose: To evaluate physiological alterations that occur in ST and its outer membrane as a result of exposure to GA and PA.

Methods: Bacterial outer membrane integrity after treatment with lethal concentrations of GA or PA was measured through the use of the LIVE/DEAD® BacLight™ Bacterial Viability Kit (L/D) protocol for fluorescent microplate reads. Changes in outer membrane protein concentration were also measured using a Bicinchoninic Acid (BCA) assay. The relative expression of other genes related to physiological functions of ST (*aaeB*, *acrA*, *lapB*, *murD* and *rpoS*) were also evaluated. Student's *t*-test was used for statistical analysis.

Results: L/D results demonstrated a significant ($P < 0.05$) influx of fluorescent dyes in pre-treated ST, in a concentration dependent manner, beginning at a relative fluorescence intensity unit (RFU) of 5,466 for control and increasing up to 16,176 and 20,887 at 4 mg/mL of GA and PA, respectively. Pre-treated bacteria also demonstrated a significantly ($P < 0.05$) greater release of outer membrane proteins, with control containing 165.2 mg/mL, while GA and PA contained 1,726.7 mg/mL and 1,014.2 mg/mL, respectively. Relative gene expression in ST was significantly ($P < 0.05$) reduced during GA treatment by 1.37, 1.32, 0.83, 0.96 and 0.97 for genes *aaeB*, *acrA*, *lapB*, *murD* and *rpoS*, respectively, while PA treatment reduced relative gene expression by 0.63, 0.65, 0.58, 0.67 and 0.84 for *aaeB*, *acrA*, *lapB*, *murD* and *rpoS*, respectively.

Significance: This work suggests a potential mechanism of action of GA and PA against the outer membrane integrity of ST, making it an important target that leads to increased permeability, protein loss and overall cellular stress.

P3-31 Efficacy of Natural and Synthetic Antimicrobials to Inhibit Adhesion of EHEC, EAEC and Serotype O104:H4 to HEp-2 Cells

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❖ ***Developing Scientist Entrant***

Introduction: In general, pathogenic *E. coli* are good colonizers of human gut, and their enhanced adherence to intestinal epithelial cells might facilitate systemic absorption of toxins such as stx, increasing the risk of diseases and severity.

Purpose: To analyze the effect of natural and synthetic antimicrobials on the adhesion of isolates of EHEC, EAEC and the hybrid serotype O104 of *E. coli* to HEp-2 cells

Methods: Carvacrol, extract of oregano and rifaximin were used at sublethal concentrations. The experimental design was performed to analyze if a) antimicrobials blocked bacterial ability to attach eukaryotic (HEp-2) cells, b) antimicrobials acted on HEp-2 cells, preventing the adhesion of bacteria, c) antimicrobials acted on both bacteria and HEp-2 cells, or d) antimicrobials detached adhered bacteria from HEp-2 cells. The adhesion assays were conducted for 3 h at a ratio of 100:1 (*E. coli*:HEp-2 cells), and the number of adhered bacteria determined by plate account. After adhesion, expression of attachment- and virulence-associated genes were analyzed by qPCR. The experiments were performed at least three times, each tested in duplicate.

Results: Oregano extract and rifaximin when exposed to bacteria reduced 40% their adhesion to the cells, and carvacrol reduced 65% ($P \leq 0.05$). Carvacrol altered the "stacked-brick" like structure adhesion pattern of serotype O104:H4, showing a total separation between bacteria. The antimicrobials did not detach the bacteria from the cells. An undefined pattern of expression of the genes *aggR, aap, aggA, pic, eae,* and *stx2* was observed; however, carvacrol decreased the expression of all genes except *AggR* and *aggA* ($P \leq 0.05$) in EAEC and O104:H4, respectively; whereas, the oregano extract overexpressed most of the genes.

Significance: The antimicrobials analyzed were able to reduce the adhesion of *E. coli* to HEp-2 cells and modified the expression of bacterial virulence-associated genes. Carvacrol provoked a diffuse adherent pattern in the O104:H4 isolate, suggesting this compound act on the aggregative adherence plasmid (pAA) or over related proteins.

P3-32 Prevalence of Triazole-Resistance *Aspergillus fumigatus* Isolated from a Tomato Production Environment

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❖ ***Developing Scientist Entrant***

Introduction: *Aspergillus fumigatus* is one of the most prevalent airborne fungal pathogens, causing severe allergic, chronic and invasive aspergillosis in immunocompromised patients. It is commonly found in agricultural environments including soil and decaying vegetation. Triazole antifungal agents (azoles) are the main medical treatment of aspergillosis in humans and are also frequently used for crop protection in agriculture. Prolonged azole treatments to control aspergillosis have been associated with azole resistance development in *A. fumigatus*. However, resistance has also been reported in azole-naive patients. This situation suggests an additional route of resistance development to clinical isolates through environmental exposure to azole fungicides used in agriculture.

Purpose: The purpose of this research was to determine the prevalence of azole-resistant *A. fumigatus* isolates in the tomato production environment following exposure to azole fungicides and the mechanism(s) of resistance in these isolates.

Methods: A tomato field was established in 2019 and propiconazole (147.87 mL/acre) was applied weekly until harvest. Soil and leaf samples were collected prior to and following each application of propiconazole. Fruit samples were collected at harvest. Isolations were done on potato dextrose and chloramphenicol-amended Sabourand dextrose media. Putative *Aspergillus* spp. isolates were identified based on 18S rDNA gene sequencing and screened for resistance to three clinical azoles – itraconazole (4 mg/L), posaconazole (0.5 mg/L), and voriconazole (2 mg/L).

Results: In total, 260 isolates of putative *Aspergillus* spp. were recovered prior to the first application of propiconazole and 558 were collected during the season for a total of 818 isolates. Preliminary data collected using baseline representative isolates indicated that 79% were *A. fumigatus* and resistant to itraconazole only. The mechanism of resistance is currently under investigation.

Significance: These data indicate that isolates of *A. fumigatus* in the environment are resistant to itraconazole and could contribute to the antimicrobial resistance public health concern.

P3-33 Ready-to-use Disinfectant Wipes Retain High Levels of *Staphylococcus aureus* and *Pseudomonas aeruginosa* after Use

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✦ ***Undergraduate Student Award Entrant***

Introduction: *S. aureus* and *P. aeruginosa* contaminate food and environmental surfaces, and can cause disease outbreaks and food spoilage, respectively.

Purpose: The purpose of this study was to evaluate the cross-contamination risk by disinfectant towelettes used on a food contact surface contaminated with *S. aureus* and *P. aeruginosa*.

Methods: Formica sheets were inoculated with 50µl of 5.0 x 10^8 CFU *S. aureus* ATCC CRM-6538 or *P. aeruginosa* strain (i-zone); sheets were marked at 0.5 m^2, 1 m^2, 1.5 m^2, and 2 m^2. Three quaternary ammonium (QA) and three hydrogen peroxide (HP) were tested. A disinfectant towelette was used to wipe the entire 2 m^2 Formica sheet and the disinfectant liquid released allowed to stay in contact with the wiped surface for the label-defined contact time. Surface swab samples were collected from defined 0.1 m^2 within each marked area and used towelettes were placed in neutralizing buffer. *S. aureus* and *P. aeruginosa* were enumerated from swab samples and used towelettes following EPA MLB SOP-MB-33.

Results: Overall, disinfectant wipes transferred 1.17 ± 0.36 logs of *S. aureus* and 2.34 ± 1.09 logs of *P. aeruginosa* from the i-zone to all other test surfaces up to 2 m^2. There were no significant differences in the pathogen loads transferred from the i-zone to other surfaces when comparing HPs to QAs for both pathogens ($P > 0.05$). Post disinfection, used wipes retained on average 1.59 ± 0.71 CFU of *S. aureus* and 2.11 ± 0.65 CFU of *P. aeruginosa* with no overall significant differences between QAs and HPs ($P > 0.05$).

Significance: Ready-to-use disinfectant towelettes may contribute to the risk of spreading *S. aureus* and *P. aeruginosa* from contaminated environmental surfaces.

P3-34 All Surfaces are Not Created Equal: Inactivation of RNase a on Food-contact Surfaces Using Hi-intensity 278 Nm UV LED

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Poster

Introduction: The wide variety of food contact surfaces may not respond similarly to Hi-intensity 278 nm UV LED exposure, requiring different doses to achieve enzyme (as a surrogate for microbial) inactivation.

Purpose: To demonstrate differential responses of food contact surfaces to identical doses of 278 nm UV LED, as determined by an enzyme assay surrogate for microbial contamination.

Methods: RNase A was inoculated onto (2 µL, 0.02 units/mL, Worthington Biochemical) various types of food contact surfaces (ceramic, stainless steel, aluminum foil, glass, brushed aluminum, Teflon, polyolefin, silicone, styrofoam, PETE, paper) and allowed to dry. Surfaces were exposed to 278 nm UV LED (330 mW/cm^2 at the window) from 25 mm (9.9 J/cm^2 dose). Exposure time (and therefore dose) was computer controlled. RNase A was recovered from each surface in RNase-free water and the suspension was fluorometrically assayed for RNase activity, one hour for enzyme kinetic assay (RNaseAlert IDT).

Results: Exposure doses were intentionally chosen to allow only partial enzyme inactivation (targeted to 50%) in the time frame of the assay. Data (expressed in relative fluorescence units) for exposed samples were normalized to unexposed control samples recovered from the same surfaces. Unexpectedly, a comparison of enzyme activity for each of the surfaces differed based on whether the surface was UV reflective or non-reflective. Glass, aluminum (foil and brushed), and PETE were consistent with the expected decrease in enzyme activity. Surprisingly, on the stainless steel surface the enzyme did not inactivate. However, this may be consistent with the lower UV reflectivity as compared to aluminum.

Significance: The type of food contact surface being exposed to 278 UV LED can affect the dose required to inactivate RNase A, suggesting that the same may be true for microbial contamination. Therefore, non-UV reflective surfaces require a higher 278 nm UV LED dose than reflective surfaces.

P3-35 Antilisterial Efficacy of Cranberry Extract in Produce Wash Treatments

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Introduction: Cranberry has been shown for its antimicrobial properties against foodborne organisms.

Purpose: As fresh produce remains one of the most outbreak-implicated food vehicles, this study aimed to evaluate the antimicrobial effect of cranberry extract (CE) in produce wash treatments to improve food safety.

Methods: CE at 5-10%, and a commercial plant-based all-natural produce wash (PW) at 5% were tested *in vitro* individually and in combination against *Listeria innocua* at pH 3.2, 25°C for 0, 5 and 15 mins. Further antimicrobial effect evaluation of 5-10% CE, 5-100% PW, 5% PW+5% CE and 5% PW+10% CE was carried out in a 2-min wash model using tomatoes with *L. innocua* surface inoculation, at 25°C. Water was used as a wash solution control. Washed tomatoes and spent wash solutions (as potential sources of cross-contamination in wash process) were tested for their listerial loads. Each experiment was replicated twice.

Results: *In vitro*, 5% PW showed significant *Listeria* reduction by up to 4.2 log CFU/mL after 15-min exposure, while 5-10% CE was not effective individually. When 5% PW+5% CE was used, 4- and 5.3-log reduction was shown at 5 and 15-min, respectively. In the wash model, 5-10% CE reduced >1 log *Listeria* on tomatoes, and >1.5-log reduction was achieved by 100% PW. Combinations of 5% PW+5% CE and 5% PW+10% CE did not improve wash effectiveness, with no further decrease of *Listeria* on tomatoes. *Listeria* load in spent CE-containing wash solutions was lower than 5-100% PW solutions. Additional reduction of *Listeria* by 1.5 and 2 log CFU/mL in spent wash solutions was evidenced in 5-10% CE and 5% PW+10% CE, respectively, when compared to 100% PW.

Significance: CE has great potential for use in produce wash formulation as it could reduce *Listeria* levels on tomato surface. Additionally, CE could lower cross-contamination chances in the wash process by decreasing organism load in spent wash solution when used alone or in combination with commercial PW.

P3-36 Plant-based Antimicrobials Inactivate *Salmonella enterica* and *Listeria monocytogenes* on Melon Rinds

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Introduction: In the past few years melons have been involved in several foodborne outbreaks. Effective control measures are needed to improve melon safety.

Purpose: The objective of this study was to evaluate the efficacy of plant-based antimicrobials against *Salmonella* Newport and *Listeria monocytogenes* on melon rinds.

Methods: The tested melons included 5 cantaloupe varieties, 3 honeydew varieties and 7 cantaloupe hybrids grown in 6 states. Sixty-three melon samples were analyzed for *S*. Newport and *L. monocytogenes*. Melon rinds were cut into 10 g pieces and inoculated with 10^6 CFU/mL of *S*. Newport or *L. monocytogenes* culture. Rind samples were then immersed in 5% olive extract or 0.5% oregano oil antimicrobial solution and gently agitated for 2 min. Phosphate buffered saline was used as a control. After treatment, the surviving populations of *S*. Newport and *L. monocytogenes* were enumerated at Day 0 and 3. The rind samples were mixed with 90 mL of buffered peptone water and stomached for 2 min. Serial dilutions were plated on xylose lysine desoxycholate agar and Modified Oxford agar for enumeration of *S*. Newport and *L. monocytogenes*, respectively. Experiments were repeated three times.

Results: The plant-based antimicrobials reduced *S*. Newport and *L. monocytogenes* population on all rind samples, regardless of the melon types, varieties or growing locations. Compared to PBS control, the plant-based antimicrobial treatments caused 2 to 3.6- and 1.6 to 3.7-log reductions in populations of *Salmonella* and *L. monocytogenes*, respectively. In most cases, the plant-based antimicrobial treatments reduced pathogen population to below the detection limit (1 CFU/g) at Day 3. In general, oregano oil had better antimicrobial activity than olive extract. The antimicrobial treatments were more effective on *Salmonella* than on *L. monocytogenes.* The antimicrobial treatments exhibited better reductions on honeydews than on cantaloupes.

Significance: The results showed that plant-based antimicrobials have the potential to be used as sanitizers for decontaminating melons.

P3-37 Green Sanitizers: Improved Safety and Shelf-life of Iceberg Lettuce Washed with Plant-based Antimicrobial Microemulsions

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❖ ***Developing Scientist Entrant***

Introduction: The produce industry currently uses chlorine to wash vegetables post-harvest. However, chlorine could be disadvantageous because it is not sustainable to the environment, not user friendly, corrosive to equipment, and loses efficacy with organic matter. Alternatives to chlorine are needed.

Purpose: The objective was to evaluate alternatives to wash water sanitizers for lettuce that better meet the needs of consumers and improve food safety. Plant-based antimicrobials in the wash water were evaluated for their efficacy against foodborne pathogens *Salmonella enterica* serotype Newport, *Escherichia coli* O157:H7, and a spoilage organism *Lactobacillus casei* on Iceberg lettuce.

Methods: Wash water microemulsions evaluated included oregano, lemongrass, and cinnamon essential oils along with a plant-based emulsifier for improved solubility of the oil in water. Iceberg lettuce (10 g) was washed thoroughly and inoculated with either *S. enterica* Newport (5.0 log CFU/g), *E. coli* O157:H7 (5.0 log CFU/g), or *L. casei* (6.0 log CFU/g). Leaves were treated by immersing in 0.1%, 0.3%, or 0.5% of one of the treatment solutions for 2 min with gentle agitation, stored at 4°C, visually observed, and analyzed for surviving populations of *S.* Newport, *E. coli* O157:H7, and *L. casei* on days 0, 3, 7, 10, 14, 21, and 28. Samples were serially diluted in 0.1% peptone water and plated on media appropriate for each test organism.

Results: The efficacies of antimicrobials were concentration- and storage-time dependent. While the efficacies of essential oil microemulsions varied among the test bacteria, they exhibited 2.0 to 5.0-, 0.22 to 5.0- and 0.16 to 5.0-log CFU/g reductions in populations of *S.* Newport, *E. coli* O157:H7, and *L. casei,* respectively, during days 0-28. The inoculated leaves (all 3 bacteria) from the two most effective treatments – 0.3% and 0.5% oregano and cinnamon oils, respectively, also showed better visual appearance on days 0-28 than their respective controls.

Significance: The essential oil microemulsions with lasting residual activity provide natural, eco-friendly, and effective alternatives in small concentrations to chemicals for produce decontamination.

P3-38 Inhibition of Surface Spoilage Bacteria on Refrigerated Catfish Fillets by Various Chitosan Applications

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Introduction: Consumers have increased their demands for more natural preservatives used in food products. Chitosan, a complex polysaccharide, has been shown to possess antimicrobial properties and has potential to be used as an alternate to synthetic preservatives commonly used in foods. Fish, unlike beef and pork, are very delicate and spoil easily, with limited shelf life once thawed.

Purpose: The objective of this experiment was to determine if various commercial applications of a chitosan solution would extend the shelf life of refrigerated catfish fillets.

Methods: Non-frozen, refrigerated catfish fillets were purchased from a local grocery store and aseptically cubed into 25-g samples. The cubes were then separated and subjected to different treatment applications of a patent pending chitosan solution: control (CT), chitosan steam (ST), chitosan dipping (DP), and chitosan electrostatic spray (ES). Samples were stored at 4°C and microbiological analysis occurred intermittently on days 0, 2, 5, and 8. Samples were plated on APC Petrifilm, to determine total plate count, and incubated at 37°C for 24 hours.

Results: Initial microflora for all samples, including the control, had average counts of 4.87 log CFU/g. By day 2 all samples excluding the dip treated cubes had significantly ($P < 0.05$) increased microbial growth. Microbial counts for all samples increased by the final day. On day 8 both the dip treated, and electrostatic spray treated samples had significantly ($P < 0.05$) lower microbial growth compared to the control, 2 log CFU/g and 1 log CFU/g, respectively.

Significance: Both the dipping and electrostatic spray method for applying chitosan proved to be suitable applications to extend the shelf life of refrigerated catfish fillets.

P3-39 Inhibition of *Listeria* Biofilms By Cranberry Extract

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Introduction: *Listeria* survives in food processing environments despite cleaning and sanitization. *Listeria* can survive in protected, multi-species biofilms and some strains are resistant to alkaline and oxidative stress.

Purpose: The purpose of this study was to demonstrate antimicrobial activity of cranberry extract (CE) against *Listeria innocua*.

Methods: To examine survivorship, *L. innocua* was suspended (5 logs/mL) in serial dilutions of CE neutralized to pH 7.0. Samples were collected periodically and plated on BHI agar. Subsequently, *L. innocua* biofilms were grown on glass beads submerged in BHI broth or 12.5% neutralized CE in BHI (3 replicates each). *L. innocua* was inoculated (5 logs/mL) and incubated at room temperature for 48 hours. Beads were rinsed in deionized water and sonicated to remove biofilm. Samples were filtered, Live-Dead stained (Molecular Probes BacLight), and enumerated.

Results: In suspension, a 4-log reduction in *L. innocua* was observed after 18 hours in 12.5% CE and 25% CE. Smaller log reductions were observed at lower concentrations (2 logs at 6.25% CE and 1 log at 3.13% CE). In the biofilm experiment, there was a > 1-log reduction in both live and dead cells in biofilms grown in 12.5% CE. The formation of live microcells in the presence of CE was evidence of a stress response; poorly defined dead cells and debris were apparent with the dead stain.

Significance: This study demonstrated inhibition of *L. innocua* by CE in suspension and biofilms. It did not distinguish between physiological stress and other mechanisms of biofilm inhibition (e.g., preventing attachment or extracellular matrix production). CE may be developed as an addition to cleaning and sanitation regimes for *Listeria* control. Future studies will examine mechanisms of biofilm inhibition and impact of CE on *L. innocua* in multispecies biofilms.

P3-40 Assessing the Efficacy of Addition of Sodium Bisulfate (SBS) in Stored Wheat Grains to Control *Aspergillus flavus* (ATCC 15548)

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Introduction: Grains are an important part of human food and livestock feed. Yeast and molds are naturally occurring spoilage organisms in grains and grain-derived products and can produce mold toxins which are harmful to human and animal health. Tempering of wheat before milling is done to ease the bran and endosperm separation and may provide an avenue to reduce mold spores.

Purpose: To determine the effect of sodium bisulfate (SBS) against *Aspergillus flavus* (ATCC 15548) in tempered wheat.

Methods: Minimum inhibitory concentration (MIC) of SBS against *A. flavus* was determined using the broth micro-dilution assay. The *A. flavus* was inoculated in wheat and given an attachment time of 30 mins followed by the treatment with antimicrobial. The final moisture of the tempered wheat was adjusted to 16% wet weight basis. The treated wheat samples were stored at 25°C for 48 h. Microbiological analysis of wheat were performed at different time intervals (0, 6, 12, 18, 24, and 48 h) by diluting and plating on potato dextrose agar and incubating at 25°C for 72 h. The experiment was conducted in triplicate.

Results: The MIC of SBS against *A. flavus* was found to be 0.3125%. On wheat, 0.5% led to1.7-log reduction ($P < 0.05$) at 48 h, whereas, 1.0% and 1.5% SBS reduced *A. flavus* by ~1.5 logs ($P < 0.05$) within 18 h. The *Aspergillus* recovery from positive control (no SBS) wheat was ~5logs. Across different time points, 0.5% SBS was most effective ($P < 0.05$) with 1.3-log reduction at 48 h; whereas, 1.0% and 1.5% SB were equally potent throughout the storage time.

Significance: This study suggests that the use of SBS during wheat tempering may reduce the mold load in wheat which might decrease the potential for mycotoxin production.

P3-41 Effect of Sub-inhibitory Concentrations of Antimicrobials on Listeria monocytogenes Motility and Its Ability to Adhere to and Invade Caco-2 Cells

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❖ ***Developing Scientist Entrant***

Introduction: Exposure to sub-inhibitory concentrations (SICs) of antimicrobials on foods may alter pathogen virulence by reducing expression of important virulence factors or inducing stress tolerance responses. Hydrogen peroxide (HP), ε-polylysine (EPL), and lauric arginate ethyl ester (LAE) have been shown to inhibit the growth of *Listeria monocytogenes* (*Lm)* in various foods but the potential effects of exposure to SICs of these antimicrobials is not known.

Purpose: The purpose of this study was to determine the effects of SICs of EPL, HP, and LAE on *Lm* motility and adhesion and invasion of Caco-2 cells.

Methods: *Lm* strains Scott A and 2014L-6025 were grown to mid-log phase (5 h) in the presence of SICs of each antimicrobial in brain heart infusion (BHI) broth at 37°C prior to use. Resulting cultures were spot inoculated onto BHI agar (0.3% agar) and motility was measured daily over 48 h at 20°C, 30°C, and 37°C. Caco-2 monolayers in 12-well plates were inoculated with 6 log CFU (10 MOI) of *Lm.* Adhesion was determined by harvesting cells after 1 h at 37°C. For invasion assays, gentamicin was added to Caco-2 monolayers after 1 h of adhesion and incubated for another hour prior to harvest and enumeration.

Results: No significant differences were observed between any of the treatments compared to controls as ~5 log CFU/mL of *Lm* were able to attach to Caco-2 cells and ~3 log CFU/mL were able to invade regardless of strain or antimicrobial used. Observed motility was highest at 30°C followed by 37°C but was not affected at any temperature following pre-exposure to SICs of antimicrobials.

Significance: These data demonstrate strain variability when *Lm* is exposed to SICs of antimicrobials but suggest that exposure to SICs of EPL, HP, and LAE may not affect *Lm* virulence based on motility or the ability to adhere to and invade Caco-2 cells under the conditions tested.

P3-42 Inactivation of Biofilms of Multiple Foodborne Pathogens Using Antimicrobial Nanoconjugates

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❖ ***Developing Scientist Entrant***

Introduction: Capacities of foodborne pathogens to form biofilm help their persistence in the food processing environment and repeated product contamination.

Purpose: This study aims to develop an eco-friendly and biodegradable food-grade antibacterial nanoconjugate to inactivate biofilm of multiple foodborne pathogens in the food processing and slaughterhouse environment.

Methods: We prepared the nanoconjugates by synthesizing chitosan nanoparticles (ChNP) conjugated to a broad-spectrum antimicrobial polypeptide, ε-poly-L-lysine (PL) using ionic gelation method. Sodium triphosphate was applied as a polyanionic linker for nanoparticle formation. Synthesized ChNP-PL

was further processed by sonication and ultrafiltration to improve overall size distribution which was measured using Malvern Zetasizer. ChNP-PL activity (prevention or inactivation of biofilm) was tested against biofilms (on polystyrene multi-well plates after 48 h) produced by mono or mixed cultures including *Listeria*, *Pseudomonas*, *Staphylococcus*, *E. coli* and *Salmonella* by plate counting and Crystal violet staining methods.

Results: The average diameter of ChNP-PL was determined to be around 100 nm. Analyzing bacterial inhibition zone caused by ChNP-PL with or without removing unbound PL revealed that over 90% of PL was successfully conjugated. Minimal inhibitory concentration test showed that PL conjugation significantly ($P < 0.05$) increased the antimicrobial activity of ChNP. The ChNP-PL preparation prevented biofilm formation of all five bacterial pathogens to the undetectable level while significantly inactivated pre-formed (24- or 48-h old) biofilms when compared with ChNP-treated samples. Crystal violet staining further confirmed biofilm prevention and inactivation by ChNP-PL.

Significance: These findings suggest the potential application of biodegradable eco-friendly antimicrobial nanoparticles (ChNP-PL) as a substitute for harsh cleaning agents for inactivation or prevention of biofilms in food production/processing environments. This research was supported by a grant from NAS and USAID (AID-263-A-15-00002) and the conclusions or recommendations expressed here are those of the author(s) and do not necessarily reflect the view of the USAID or NAS.

P3-43 Microbial Diversity, Antimicrobial Resistance and Virulence Genes in Small-scale Poultry and Cattle Farms

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Introduction: Antimicrobial resistance (AMR) is a growing threat of public concern. AMR in food animals has mainly focused on large-scale production systems as compared to small-scaled animal farms.

Purpose: This study aimed to characterize the microbiomes and resistomes from different components in small-scaled poultry and cattle farms.

Methods: Metagenomic Taxonomic approach was applied to characterize microbial diversity, antibiogram and virulence genes of bacteria from poultry and cattle farms. DNA samples from feces ($n = 22$), soil ($n = 18$), water ($n = 13$), and soil from natural land ($n = 2$; control) was analyzed. Unassembled sequencing reads were directly evaluated by CosmosID metagenomic software to reveal associated microbial community, resistance and virulence genes composition.

Results: Relative abundance of genes were significantly high in *Bacteroides fragilis* (42.3% and 76.9%), followed by *Klebsiella pneumonia* (11.6% and 3.28%), *Enterococcus faecium* (10.09% and 2.33%), *Proteus mirabilis* (9.02% and 1.44%), *Staphylococcus lentus* (5.7% and 0.2%), *Streptococcus pyogenes* (2.87% and 5.73%), *Salmonella* GENE tetR (0.4 % and 1.3%), and *Salmonella* Typhimurium (0.3% and 1.3%) from poultry and cattle farms, respectively. Results show that resistance was significantly ($P < 0.05$) higher in tetracycline with relative abundance of 33.4% followed by beta-lactam-resistance (30.3%), macrolide (9.3%), aminoglycoside (8.3%) and repressor-for-mdr-efflux-pump (4.1%). Very low relative abundance of genes conferred resistance to trimethoprim (2.5%), sulphonamide sul2 (1.5%), MDR-Efflux-pump (1.0%), phenicol (0.6%), efflux-pump (0.4%), among others. A total of 147 virulence genes were identified and significantly ($P < 0.05$) higher in *Enterococcus faecalis* (22, 45.5, 30.8%) as compared to *Morganella morganii* (2, 0, 15.4%), followed by *Salmonella* GENE tetR (0, 40.9, 23.1%), *Salmonella* Infantis (5.6, 0, 7.7%), *Salmonella* Typhimurium (0, 40.9, 15.4%), *Shigella flexneri*, (0.0, 0, 7.7%) from soil, manure, and water, respectively.

Significance: Our data provide input to monitor and elucidate antimicrobial resistant and virulence genes dissemination between and among microbial communities in food animal farming systems.

P3-45 Lytic Bacteriophage Help to Reduce *Salmonella* Typhimurium from Raw Chicken Breast

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Introduction: Out of 2,300 serotypes, *Salmonella* Enteritidis and *Salmonella* Typhimurium account for almost half of all human infections in the US. Risk groups include all ages, however immune-compromised, elderly, and infants are at a higher risk of contracting *Salmonella* infection. In addition, strains of antibiotics-resistant *Salmonella* are on the rise in the last few decades.

Purpose: The purpose of this study was to evaluate the effect of lytic bacteriophages (phage) and Lactobionic acid (LBA) as an antimicrobial in reducing the *Salmonella* Typhimurium DT 104 present on raw chicken breasts.

Methods: *Salmonella* Typhimurium DT 104 was maintained on Luria-Bertani (LB) broth throughout the experiment. Raw chicken breast (5 cm X 5 cm, $N = 84$) were experimentally inoculated with *S.* Typhimurium strains (10^6 CFU/mL for 30 min attachment at room temperature). Raw chicken breasts were randomly allotted to one of the seven treatments per replication (Control, DI water, 1% phage, 5% phage, 10 mg/mL LBA, 20 mg/mL LBA, 5% phage + 20 mg/mL LBA). The concentrations were made based upon the manufacturer's recommendation. SPSS Univariate Analysis of Variance was used for the data analysis.

Results: The application of lytic bacteriophage (1% and 5%) on chicken breast results in the significant reduction ($P < 0.05$) of *S*. Typhimurium counts, however, there was no treatment*time interaction. Likewise, a significant reduction ($P < 0.05$) of *S*. Typhimurium was observed when 5% of phage and 20 mg/mL LBA were used in combination on chicken breast. Out of all seven different treatments, the highest log reduction was shown by 5% phage, which reduced the pathogen by 2.17 log CFU/cm^2.

Significance: These data suggest that the application of lytic bacteriophage significantly reduces the presence of *S*. Typhimurium on raw chicken breasts.

P3-46 Inactivation of *Campylobacter jejuni* on Artificially Inoculated Chicken Skin by Organic Acids Alone or Combined with *Yucca* Extract

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Introduction: *Campylobacter jejuni* on poultry products is a major food safety concern to regulatory agencies, meat processors and consumers. Because poultry skin is hydrophobic and can resist thorough wetting by commonly used water-based antimicrobials, there is an urgent need for effective interventions to destroy them during processing of raw poultry products.

Purpose: The purpose of this study was to determine the antibacterial effectiveness of lactic/citric acid along and in combination with *Yucca* extract (YEX) against *C. jejuni* chicken skin.

Methods: The antibacterial effectiveness of a lactic/citric acid mixture (1.5% and 2.5%) or acetic acid (1.0% and 2.0%), alone or combined with YEX (0.25% and 0.5%), was evaluated against *Campylobacter jejuni* (10^7 CFU/mL) in vitro (0.85% NaCl) and on raw chicken skin. The 0.85% NaCl (saline) without organic acids or added YEX served as controls. Pathogen survivors were evaluated after 0, 30, 60 and 90 s of in vitro exposure to treatment solutions. Survivors on chicken skin were evaluated after 2.0 min of immersing inoculated chicken skin in treatment solutions. Survivors were determined by plating samples on Mueller Hinton agar with added *Campylobacter* growth supplement and Preston *Campylobacter* selective supplement. The inoculated agar plates were

incubated at 42°C under microaerophilic atmosphere. Bacterial colonies were counted after 48 h. Means were separated using LSMEANS and significant differences were defined at $P < 0.05$.

Results: Lactic/citric (2.5%) or 2.0% acetic acid with added 0.5% YEX exhibited the largest reduction in populations of *C. jejuni* in vitro as well as on chicken skin at 90 s ($P < 0.05$). The addition of YEX (0.5%) to Lactic/citric acid (2.5%) or to acetic acid (2.0%) can enhance the antibacterial activity of those organic acids against *C. jejuni* on raw chicken skin.

Significance: These findings offer a strategy for the inactivation of *C. jejuni* using naturally derived compounds combined with organic acids.

P3-47 Efficacy of Sodium Bisulfate (SBS) in Reducing the Shiga Toxin-producing *E. coli* STEC (O121) Load of Wheat During Tempering

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❖ ***Developing Scientist Entrant***

Introduction: There have been multiple STEC (O121 and O26)-linked recalls involving wheat flours. These products were previously not considered as a route for foodborne illness in humans but now a solution to control *E. coli* contamination in wheat is needed. Tempering adds water to the wheat before milling improving its yield and flour quality. Modifying this step may potentially provide a route to control STEC.

Purpose: To evaluate the efficacy of SBS in reducing *E. coli* O121 (ATCC-2219) levels in wheat during tempering.

Methods: The minimum inhibitory concentration (MIC) of SBS was determined using broth-microdilution assay. Autoclaved wheat samples were tempered to 17% moisture content (wet basis) with liquid inoculum and SBS solutions (1:1 ratio). Wheat samples were inoculated with *E. coli* (~6 log CFU/g wheat) and incubated (37°C; 30 minutes) followed by the SBS treatments (0.5, 0.75, 1.0, 1.25, and 1.5% wheat weight). Negative (uninoculated wheat + water) and positive (inoculated wheat + water) were produced. Treatments were tempered for 24 hours with analyses conducted at different time intervals (0.5, 2, 6, 12, 18, and 24 h) by serial dilution and plating on tryptic soy agar. Plates were incubated (37°C; 24 hours) and enumerated for *E. coli*. Experiments were conducted in triplicates.

Results: The MIC of SBS against *E. coli* O121 was found to be 0.32%. At 0.5 h, all SBS treatments reduced *E. coli* O121 levels in wheat by ≥ 1 log with maximum reduction (~2 logs; $P < 0.05$) for 1.5% SBS. By 24 h, all SBS treatments reduced *E. coli* by ≥2 logs ($P < 0.05$) with the 1.5% SBS reducing *E. coli* to a non-detectable level suggesting complete elimination of *E. coli*.

Significance: The results indicate that adding SBS to the tempering water can effectively reduce the *E. coli* load of wheat during the milling process.

P3-48 Antimicrobial Properties of Proanthocyanidins in *Ohelo* Berry (*Vaccinium calycinum*) Against *Escherichia coli* O157:H7

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❖ ***Developing Scientist Entrant***

Introduction: *Ohelo* berry (*Vaccinium calycinum*) is a Hawaiian wild relative of cranberry (*Vaccinium macrocarpon*). The concentration of proanthocyanidins in *ohelo* berry is approximately twice as high as that in cranberry, which is known to have antimicrobial activities against pathogenic bacteria.

Purpose: This study aimed to investigate the antimicrobial, antibiofilm, and antimotility effects of *ohelo* berry proanthocyanidins (oPAC) on *Escherichia coli* O157:H7.

Methods: Extracts of proanthocyanidins were prepared from freeze-dried ohelo berries. The minimum inhibitory concentration (MIC) of oPAC was determined against *E. coli* O157: H7 and probiotic *Lactobacillus rhamnosus* GG. The antibiofilm and antimotility activities of oPAC against *E. coli* O157:H7 were evaluated using the standard microtiter plate assay and semisolid agar assay, respectively.

Results: The MIC of oPAC against *E. coli* O157:H7 was 300 μg/mL, while the growth of probiotic *L. rhamnosus* GG was not affected even at the highest tested oPAC concentration (800 μg/mL). A sublethal concentration of oPAC (100 μg/mL) significantly inhibited biofilm formation of *E. coli* O157:H7 by 62%. In addition, it significantly impaired flagella-mediated motility of *E. coli* O157:H7. The colony diameter was 3 mm compared to 33 mm for the untreated cells.

Significance: These results indicated that proanthocyanidins in ohelo berry have the potential to conserve probiotic bacteria while inhibiting pathogenic bacteria. It may be useful as a natural antimicrobial for preserving food, enhancing gut health, and treating urinary tract infection.

P3-49 Effect of Neem Oil Nanoparticles on the Growth Inhibition of Peanut Mold*, Aspergillus flavus*

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❖ ***Developing Scientist Entrant***

Introduction: Aflatoxin production occurs when *Aspergillus flavus* invades peanuts, cotton seed, corn and certain nuts under favorable conditions of temperature and humidity. Under improper storage conditions, it is capable of growing and forming aflatoxin in almost any crop seed. Most of the time it is challenging to prevent the growth of the fungus and aflatoxin production. Therefore, it is essential to apply antifungal agents to prevent the growth of *Aspergillus flavus* and control its aflatoxin production.

Purpose: The aim of this study was to develop and characterize neem oil-loaded polymeric nanoparticles (NPs) that can be used to effectively control the growth of *Aspergillus flavus*.

Methods: A rapid nano-precipitation method was used for the preparation of neem oil NPs. Particle size distribution of neem oil-loaded NPs was measured by dynamic light scattering (DLS). Changes in concentration of free neem oil were tracked by spectroscopy using a UV-1800 UV/Visible scanning spectrophotometer. Antifungal activity of both neem oil-loaded NPs and neem oil were tested for their 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 10 wt.%. Treated samples and control samples were stored up to 7 day at 25**°**C, after which samples were counted for mold growth.

Results: Neem oil-loaded NPs were characterized for size, zeta-potential, release, encapsulation efficiency, and inhibition of *Aspergillus flavus*. The NPs showed a size 300.6 nm, zeta potential -16 mV, with an encapsulation efficiency 84.8 ± 3.3 wt.%. Neem oil-loaded NPs inhibited *Aspergillus flavus* growth at much lower concentrations as compared to unencapsulated neem oil. Neem oil-loaded NPs displayed sustained release with a time constant of 120 h, maintaining their anti-pathogenic properties over a prolonged time period.

Significance: This study demonstrates neem oil can be loaded into polymeric nanoparticles with sustainable release profile. Nano-encapsulation of hydrophobic antimicrobials could improve their solubility, stabilization, and antimicrobial activity. Neem oil nanoparticles with lower neem oil concentration were significantly more effective than pure neem oil in inactivation of *Aspergillus flavus*. Antifungal NPs may be useful decontamination of various crops.

P3-50 Antimicrobial, Physical and Mechanical Properties of Polyvinyl Alcohol Films Incorporated with Modified Bacterial Nanocellulose

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Introduction: Biopolymers, such as bacterial nanocellulose (BNC), have a great potential to enhance the functional properties of packaging films. Active packaging can improve the quality, shelf life and safety of food products.

Purpose: This research aimed to study the effect of modified BNC (mBNC) on the antimicrobial and physical properties of polyvinyl alcohol (PVA) films for food packaging applications.

Methods: BNC, produced by *Gluconobacter xylinum*, was modified and functionalized with aminoalkyl groups using N-[3-(trimethoxysilyl)propyl]ethylenediamine. The mBNC, at concentrations of up to 10 wt%, were added to 5 wt% PVA aqueous solutions. The PVA/mBNC nanocomposite films were fabricated using a solution-casting method. The antimicrobial properties of the composite films were examined by exposing a cocktail (≈10^5 CFU/g) of five strains each of *Escherichia coli* O157:H7 and *Salmonella* to the films (1.5 cm × 3 cm) for 24 h. Film (1 cm × 5 cm) tensile strength (TS) and elongation at break (%E) were measured using a texture analyzer. The moisture content of the films (2.5 cm × 2.5 cm) was measured using a series of published methods. Color variations of the films were studied using a colorimeter.

Results: PVA/mBNC nanocomposite films were successfully fabricated with various percentages of mBNC. Growth of *E. coli* O157:H7 and *Salmonella* was significantly ($P \leq 0.05$) reduced by films containing 10% mBNC after 8 h and 4 h of incubation, respectively. Upon incorporation of 10% mBNC, the TS and %E of the films significantly ($P \leq 0.05$) increased by 3.2% and 49.7%, respectively, while the moisture content of the films decreased by 28.7%. The color of the films became darker, greener and yellower with increasing concentrations of mBNC.

Significance: Incorporation of mBNC into PVA films may enhance the antimicrobial activity, and physical and mechanical properties of active packaging films with potential for food packaging applications.

P3-51 Evaluation of Natural Chelating Compounds for Use As Enhancers of Quaternary Ammonium Compound Efficacy

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Introduction: Quaternary ammonium sanitizers (quats) have a long history of use by industry, but may also be perceived as being environmentally unfriendly and not possessing a clean or clear label. New strategies are needed to enhance the efficacy of quat-based sanitizers, promoting the development of effective yet label-friendly formulations.

Purpose: In this study, we sought to develop modified quat-based sanitizer formulations using natural, functional food ingredients, specifically chelators, as enhancers of quat efficacy. The resulting formulations are expected to enhance the killing of foodborne pathogens, potentially allowing the use of quat compounds at lower effective doses.

Methods: An initial literature search identified candidate food ingredients having previously reported or potential chelating activities. A colorimetric assay for chelation activity was used to screen for and measure their metal chelation capacity, using EDTA as a chelation standard. A subset of the most promising compounds was identified and baseline minimum inhibitory concentration (MIC) data were collected so that sub-inhibitory levels could be evaluated via broth microdilution against target pathogens in combination with the model quat cetylpyridinium chloride.

Results: Chelation-positive compounds included long-chain polyphosphates, phytic acid, ascorbic acid, tartaric acid, ε-polylysine and quercetin. In concept-confirming experiments, EDTA was shown to have QAC-enhancing capabilities against test organisms, including *E. coli* ATCC 25922, *S.* Typhimurium ATCC 14028 and *L. monocytogenes* ATCC Scott A. Among natural chelators examined, ascorbic acid showed additive and phytic acid synergistic effects, when combined with CPC. As an example, phytic acid (25 mM) was able to synergistically lower the MIC of CPC against *E. coli* ATCC 25922 from 16 µM to 1.25 µM.

Significance: Our results demonstrate the utility of natural functional food compounds for enhancing the activities of model sanitizer compounds, such as CPC, laying the foundation for value-added natural chelators from agricultural waste streams for development of enhanced sanitizer formulations.

P3-52 Isoeugenol Prevents Yeast Spoilage of Refrigerated Raw Pineapple Juice Containing an Extract of *Quillaja saponaria* or *Yucca schidigera*.

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❖ ***Developing Scientist Entrant***

Introduction: Poor solubility of isoeugenol (ISO) can reduce its antimicrobial efficacy in beverages such as fruit juices. *Quillaja saponaria* extract (QSE) and *Yucca schidigera* extract (YSE) are natural surfactants that can overcome such miscibility problems. There are no published reports on the antimicrobial efficacy of isoeugenol in combination with QSE or YSE against yeast and molds in raw pineapple juice.

Purpose: This study evaluated the effectiveness of isoeugenol for inhibiting growth of yeasts and molds in refrigerated (4°C) raw pineapple juice (PJ) with added QSE or YSE.

Methods: Raw pineapple juice (pH 3.47; 13.9 °BRIX) with 0.5% YSE or 0.5% QSE and containing 0 (control), 0.50, 0.75, 1.0 or 1.5 µL/mL isoeugenol was stored at 4°C. Juice without YSE, QSE and isoeugenol served as another control. Survivors of fungi were determined at set intervals by surface plating diluted (10-fold) aliquots of juice on Dichloran Rose Bengal Chloramphenicol (DRBC) agar, and counting fungal colonies after incubation (25°C; 5 days).

Results: QSE and YSE inhibited the growth of fungi in raw juice without isoeugenol. After 24 days yeast and mold count (log CFU/mL) in juice (no isoeugenol) with or without QSE reached 6.31 and 6.70, respectively. Counts in juice (no isoeugenol) with added YSE reached 4.31 log CFU/mL. After 24 days of refrigeration, 1.5 µL/mL isoeugenol with added YSE completely inactivated yeast and molds in juice, whereas, that same concentration of isoeugenol with added QSE decreased initial fungal counts by 4.20 log CFU/mL ($P < 0.05$).

Significance: Growth of yeast and molds in raw juices can cause significant economic loss due to spoilage. Isoeugenol combined with a natural surfactant such as YSE or QSE has good potential for extending the microbial shelf life of refrigerated PJ.

P3-54 Inactivation of *Listeria monocytogenes* in Cheese Brines Treated with Hydrogen Peroxide

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Introduction: Salting is an important step in cheese manufacturing to add flavor. Commercial cheese brines are used repeatedly over extended periods and can be a reservoir for salt-tolerant pathogens.

Purpose: To determine the inactivation of *Listeria monocytogenes (Lm)* in commercial cheese brines treated with hydrogen peroxide during incubation at 12.8, 7.2, and 0°C.

Methods: Eight commercial brines (representing 5 cheese types, 12-30% NaCl, pH 4.5-5.5) were inoculated with ~5.5 log CFU/mL *Lm*, treated with 0, 50, or 100 ppm H_2O_2, and stored at 12.8, 7.2, and 0°C. Triplicate samples (duplicate for 0°C) per treatment were enumerated on days 0, 1, and 7 by plating on Modified Oxford Agar. In addition, samples were assayed for lactic acid bacteria, mold/yeast, total plate count, pH, and residual H_2O_2. Each experiment was conducted three times.

Results: Populations of *Lm* were unchanged (<0.7 log reduction) through Day 7 in 6 of 8 control brines containing no H_2O_2. In contrast, *Lm* decreased ≥1 log in Feta E (pH 4.5, 16% NaCl) at 7 days and >4 log in Gorgonzola F (pH 4.9, 19% NaCl) at 1 day. H_2O_2 had no effect on *Lm* populations in Brick J (pH 5.5, 15% NaCl) due to inactivation of H_2O_2 within 30 minutes by indigenous yeasts (~3 log CFU/mL). Addition of 100 ppm H_2O_2 reduced *Lm* >4 log in all other brines stored at 7.2 or 12.8°C for 7 days, but only 3 to 4-log reduction when stored at 0°C. Addition of 50 ppm H_2O_2 had similar lethal effects at 12.8°C, but was less effective at 7.2 or 0°C.

Significance: Pathogen survival in cheese brine depends on acidity and salt levels as well as background microbiota and storage temperature. The addition of 100 ppm H_2O_2 can be used to inactivate *Lm* in cheese brines with low populations of catalase-positive microbes to reduce the risk of post-pasteurization environmental contamination.

P3-55 Inhibition of *Listeria monocytogenes* by Bacterial Species Isolated from Wooden Boards Used for Aging Semi-soft Cheese

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Introduction: In a previous study, we identified a complex microbial community attached to wooden boards with *Brevibacterium*, *Brachybacterium*, *Staphylococcus*, *Psychrobacter* and *Corynebacterium* being among the most abundant bacterial genera.

Purpose: The purpose of this study was to assess the fate of *L. monocytogenes* in the presence of bacteria isolated from wooden boards used for aging cheese.

Methods: Wooden boards were obtained from local cheese production facilities. Surface samples were collected from 5 boards by scraping, inoculated into TSB and incubated at 11°C for 48 h, at which time the bacterial concentration was approximately 10^8 CFU/mL. We then added 10^4- 10^5 CFU/mL of *L. monocytogenes* 2203 (grown at 11°C) to the cultures and incubated at 11°C for up to 8 days. At various intervals samples were removed, diluted in saline and plated on Modified Oxford agar to estimate numbers of *L. monocytogenes*.

Results: Broth cultures of microbial communities recovered from all 5 wooden boards significantly inhibited growth of *L. monocytogenes* during an 8-day incubation at 11°C. In the presence of competing microflora *L. monocytogenes* multiplied 0.2. to 1.5 log CFU/mL, with two of the microbial communities restricting multiplication of *L. monocytogenes* to 0.3 log CFU/mL or less. In contrast, *L. monocytogenes* increased 4-5 log CFU/mL in the absence of competing microflora. We identified the most abundant bacterial species recovered from one board (*Leuconostoc mesenteroides* and *Staphylococcus equorum*). When *L. monocytogenes* was added to broth cultures of *L. mesenteroides* or *S. equorum* we observed nearly complete growth inhibition of *L. monocytogenes* by *L. mesenteroides* (0.4-log CFU/mL increase in 8 days) and partial inhibition by *S. equorum* (4.0-log CFU/mL increase), compared to a 5.0-log CFU/mL increase for *L. monocytogenes* alone.

Significance: These data suggest that the microbial communities present on wooden boards used to age cheese might inhibit growth of *L. monocytogenes*.

P3-56 Growth Potential of *Listeria monocytogenes* in Soft Ripened Cheeses

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is known to grow in soft ripened cheeses (*e.g.,* Brie; SRC) due to favorable moisture and pH conditions; however, the growth potential may not be uniform across all SRC.

Purpose: The objective of this study was to compare the growth of *L. monocytogenes* across multiple SRC of different origin and milk treatments, and to determine predictors of growth potential.

Methods: Twenty-three SRC produced from raw (n = 4), pasteurized (n = 18), or thermolyzed (n = 1) milk were tested. Cheeses were sub-divided into 3.0 g portions which were inoculated with ~3.0 log CFU/g of *L. monocytogenes* BCCDC-A3 (serogroup 4b/4d/4e). Growth was monitored by spread plating of triplicate portions of each cheese on PALCAM agar. Microbial enumerations of uninoculated portions of each cheese were conducted on M-17, MRS pH 5.4, and tryptic soy agars, and each cheese was analyzed for pH and water activity.

Results: The growth of *L. monocytogenes* differed significantly between cheeses (ANOVA; $P < 0.001$), increasing an average of 2.67 ± 1.38 log CFU/g over the 14-day period. Raw milk cheeses showed significantly lower growth than pasteurized milk cheeses (Wilcoxon test; $P = 0.042$), with an average increases of 1.49 ± 0.79 log CFU/g and 2.87 ± 1.39 log CFU/g over the 14 days, respectively. Paradoxically, the lowest growth of *L. monocytogenes* was observed in two pasteurized milk cheeses, suggesting other factors may be important. No significant correlation was observed between *L. monocytogenes* growth potential and microbial enumerations, pH or water activity. Ongoing microbiome analysis of the cheeses is expected to provide further insight.

Significance: The growth potential of *L. monocytogenes* differs between different SRCs. A better understanding of what factors contribute to *L. monocytogenes* survival may help producers reduce the risk of pathogen growth in their cheeses.

P3-57 Effect of pH, Salt, Temperature, and Hydrogen Peroxide on the Survival of *Listeria monocytogenes* in Model Cheese Brines

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Introduction: Commercial cheese brines are used repeatedly over extended periods and can serve as reservoir for salt-tolerant pathogens.

Purpose: To determine the effect of pH, salt, and hydrogen peroxide on the survival of *Listeria monocytogenes (Lm)* in model cheese brines held at 10 and 15.6°C.

Methods: Four model cheese brines were created to represent commercial brines with 10 and 20% salt at target pHs 4.6 and 5.4. Brines were inoculated with ~6 log CFU/mL *Lm* (5-strain mixture), treated with 0, 50, or 100-ppm H_2O_2, and stored at 10 and 15.6°C. Triplicate samples per treatment were enumerated on Day 0, 1, and 7 by plating on Modified Oxford Agar. Each experiment was conducted twice.

Results: Populations of *Lm* were unchanged (<0.5-log difference) in brines without H_2O_2 at Day-1, and 0.4 to 1.0 log lower than inoculated levels at Day 7. Addition of ≥50-ppm H_2O_2 inactivated >5 log *Lm* for both temperatures in all model brines assayed at Day 7. In 24 h, 100-ppm H_2O_2 inactivated >5 log *Lm* in all brines stored at 15.6°C except 10% salt-low pH brine, but not at 10°C. Fifty ppm H_2O_2 reduced *Lm* by 1-5 log with greatest inactivation in 20% salt-low pH brine at 15.6°C. The effect of H_2O_2 was validated in 4 commercial brines (unfiltered, pH 4.6-5.7; NaCl 19-30.5%). 100-ppm H_2O_2 inactivated >5 log *Lm* in all brines at Day 7. Greater than or equal to 50-ppm H_2O_2 inactivated >5 log *Lm* in pH 5.7-30.5% NaCl brine within 24-hours, but <1.5 log in others. The lowest inactivation rate (<3-log reduction at Day 7) occurred in pH 4.6-19% NaCl brine, which correspondingly had the highest background yeast populations.

Significance: This study confirmed that low pH, high salt, warmer temperature and 100-ppm H_2O_2 accelerate the inactivation of *Lm* in cheese brines. Data also suggest that presence of certain indigenous microbes may counteract the effect of H_2O_2.

P3-58 The Effect of pH and Cultured Sugar-Vinegar on the Growth of *Listeria monocytogenes* in a Model High-moisture Cheese

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❖ ***Developing Scientist Entrant***

Introduction: High-moisture, low-acid cheeses, like Hispanic-style cheeses, are susceptible to post-process contamination, and can support the growth of *Listeria monocytogenes* during refrigerated storage. Adding "clean-label" ingredients, such as bacterial fermentates may inhibit bacterial growth.

Purpose: This study determined the effectiveness of a commercial fermentate (cultured sugar-vinegar blend; CSV) to prevent *L. monocytogenes* growth in a model soft cheese at various pH values.

Methods: A model cheese (56% moisture, 1.25% salt, including pasteurized cream, water, micellar casein, lactose, sodium chloride; no starter) was prepared at pH 5.25, 5.50, 5.75, and 6.00 using lactic acid. Control cheeses had no CSV whereas test treatments at each pH contained 0.5% CSV (w/w). Formulations were inoculated with 3-log CFU/g *L. monocytogenes* (5-strain mix). Samples were aseptically portioned, vacuum-sealed, and stored at 4°C for eight weeks. Triplicate samples were assayed weekly for changes in populations of *L. monocytogenes* by surface plating on Modified Oxford agar. In addition, spoilage was monitored for changes in pH, total plate counts (aerobic plate count agar), mold/yeasts (acidified PDA), and lactic acid bacteria (MRS agar). The study was conducted twice.

Results: The addition of 0.5% CSV significantly inhibited *L. monocytogenes* growth in all model cheese formulations compared to the control ($P < 0.05$). Control cheeses at pH 6.0 and 5.75 supported a ≥1-log increase in *L. monocytogenes* at 1 week, whereas the 0.5% CSV treatments delayed growth through three and five weeks, respectively. Control cheese at pH 5.50 supported bacterial growth at 4 weeks, whereas the CSV addition delayed growth through eight weeks. No growth was observed at pH 5.25, regardless of addition of antimicrobial.

Significance: This study confirmed that clean label antimicrobials, such as cultured sugar-vinegar, inhibit growth of *L. monocytogenes* in soft cheeses, particularly at controlled pH. This strategy can be a practical barrier to enhance the safety of soft cheeses.

P3-59 Control of *Listeria monocytogenes* in High-moisture Mexican Queso Fresco Style Cheese

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Introduction: *L. monocytogenes* in refrigerated, ready-to-eat (RTE) natural cheese products can be controlled by formulation that contains one or more inhibitory substances or through processes such as fermentation or culturing. Traditional Mexican Queso Fresco are not fermented or cultured. Therefore, there is a risk for microbial growth including pathogens or spoilage organisms. The products evaluated in this study were made with starter cultures and a challenge study was conducted to demonstrate the control of *L. monocytogenes* in the formulation.

Purpose: The purpose of this study was to evaluate the control of *L. monocytogenes* in Mexican Queso Fresco style cheese formulations during storage at 4°C for 6-12 months.

Methods: Eight Mexican Queso Fresco style cheeses were inoculated with *L. monocytogenes* to achieve an initial inoculum level of 2-3 log CFU/g. The cheeses were held at 4°C for up to 12 months depending on the product shelf life. Four of the eight cheese formulations had average pH values of 5.25 and moisture levels of 49.6%. Three cheese formulations contained 0.1-0.3% sorbic acid and had average pH values of 5.6 and moisture values of 55.6%. One cheese formulation was the control with a pH of 5.5 and a moisture level of 52.27% without sorbic acid. The acceptance criteria was greater than 1-log increase of *L. monocytogenes* over initial inoculum levels during storage.

Results: With the exception of the control and 0.1% sorbic acid cheeses, *L. monocytogenes* counts did not increase >1 log CFU/g over the shelf life during storage at 4°C.

Significance: The use of starter cultures, maintaining target analyticals (pH 5.25 and moisture 49.6%), and/ or application of 0.2% sorbic acid will prevent the growth of *L. monocytogenes* in RTE Mexican Queso Fresco style cheese when stored at 4°C for up to 12 months.

P3-60 Rapid Detection of Microbial Contaminants in UHT Milk and Other Aseptic Dairy Products by an Application of the Bact/Alert 3D System for Industrial Sterility Testing

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Introduction: Ensuring fast and accurate microbial detection of contaminants such as bacteria is vital to produce safe products and meet quality standards. Automated microbial detection systems are useful for the dairy industry to take quick, reproducible and traceable decisions on the process and to release aseptic products in less time, while lowering inventory housing costs.

Purpose: To evaluate the effectiveness of BacT/ALERT 3D System for the detection of microbial contamination, four different strains (*Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus sporothermodurans*) were inoculated at different concentrations in UHT milk packs (1 L).

Methods: To evaluate UHT milk product compatibility with BacT/ALERT 3D System, ten sample types of shelf-stable and low-acid UHT milk products were evaluated for a total of 150 tests. In addition, shelf-stable whole milk and skimmed milk and lactose-free milk were inoculated with the different organisms and a comparison was performed between conventional analysis (petri dishes) and BacT/ALERT. For each species, five batches, five concentrations (ranging from 10 – 100,000 CFU), were evaluated in triplicate for reproducibility testing. The specificity of the positive culture bottles was verified by Gram staining and then subcultured to identify the microorganism grown.

Results: Product compatibility was established and all un-inoculated samples were reported negative. BacT/ALERT generally detected bacterial contamination between 3 and 14 hours depending on the concentration and type of bacteria inoculated except for the highly heat resistant bacteria, *Bacillus sporothermodurans* which was detected in >48 hours.

Significance: The experiments show that conventional microbial detection could be substituted by this rapid and automated technique, obtaining results with BacT/ALERT 3D in less than 1 day vs. 6 days for microbial contaminants of UHT milk products, demonstrating a novel application for quality control testing in the dairy industry.

P3-61 Evaluation of a Rapid Technology to Detect UHT Microbial Contamination in Milk and Dairy Products

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Introduction: In the production of commercial milk, ultra-high-temperature (UHT) pasteurization is a common technique used to eliminate spore-forming bacteria as well as vegetative microbial cells to render it into a commercially sterile product. Verifying commercial sterility of UHT products is critical to enable distribution and commercialization by milk and dairy producers.

Purpose: To evaluate the performance of a rapid microbial ATP-detection method in comparison to growth on agar and pH measurement for testing commercial sterility in UHT dairy products.

Methods: Twenty-four different UHT dairy products (ranging in volume from 290 mL to 1 L) were inoculated with each of the following microorganisms, *Bacillus licheniformis*, *Bacillus subtilis*, *Escherichia coli* and *Enterococcus faecalis* from a range of 10 to 10,000 CFU per sample. Three samples were used for each combination (product-microorganism-inoculum level). Each sample was evaluated using the rapid microbial ATP detection method, pH measurement, agar streaking (presence/absence) and microbial enumeration in agar. Ten samples were used as negative control for each matrix.

Results: Using a threshold value for microbial ATP detection with non-contaminated UHT containers, it was possible to detect microbial contamination when microbial populations reached an average of 5.58 x 10^5 CFU/brick, 2.07 x 10^5 CFU/brick, 6.98 x 10^5 CFU/brick, and 7.61 x 10^5 CFU/brick for samples contaminated with *B. licheniformis*, *B. subtilis, E. coli* and *E. faecalis*, respectively. All positive results and results from non-contaminated bricks from the alternative method, were confirmed on agar. Both sensitivity and specificity was 100% for the alternate method. However, there was no change in pH for all the matrices tested.

Significance: The microbial-ATP based detection method provided accurate and faster results when compared to agar or pH to detect microbial contamination in UHT products.

P3-62 Microbial Community Shift during Cheddar Cheese Making Process

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Introduction: Understanding the microbiome of cheese is important in the dairy industry since the microbiota contribute to the physicochemistry, quality, and safety of cheese.

Purpose: The purpose of this study is to understand and compare the composition of the microbial population throughout the Cheddar cheese-making process.

Methods: Three different batches of Cheddar cheese (April 26th, June 6th, and June 26th, 2018) produced at the Oregon State University Creamery were collected. To understand the shift of the microbial communities during cheese making, samples were collected from the raw milk, making process, and aging (weekly up to 6 months). Cheese products were sampled from each batch at 36 stages (one sample per each stage), so a total of 108 samples were collected from three batches. Genomic DNA isolated from all cheese and raw milk samples were subjected to sequencing based on the V4 region of the 16S rRNA gene via a Miseq platform (Illumina) to investigate the bacterial population differences.

Results: At the taxonomic level, all the cheese samples were predominated by *Streptococcus* and *Lactococcus,* which are commonly found in the starter lactic acid bacteria (SLAB) used in Cheddar cheese production. Both raw and pasteurized milk (before the addition of SLAB) showed a diverse microbial composition. The microbial richness was significantly decreased and changed microbial composition among cheese samples after SLAB addition. The similarity of the microbial community (beta-diversity) among samples after the addition of SLAB showed similar microbial composition. According to the functional properties of cheese microbiota, cheese manufacturing and aging exhibited similar properties while cheese samples before adding SLAB showed significant different functional properties.

Significance: The addition of SLAB reduced the microbial richness and changed the microbial properties to represent the similar composition and functional properties.

P3-63 Microbial and Chemical Properties of Fructooligosaccharides (FOS) or Inulin Supplemented Cheddar Cheese

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Introduction: Understanding the microbial and chemical properties of cheese is important in the dairy industry since it can impact the cheese quality. Prebiotics can be used as an energy source by lactic acid bacteria (LAB) in cheese, which can change the microbial community of cheese.

Purpose: In this study, we evaluated the microbial and chemical properties of prebiotics (fructooligosaccharides (FOS) and inulin) supplemented Cheddar cheese.

Methods: Two batches of lab-scale Cheddar cheese supplemented with 0 (control), 0.5, 1.0, and 2.0 (w/w)% of FOS or inulin were produced with 4 gallons of pasteurized milk. A total of 210 samples (15 samples per batch and prebiotic concentration) were collected from the cheese-making procedure and aging (10, 20, 30, 60, and 90 days after the cheese-making) period. Both microbiological (aerobic, coliform, and LAB) and chemical (carbohydrate, fat, protein, water, and ash) assays from each cheese sample were measured using the 90-day aged cheese.

Results: The prevalence of LAB (log CFU/mL) in Cheddar cheese supplemented with FOS (6.34 ± 0.11 and 8.99 ± 0.46 in whey and curd, respectively) or inulin (6.02 ± 0.79 and 9.08 ± 1.00 in whey and curd, respectively) was significantly increased than ($P < 0.05$) the control (5.84 ± 0.27 and 8.48 ± 0.06 in whey and curd, respectively) during the cheese-making process. FOS-supplemented Cheddar cheese exhibited similar chemical properties to the control Cheddar cheese. However, the moisture content of inulin-supplemented Cheddar cheese (45.10 ± 2.61%) was significantly higher than ($P < 0.05$) the control (40.59 ± 0.38%) and FOS supplemented Cheddar cheese (40.25 ± 1.82%).

Significance: The addition of prebiotics to cheese accelerates the LAB growth rapidly during the cheese-making process and the addition of inulin increases the moisture content of the cheese. Prebiotic supplementation increased the growth of bacteria during the cheese-making procedure, but coliform bacteria were not detected.

P3-64 Effect of Water Activity on Thermal Resistance of *Salmonella* in Dairy Powders

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* persistence in dairy powders has caused several foodborne outbreaks. The determination of proper pasteurization processing conditions requires identification of the thermal inactivation kinetics of *Salmonella* in dairy powders. However, there is a lack of knowledge related to the effect of water activity (a_w) and fat content on *Salmonella* inactivation in dairy powder during thermal processing.

Purpose: The objectives of this study were to 1) determine the moisture sorption isotherm of dairy powders; 2) evaluate the effect of a_w on microbial inactivation kinetics of *Salmonella* in dairy powders; and 3) assess the effect of fat content on microbial inactivation kinetics.

Methods: Two types of dairy powders, nonfat dry milk and whole milk powder, with different fat contents (0.62% and 29.46% w/w) were inoculated with a 5-strain *Salmonella* cocktail and equilibrated to three a_w levels (0.10, 0.20 and 0.30) for the isothermal treatment at 75, 80 and 85°C to obtain the *D*- and *z*-values. The custom design thermal sandwich was used to conduct the isothermal treatments. The moisture sorption isotherm of each dairy powder product was generated at 20°C.

Results: The moisture sorption isotherm was measured to understand the relationship between a_w and moisture content of dairy powders. The thermal resistance of *Salmonella* significantly increased ($P < 0.05$) as a_w decreased, which suggested that a higher temperature or longer processing time would be required to achieve the desired inactivation of *Salmonella* at lower a_w. A universal response surface model for both dairy powders was developed to identify conditions that would deliver a desired *D*-value or log reduction. Both temperature and a_w showed significant linear ($P < 0.05$) and quadratic ($P < 0.05$) effects.

Significance: This study provides guidance to the dairy industry to understand the influence of temperature and a_w on the thermal inactivation of *Salmonella* in dairy powders and identify the proper temperature and time combinations for the development and implementation of pasteurization process to ensure food safety.

P3-66 Thermal Lethality of *Listeria monocytogenes* to Improve the Safety of Cheeses Made with Unpasteurized Cheesemilk

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❖ ***Developing Scientist Entrant***

Introduction: Forty-seven US cheese varieties can be legally produced using raw or unpasteurized milk, including soft-ripened varieties capable of supporting *L. monocytogenes* growth (e.g., Brie, Camembert). Risk assessors have suggested that a 3-log decrease in *L. monocytogenes* achieved via thermization of cheesemilk (e.g., heat treatment below US milk pasteurization requirements) would reduce listeriosis risk up to 10-fold in soft cheeses; however, no standard definition for thermization exists.

Purpose: To determine the D-values of *L. monocytogenes* in cheesemilk at 3 thermization temperatures (60.0, 62.8, 65.6°C; 140, 145, 150°F) and validate with published thermal inactivation rates in fluid whole bovine milk.

Methods: Non-homogenized, pasteurized whole milk was inoculated with 8-log CFU/mL *L. monocytogenes* (5-strain mixture). Samples (1 mL) were vacuum-sealed in moisture-impermeable pouches and heated to 60.0, 62.8, or 65.6°C by submersion in a water bath. Triplicate samples were removed from heating at appropriate time intervals and immediately cooled in an ice bath. Surviving *L. monocytogenes* were enumerated by plating on Modified Oxford agar overlaid with Tryptic Soy agar to aid in the recovery of heat-injured cells. Duplicate trials were conducted and survival data used to calculate thermal inactivation rates.

Results: D-values of 145.2±61.4, 40.5±0.8, and 17.1±0.3 sec were achieved with heating at 60.0, 62.8, and 65.6°C, respectively, corresponding to a 3-log reduction of *L. monocytogenes* in cheesemilk in 7:16, 2:02, and 0:51 (min:sec), and an overall Z-value of 6.2°C ($R^2 = 0.95$). Comparatively, a Z-value of 6.1°C ($R^2 = 0.87$) was calculated from data derived from 19 scientific studies utilizing 177 inoculated fluid whole milks heated to temperatures below pasteurization (<72°C). Based on the collated published values, a 3-log reduction of *L. monocytogenes* is achieved in cheesemilk in 6:51, 2:24, and 0:50 for temperatures 60.0, 62.8, and 65.6°C, respectively.

Significance: These data can be used to create a flexible thermal process for artisanal cheesemakers.

P3-69 Metagenomic Study of the Impact of Novel Packaging Types on Meat Microflora

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Introduction: Maximizing the shelf life of perishable foods is a key driver for producers. Any techniques used to prolong the shelf life need to meet food safety regulatory criteria as well as being acceptable to the consumer. The fresh meat industry predominantly uses packaging and temperature control to extend shelf life.

Purpose: This study shows the use of metagenomic analysis to evaluate the impact of novel meat packaging formats on the product microflora and its evolution during the shelf life.

Methods: Several different packaging formats, including standard vacuum & skin packs, modified atmosphere pack and a novel close-contact film pack, were used to package cuts of beef and were stored according to a standard shelf life protocol by Dunbia. The products were held in the various retail packs for up to 25 days with samples taken at the start of retail life and every 5 days thereafter. Samples were analyzed by Campden BRI using the Thermo Scientific™ Ion GeneStudio™ S5 Food Protection System to determine the metagenomic profile. The outputs from the sequencing were compared to ascertain the effect of the novel packaging format on the natural microflora of the product.

Results: The metagenomic profiles generated have shown that there was no variation between the novel packaging format and the standard vacuum or skin packs, however, the modified atmosphere pack showed a different microflora to all the other packaging types tested.

Significance: The use of Next Generation Sequencing to determine the microflora of meat products is a valuable technique for assessing the impact of novel packaging formats on shelf life. In the future, metagenomic profiling has the potential to enhance shelf life management through a better understanding of product microflora and spoilage organism evolution.

P3-70 Advanced Microbial Profiling – Helping Determine Safe Shelf Life for Cold Smoked Salmon

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Introduction: The use of Next Generation Sequencing to analyze food microflora has greatly increased the industry's understanding of the contribution that natural microflora can have to inhibiting pathogenic organisms in perishable products.

Purpose: Cold smoked salmon is of interest because the primary pathogen of concern, *Listeria monocytogenes*, is likely to be present on the product with a high frequency. This study determines how the wider microflora and the primary pathogen grow as the shelf life progresses.

Methods: In this study smoked salmon from two producers was deliberately inoculated with the pathogenic organisms of concern, as guided by the regulatory specifications for the product. Samples were taken periodically during the shelf life, which were sequenced using the Thermo Scientific™ Ion GeneStudio™ S5 Food Protection System to describe the microflora.

Results: *L. monocytogenes* was shown to grow in samples from both producers as the shelf life progressed. However, one product was found to be 'cleaner' with a lower microbial load compared to the other product that exhibited a higher microbial load. The product with the lower microbial load was found to support the growth of *L. monocytogenes* earlier than in the product with a higher load. These results suggest that there is an effect from natural microflora. 16S rRNA gene based metagenomics revealed that in both cases, the onset of *L. monocytogenes* growth coincides with an increased abundance of reads identified in the Order Vibrionales. In addition, the microbial profiles from the agar plates, used to determine total viable count, were found to differ significantly from those generated directly from the product.

Significance: This study is the first time that Vibrionales has been highlighted as relevant to cold smoked salmon, and more widely it questions the suitability of current microbiological specifications for accurately indicating the end of product shelf life.

P3-71 Effect of Salinity, Alcohol and Heat Treatment on the Fate of *Bacillus cereus* Spores in Soybean Paste during Aging

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Introduction: The potential risk of food poisoning occurrence by *B. cereus* in traditional Korean soybean paste has been a concern for a long time, but few treatment methods have been evaluated to control the survival of the bacteria in paste.

Purpose: This study was to investigate the individual and combined effects of salinity, alcohol and heat treatment on the germination and survival of *B. cereus* spores in soybean paste during storage.

Methods: A 16% salinity soybean paste, and boiled soybeans were mixed to adjust the salinity to 10 and 12%, and a cocktail of three strains of *B. cereus* spores were inoculated 4 to 5 log CFU/g into paste. After treatment with alcohol (1 and 3%) and/or heating at 65°C for 20 min, the numbers of bacteria were analyzed every seven days while storing at room temperature. The soybean paste was autoclaved at 90°C for 30 min to evaluate the germination rate of *B. cereus* spores, and the effects of treatment on the germination and survival ratio were evaluated.

Results: Spore germination rate (46.3 to 100%) was increased steadily during the 45-day storage period and vegetative cells were observed to disappear (up to 100%). The heat treatment increased the germination rate of the spores (59.2 to 100%) and increased the effect of reducing *B. cereus* compared to the non-heat treatment (46.3 to 100%). In addition, the higher the salinity and alcohol concentration, the faster the spore germination rate and the cell inactivation.

Significance: These results suggest that the treatment conditions would be the factors to promote the germination of spores and also to accelerate the death of cells, and thus individual or combination treatments of heating and alcohol adding could be used to control microorganisms in soybean paste.

P3-72 Estimation of *Alicyclobacillus* spp. Spoilage Potential in Plant-based Dairy Products

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Introduction: *Alicyclobacillus* spp. is a spoilage organism that may pose a critical burden to the fruit juice industry, since they produce endospores, highly resistant to pasteurization and acidic environments, capable of germinating and multiplying during storage and distribution at ambient temperatures.

Purpose: To assess the microbiological spoilage and quality deterioration caused by specific *Alicyclobacilli* species in pasteurized plant-based dairy products under extreme storage temperatures.

Methods: Commercial bottles (250 mL) of 3 different types of pasteurized plant-based dairy products (Coconut & Berry–CB; Almond, Mango, & Passionfruit–MP; Oat, Strawberry, & Banana–OS) were inoculated with 10 spores/mL or 2 x 10^3 spores/mL of either *A. acidoterrestris*, *A. fastidiosus*, or *A. acidocaldarius* strain composites. Non–inoculated samples served as controls. Bottles headspace was flushed with N_2 to mimic the commercial atmosphere. Samples inoculated with *A. acidoterrestris* and *A. fastidiosus* were stored at 30°C and 45°C, while in case of *A. acidocaldarius*, storage took place at 50°C for 8 months. Gas composition, populations of *Alicyclobacillus* spp., Total Viable Counts, pH, a_w, color and guaiacol concentration (via HPLC- UV) were monitored.

Results: CB and MP supported growth of *A. acidoterrestris* and *A. fastidiosus* to 4.0 – 5.0 log CFU/mL at both temperatures and inoculation levels. In OS, populations of the latter *Alicyclobacilli* remained stable for 8-months storage at 30°C, while at 45°C, the populations declined close to 1 CFU/ mL. Growth of *A. acidocaldarius* was supported only in CB samples (*ca.* 3.0 log CFU/mL) at 50°C, regardless of initial level. Guaiacol production was detected (also organoleptically) only in *A. acidoterrestris* inoculated samples of CB and MP after 30 days at 30°C and 14 days at 45°C.

Significance: Spoilage assessment of different *Alicyclobacillus* species for such a highly emerging product category may provide significant information to the food industry related to raw material specifications development and quality control plans.

P3-73 Influence of Using Biological Soil Amendments of Animal Origin (Dairy and Poultry Manure) on the Prevalence of *Campylobacter, E. coli O157, Listeria monocytogenes* and *Salmonella* on Fresh Produce

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Introduction: There are critical gaps in data concerning the risk factors for vegetable contamination with foodborne human pathogens on produce grown in fields amended with untreated biological soil amendments of animal origin (BSAAO).

Purpose: To determine the influence of using different BSAAO amendments on the prevalence of foodborne human pathogens such as *Campylobacter, E. coli O157, Listeria monocytogenes* and *Salmonella* on produce.

Methods: Monthly samples of manure (N = 353) and soil (N = 430) were collected from farms utilizing BSAAO applications of dairy and poultry manure and from fields (N = 164) using no BSAAO. Produce grown on both the BSAAO amended fields (N = 220) as well as the non-amended fields (N = 102) were also collected. Samples were collected during 2017 to 2019 growing seasons. All samples were tested by enrichment culturing for the presence of *Campylobacter, E. coli O157, L. monocytogenes* and *Salmonella* and confirmed with PCR.

Results: The prevalence of foodborne pathogens in BSAAO amended soils was: *Campylobacter* (22/430); *E. coli O157* (3/430); *L. monocytogenes* (25/430) and *Salmonella* (3/430); while in BSAAO amended produce the prevalence was: *Campylobacter* (4/220); *E. coli O157* (4/220); *L. monocytogenes* (18/220) and *Salmonella* (1/220). In the Non-BSAAO amended soils, the prevalence was: *Campylobacter* (2/164); *E. coli O157* (3/164) and *L. monocytogenes* (4/164) and no *Salmonella* was isolated; while in the non-BSAAO amended produce the prevalence was: *E. coli O157* (2/102) and *Listeria monocytogenes* (2/102) and no *Campylobacter* or *Salmonella* were isolated.

Significance: Although less prevalent, foodborne human pathogens were isolated in soils and produce not amended with BSAAO; thus, more research is needed to determine other sources that may contribute to contamination of produce grown on non-amended fields.

P3-74 Precipitation and Soil Moisture Effects on Survival and Transfer of *Escherichia coli* to Fresh Produce in Manure-amended Certified Organic

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Introduction: Precipitation and soil moisture effects on manure pathogen survival and crop contamination remains unclear for the 90-120-day National Organic Program (NOP) manure-to-harvest wait-period for fresh produce.

Purpose: Evaluate precipitation, soil moisture, and raw manure effects on *E. coli* survival in NOP-certified soil and its transfer to fresh produce.

Methods: A randomized complete block study was conducted on Delmarva NOP-certified plots over six growing seasons (2017-2018). Survival of generic *E. coli*$^{rif\text{-}R}$ (EC) on soil, tomatoes, radish, and spinach was determined over 90-120(-180)-days for dairy manure (DM), horse manure (HM), poultry litter (PL), and unamended (UnA) plots. Precipitation, soil, and weather conditions were measured relative to *E. coli* population changes.

Results: All soil treatments were 100% positive for EC (5.85 log MPN/g) after inoculation. Temperature, growing degree days (GDD), and precipitation during 120-day spinach and radish crop trials in 2017 and 2018 were distinctive. Mean temperature was 8°C higher in 2018 than in 2017. Total precipitation was significantly ($P < 0.05$) greater at ~56 cm during 2018 than 26 cm in 2017. GDD = 3,254 during 2018 was nearly twice that in 2017 (GDD = 1,954). In 2017 radish soil, EC presence declined to 0 to 12 of 64 for all treatments by 90&120-day, yet all 240 bulbs were EC positive in manured plots. In 2018 by 120-day, 8/12 radish plots (5 bulbs/plot) PL and DM were EC positive. In 2017 and 2018 90-day tomato soils, only 2/12 manured plots were EC positive, but by 120-day 30-40% were positive in DM and PL 2017, respectively. In 2018, EC declined to 10-20% by 90&120-day with no transfer to tomato fruits. However, EC varied substantially during 2017, 2018 growing seasons; with 100% transfer to spinach.

Significance: Results show weather, precipitation, manure and crop type influence EC survival in manured soil and transfer to crops. Reliance on wait-time intervals alone needs further evaluation.

P3-75 Inactivation of *Salmonella* Typhimurium in Urea and Ammonia Solutions

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❖ ***Developing Scientist Entrant***

Introduction: Studies on poultry litter suggest that urea and ammonia (NH_3) may affect the survival of *Salmonella*, but their direct impact is not well studied.

Purpose: This study was designed to evaluate the effects of urea and NH_3 solutions on the survival of *Salmonella* Typhimurium.

Methods: Rifampicin-resistant *Salmonella* Typhimurium was inoculated (8 log CFU/mL) into phosphate buffered saline (PBS) tubes with urea and NH_3, and pH adjusted to 9.0. Triplicate samples were prepared at 0, 6, 12, 18, 24 h and incubated at 30°C. Four urea treatments (1% and 2% urea; 1% and 2% urea with urease), five NH_3 treatments (0.05, 0.1, 0.2, 0.3, and 0.4 M) and two control treatments (PBS and PBS with urease) were tested. At each sampling time, aliquots were serially diluted and plated onto tryptic soy agar supplemented with 80 ppm rifampicin.

Results: The 1% urea with urease and 0.3 M NH_3 treatments had the highest recorded linear death rates (log CFU mL^{-1} h^{-1}) of 0.36 and 0.35, respectively. Treatments with 0.2 M, 0.3 M, and 0.4 M NH_3 showed significantly greater ($P < 0.05$) inactivation of *Salmonella* compared to the 0.05 M, 0.1 M treatments, and the controls after 6 h. Both 1% and 2% urea with urease showed significantly greater ($P < 0.05$) inactivation than the urea-only treatments after 6 h.

Salmonella was undetectable in 1% urea with urease after 24 h. In 2% urea with urease, 0.2 M, 0.3 M, and 0.4 M NH_3 treatments, counts were 0.60, 0.49, 0.47, 0.56 log CFU mL^{-1} after 24 h, respectively. After 24 h, 1% and 2% urea with urease treatments reached concentrations of 0.16 M and 0.33 M NH_3, respectively.

Significance: This study quantifies the direct effects of urea and NH_3, providing further evidence of their antimicrobial effects and potential for reducing *Salmonella* contamination in poultry litter used as a biological soil amendment.

P3-76 Application of Competitive Exclusion Microorganisms to Inhibit the Growth of *Listeria monocytogenes* in Compost Extract

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Introduction: Dairy and poultry composts contain a diversity of microbial species. Thus, the compost products can be a good source for isolating compost-borne competitive exclusion (CE) microorganisms which inhibit the growth of pathogens such as *Listeria monocytogenes* (LM).

Purpose: To use various culturing methods to isolate CE microorganisms and then verify antagonistic activities of those CE cultures against LM in compost extract models.

Methods: CE strains were isolated from poultry and dairy composts using triple-agar-layer method, purified, and then identified to species level. The composts were extracted with sterile tap water with ratios of 1:5 and 1:10 (w/v). The filter-sterilized compost extracts inoculated with a cocktail of three LM strains (ca. 3 log CFU/g) were inoculated with or without CE (ca. 7 log CFU/g), and incubated at room temperature and 35°C up to 168 h. At selected intervals, the samples were enumerated for LM and CE population, respectively.

Results: A total of 40 CE strains were isolated and used in this study. During growth inhibition study, no change ($P > 0.05$) was observed for CE population in poultry compost extract (PCE), but CE population increased 0.27 – 0.56 logs in the dairy compost extract (DCE). After 168 h incubation, the reductions of LM in PCE with extraction ratios of 1:5 and 1:10 were 1.09 and 1.01 logs or 0.85 and 0.73 logs at 35°C or room temperature, respectively. In contrast, the reductions of LM in DCE with extraction ratios of 1:5 and 1:10 were 2.17 and 1.68 logs or 1.61 and 1.79 logs at 35°C or room temperature, respectively. Clearly, the inhibition effect from CE strains increased in more concentrated dairy compost extract (1:5) at 35°C.

Significance: The compost-borne CE microorganisms with anti-*Listeria* activity could control *L. monocytogenes* contamination in biological soil amendments to ensure safe production of fresh produce.

P3-77 Inactivation of *E. coli* O157:H7 in Fresh Dairy Manure Compost by Addition of Slow-pyrolysis Walnut Biochar

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Introduction: Biochar has been added to composting materials to increase aeration and improve heating profiles of compost piles. Some biochar has also been shown to be biocidal to pathogens in soil; however, its antimicrobial properties have not been tested in contaminated compost.

Purpose: A study was conducted to determine the biocidal activity of biochar when added to dairy manure compost inoculated with *E. coli* O157:H7.

Methods: Compost was composed of fresh dairy manure, collected from a regional dairy farm, combined with 7% added wheat straw and mixed thoroughly. Two types of slow-pyrolysis biochar ([1] high temperature walnut biochar and [2] walnut cyclone biochar, provided by All Power Labs, Berkeley, CA) were, respectively, added (10%, weight:weight) to fresh dairy manure compost and mixed thoroughly. Samples were then inoculated with 7.84 log CFU/g of a four-strain composite of nontoxigenic *E. coli* O157:H7 (EC), mixed thoroughly, and stored at 21°C. Samples were analyzed weekly and EC populations determined.

Results: When fresh dairy manure compost was supplemented with 10% biochar, the no-biochar control compost maintained high levels of EC up to week seven (8.51 log CFU/g). However, no *E. coli* was detected in the walnut cyclone biochar-supplemented compost throughout 7 weeks of storage. When compost was supplemented with 10% high temperature walnut biochar, populations remained as high as 9.05 log CFU/g up to week 4. Further testing in soil reveal that when 3.5% of the high temperature walnut biochar was added to soil, EC populations were reduced to 4.42 log CFU/g as compared with the no-biochar control, which was 6.46 log CFU/g. This indicates that biochar may exhibit greater antimicrobial activity in soil than in higher moisture dairy manure compost.

Significance: Results should provide guidance on the application of biochar added to compost to inactivate foodborne pathogens.

P3-78 *Escherichia coli* O157 Survival in Liquid Culture and Artificial Soil Microcosms with Different pH, Humic Acid, and Clay Levels

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❖ ***Developing Scientist Entrant***

Introduction: Many factors can influence pathogen survival in soil, and artificial soil microcosms provide a controlled environment to evaluate microbial response in the absence of biotic factors.

Purpose: The objective of this research was to determine the survival of *E. coli* O157 in liquid culture and artificial soils with different physicochemical characteristics.

Methods: *E. coli* O157 survival in liquid culture (10 mL) at pH 7.4, 8.6, 9.6, and 10.6 for 0, 1,000, 5,000, and 20,000 ppm humic acid at 0, 4, 8, 12, 24, and 72 h at 37ºC was assessed by diluting and plating to determine log CFU mL^{-1}. Additionally, *E. coli* O157 survival was performed in artificial soil microcosms (50 g) at 30ºC by diluting and plating 1.0 g of soil to determine log CFU g^{-1} on days 0, 3, 7, 14, 21, 28, 56, and 84. Microcosms consisting of 1.5, 7.5, and 15% clay with 0, 1,000, and 5,000 ppm humic acid were evaluated. The estimated marginal means were measured at each sampling interval to determine the influence of each variable on *E. coli* O157 log CFU mL^{-1} or g^{-1}. When statistical significance was observed ($P < 0.05$), a Tukey's multiple comparisons of means test was performed to determine which samples were significantly different.

Results: Significant differences ($P < 0.05$) in *E. coli* O157 log CFU mL^{-1} were observed at pH 7.4, 8.6, and 9.6 influenced by humic acid concentrations after 8 h of incubation at 37ºC. After 56 days of incubation at 30ºC, significant differences were observed between 0 and 1000 ppm ($P < 0.0001$) and 0 and 5000 ppm ($P < 0.0001$) humic acid in 1.5% clay soils, but not in 7.5 and 15% clay soils.

Significance: This study provides insights on *E. coli* O157 survival based on pH, humic acid, and clay in liquid culture and artificial soil microcosms.

P3-79 Effect of Anaerobic Soil Disinfestation on *Salmonella* Populations

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Introduction: *Salmonella* has been shown to survive in soils for extended periods. Anaerobic soil disinfestation (ASD) represents a promising alternative to fumigation used to manage soilborne diseases and pests; however, little is known about ASD's impact on *Salmonella.*

Purpose: This study investigated the survival of *Salmonella* in ASD treated soils using four different soil amendments.

Methods: Experiments were arranged in randomized complete block designs (four replications per treatment). Sandy loam soil was inoculated with a *Salmonella* cocktail (5.5 ± 0.2 log CFU/g) and amended with field-applicable rates of ryegrass (RG), brassica (BR), legume (L), or pelletized poultry litter (PPL). Soils were irrigated to saturation and covered with plastic mulch. Non-amended (anaerobic) and non-amended, non-ASD treated (aerobic) controls were performed in parallel. ASD treatment was terminated by removal of plastic (3 week). Triplicate soil samples were collected pre-ASD and 0, 1, 2, 3, 7, 10, 14, 21, 28, 31, 35, 38 and 42 d post-ASD. *Salmonella* (log CFU or MPN/g) was quantified using a modified FDA-BAM protocol. Serovar prevalence was determined by multiplex-PCR. Populations and serovar prevalence at time-points and between amendments were analyzed for significance ($P \leq 0.05$).

Results: *Salmonella* populations were significantly lower in the aerobic control compared to the anaerobic control after three-weeks ($P \leq 0.05$). No significant differences were observed in *Salmonella* populations in the controls by 42 d ($P > 0.05$). *Salmonella* survival during and post-ASD was dependent on soil amendment with significantly higher populations in PPL, L and BR amended soils, compared with RG amended and control soils ($P \leq 0.05$). PPL amended soil had the highest *Salmonella* populations at each time-point post-ASD. *Salmonella* serovar prevalence was directly linked to soil amendment with significant survival of *S*. Poona in RG, *S*. Braenderup in L and *S*. Newport in PPL ($P \leq 0.05$).

Significance: ASD is not an effective management strategy for *Salmonella* contaminated soils; instead, depending on amendment, it may enhance *Salmonella* survival.

P3-80 The Prevalence and Persistence of *Listeria monocytogenes* in the Leafy Green Produce Production Chain

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Introduction: Listeria monocytogenes (*Lm)* is a foodborne human pathogen that poses a threat to at-risk populations such as the elderly, neonates, pregnant women, and immunosuppressed individuals. *Lm* outbreaks, originally linked to meat and dairy, are now commonly associated with produce such as leafy greens. While many meat and dairy products undergo pasteurization, methods to inactivate pathogens on produce are inadequate. The route of *Lm* contamination, its preferred niches, and its mechanisms of persistence in produce production chains is not yet understood.

Purpose: This research aims to determine when and where *Lm* contamination occurs and their genomic features in terms of virulence and persistence.

Methods: Two leafy green produce farms and their processing facility were sampled from June to October 2019. Leafy green samples (25 g) and swab samples were collected from 10 areas monthly (MFLP-41), for a total of 430 samples (Farm A) and 320 samples (Farm B). The isolates were recovered using MFHPB-30 and confirmed using specific PCR (MFLP-78). Isolates will undergo whole-genome sequencing to trace *Lm* isolates along the production chain and identify (i) genetic determinants that could be associated with persistence of *Lm* strains and (ii) the points of contamination. In addition, virulence, biofilm-forming abilities, and persistence of the isolates will be examined.

Results: Farm A, an organic farm in Ontario, was positive for *Lm* in three of five months sampled (60%) in four of 10 areas (40%). *Lm* was isolated from a drain, aprons, compost bin, and a palette. Farm B, a conventional farm in Quebec, was negative for *Lm*.

Significance: This research seeks to map routes of transmission and determine methods of controlling *Lm* from the farm, to the packing facilities, and onto the end product. Preventing and controlling contamination at the source is vital to control *Lm* in RTE produce such as leafy greens.

P3-81 Pre-harvest Biocontrol of *Listeria* on Spinach by Lactic Acid Bacteria

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Introduction: Recent outbreaks and recalls linked to *Listeria*-contaminated leafy greens such as spinach highlighted the need for effective natural approaches to improve spinach safety at the pre-harvest level.

Purpose: The purpose of this study was to investigate the effect of lactic acid bacteria (LAB) including *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus johnsonii*, and *Lactobacillus acidophilus,* as pre-harvest biocontrol agents against *L. innocua,* a non-pathogenic surrogate for *Listeria monocytogenes,* on spinach cultivars grown in high tunnel.

Methods: Spinach cultivars Abundant, Butterfly, Matador, and Palco were spray-inoculated with a three-strain cocktail of rifampicin-resistant *L. innocua* (5 log CFU/g), followed by spraying with or without a mixture of aforementioned LAB cultures (8 log CFU/g). On 0, 3, and 5 days-post-inoculation (dpi), four replicate spinach samples (20 g/sample) from each group were collected and pummeled in 80 mL of buffered peptone water for 2 min, followed by spiral plating on Modified Oxford agar with rifampicin. Leaf microstructure and stomatal density of each spinach cultivar were determined by Scanning Electron Microscopy (SEM).

Results: *Listeria* attachment was not significantly affected by the stomatal density on the leaf of spinach cultivars. However, LAB treatment showed higher anti-*Listeria* activity on spinach cultivars with lower stomatal density. For example, LAB treatment significantly reduced populations of *L. innocua* by 1.5 log CFU/g on spinach cultivars Butterfly (56 stomata/mm^2) and Palco (37 stomata/mm^2) comparing to the reductions of 1.2 and 1.1 log CFU/g in *L. innocua* populations on Abundant (101 stomata/mm^2) and Matador (82 stomata/mm^2) cultivars on 0 dpi, respectively. On 3 dpi, LAB treatment significantly reduced *L. innocua* by ~ 1.5 log CFU/g on Spinach cultivars Palco, Butterfly, and Matador as compared to the control ($P < 0.05$).

Significance: Results of this study indicate that LAB can be potentially used as pre-harvest biocontrol agents against *Listeria* on spinach grown in high tunnel.

P3-82 Determination of Salmonella Javiana and Listeria monocytogenes Transfer to Sunflower Microgreens Cultivated in Soil-free Growing Media

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Introduction: Microgreens are immature shoots of edible plants often eaten as a raw salad green and are susceptible to contamination by bacterial pathogens commonly associated with produce-borne illness outbreaks.

Purpose: This study aims to measure survival of *Listeria monocytogenes* and *Salmonella enterica* subsp. Javiana on two types of soil-free growing medium (SFGM) during sunflower microgreen cultivation, as well as the degree of pathogen transfer to the edible product.

Methods: *S.* Javiana and *L. monocytogenes* FSL R2-584 were inoculated onto two types of SFGM: sphagnum peat with vermiculite and Biostrate® biodegradable mats. Following, sunflower microgreens (*Helianthus annuus* cultivar Black Oil) were cultivated on half of the inoculated tray for 10 days, with the other side left unplanted. At harvest, concentrations of the two pathogens in the growing medium at the start and completion of the growing cycle, as well as in the harvested microgreens, were determined.

Results: Overall, pathogen levels on SFGM declined more on peat than on Biostrate®, declined more without the presence of microgreen roots than when the tray is planted, and declined more for *L. monocytogenes* compared to *Salmonella* Javiana. Statistically significant differences were found on Biostrate, where *S.* Javiana growth was greater on the planted side of the tray compared to the unplanted side ($P = 0.02$). There were also survival differences between the two pathogens. On the unplanted side of Biostrate, there was a statistically significant difference between *L. monocytogenes*, which experienced a decline, and *S.* Javiana, which experienced growth.

Significance: These findings indicate that pathogen survival in microgreen cultivation systems may partially depend on the growing medium chosen. The data also show that the sunflower microgreen root environment may be a source of nutritional support for these two human pathogens.

P3-83 Understanding the Cross-contamination of Melons via Environmental Matrices under Field Conditions and Prevalence of Foodborne Pathogens

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Introduction: *Listeria monocytogenes* contaminated cantaloupes were implicated in an outbreak in 2001 causing 33 mortalities. Understanding the vehicles of *L. monocytogenes* contamination and prevalence of foodborne pathogens will assist in risk-analysis.

Purpose: Investigate soil and dust as vehicles of *L. monocytogenes* contamination and understand prevalence of foodborne pathogens in Arizona grown melons and environmental samples.

Methods: Experimental melon varieties and hybrids grown in different states (AZ, CA, TX, NC and IN) were used. Soil from melon fields in Yuma, AZ was used and sieved to create dust. Rinds were placed on soil inoculated with *L. monocytogenes* for 1 h under a bio-hood. Inoculated dust was sprayed on melon rinds in a specially designed chamber. *L. monocytogenes* was recovered from soil, dust and melon rinds, plated on selective media and enumerated to determine % transfer. From 4 fields in AZ, 339 cantaloupes, 20 rhizosphere, 20 soil, 20 air and 6 water samples were collected. Selective enrichment and plating were used to analyze the presence/absence of *Salmonella enterica* and *L. monocytogenes* and to enumerate indicator bacteria.

Results: Percent Transfer- Hybrid-cantaloupes had the greatest (0.040%) and least (0.0001%) transfer of *L. monocytogenes* from soil and dust, respectively. Honeydews had transfers of 0.0034% and 0.00033%, while cantaloupes had 0.018% and 0.0013%, for soil and dust, respectively. *L. monocytogenes* transfer was highest (0.10%±0.01%) for TH7 (AZ), and least (0.00053±0.00014) for HD252 (IN).

Prevalence- No pathogens were detected in melons and environmental samples. On cantaloupes, enterococci and coliforms ranged from 2.3 to 2.8 logs and 3.8 to 4.1 logs, respectively. Air samples had the lowest enterococci and coliform counts, <1-3.0 logs and <1-2.1 logs, respectively. Water samples had enterococci and coliforms ranging from 1.6 to 2.2 logs and 2.1 to 3.8 logs, respectively. Two samples from location-one were positive for *Escherichia coli*. All fields had signs of wildlife intrusion.

Significance: Results will help understand risk of *L. monocytogenes* contamination in field conditions and data can be used for a science-based risk analysis.

P3-84 Effect of Fumigants and Bactericides on *Salmonella* during Tomato Production

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Introduction: Several outbreaks of salmonellosis have been traced back to contaminated tomatoes. The pre-harvest environment has been proposed to be one of the possible sources of initial contamination. Little is known about the effects of pest control management applications on *Salmonella*.

Purpose: Therefore, the goal was to investigate the effect of various fumigant and bactericide applications on *Salmonella* reduction in soil and on/in tomato leaves, respectively.

Methods: To evaluate fumigants, sandy loam soils were inoculated with *Salmonella* Newport strain J1892 and Typhimurium strain ATCC 14028 to reach a population density of 6 log CFU/g, and treated with fumigants (chloropicrin, metam sodium, dimethyl disulfide, or 1,3-dichloropropene). To evaluate bactericides, 8-week tomato plants were inoculated with the same *Salmonella* strains by dipping tomato leaves in bacterial solution of 8 log CFU/mL. Inoculated plants were treated with four bactericides with different mode of actions (copper hydroxide, acibenzolar-*S*-methyl, streptomycin, and H_2O_2+peroxyacetic acid). Fumigants and bactericides were applied at equivalent maximum application levels for commercial tomato production. Sterile water was used as a control. *Salmonella* populations were measured by standard methods and transformed (log CFU/g). Fisher's LSD was used to determine significant *Salmonella* population differences by fumigant or bactericide ($P \leq 0.05$).

Results: Fumigants 1,3-dichloropropene (2.4 log CFU/g) and dimethyl disulfide (1.6 log CFU/g) significantly reduced *Salmonella* populations in soils. Bactericides H_20_2+peroxyacetic acid (0.7 log CFU/g) and streptomycin (0.6 log CFU/g) significantly reduced *Salmonella* populations on the surface of tomato leaves, while copper hydroxide significantly reduced *Salmonella* populations by 0.8 log CFU/g internally. Application of the other tested fumigants or bactericides did not significantly reduce *Salmonella* populations, compared to control samples ($P > 0.05$).

Significance: These results indicate that certain commonly applied agricultural fumigants can reduce soil *Salmonella* populations. Additionally, it was discovered that certain bactericides could influence the survivability and internalization of *Salmonella* on tomato leaves.

P3-85 White-rot Fungi Species Used as a Biocontrol Method in Bioreactors to Inhibit *Escherichia coli* for Pre-harvest Food Safety

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❖ ***Developing Scientist Entrant***

Introduction: Lignin-degrading white-rot fungi species are byproducts of mushroom production that may enhance preharvest safety in reducing risks associated with raw dairy manure.

Purpose: This study evaluated the inhibitory effects of *Pleurotus ostreatus* (PO) and *Phanerochaete chrysosporium* (PC) on *Escherichia coli*.

Methods: Controlled batch tests with PC-treated woodchips and in-line treatment tests with PO-treated woodchips were performed. In-line bioreactors were maintained on benchtop with ambient temperatures of 16-24°C and batch bioreactors were incubated at 37°C. In all, pine woodchips (260 g) were inoculated with 50 mL of PO or PC and incubated at 22°C for 4 d. Inoculated woodchips (73 g PO/PC) were then added to sterile 1L bioreactors. *E. coli* TVS355 or O157:H7 (4407) were inoculated into aqueous dairy manure, at 6 log CFU/mL and added to each bioreactor. Manure effluent (3 mL) was sampled on 0, 1, 3, 5, 7, and 10 d post-inoculation (dpi) and woodchips (30 g) on 0, 5, and 10 dpi. *E. coli* TVS355 and O157:H7 were enumerated on MacConkey agar with Rifampicin or Nalidixic Acid. Controls included bioreactors without fungi and/or bacteria. Chemical analysis included pH, moisture and ergosterol. Data were analyzed using one-way ANOVA and Student's *t*-test across 4 trials, with $n = 5$ per treatment.

Results: Across all studies, initial effluent concentrations were 6.23 log CFU/mL. At 10 dpi, all PO-treated effluent showed similar decline of *E. coli* in manure of untreated bioreactors. *E. coli* TVS355 in PC-treated effluent significantly decreased between 1 and 7 dpi ($P < 0.001$). Greater reduction was observed in *E. coli* O157:H7 (2.45 log CFU/mL) compared to *E. coli* TVS355 (5.51 log CFU/mL) at 10 dpi ($P = 0.002$). There was a significant difference in O157:H7 on PC-treated woodchips between initial and 5 dpi ($P > 0.0042$), but no statistical significance by 10 dpi ($P > 0.2195$). Bacteria were undetectable in appropriate controls throughout the study.

Significance: White-rot fungi species inhibit *E. coli,* with greater inactivation by PC; therefore, PC could serve as a biocontrol in soil amendments.

P3-86 Prevalence of *Arcobacter S*pecies in Irrigation Water from the Midwestern United States

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Introduction: Several species of the *Campylobacteraceae* family cause human gastroenteritis world-wide, with the infectious dose being as low as 500 bacteria. Human campylobacteriosis is usually associated with consumption of raw milk, contaminated water, poultry, and seafood. The objective of this longitudinal study was to analyze irrigation water from fields growing fresh produce in the Mid-Western region during the 2019 season.

Purpose: To evaluate the prevalence of *Campylobacter*-like organisms in irrigation water, which may be transferred to soil and thus become a possible source of produce contamination.

Methods: Irrigation water samples were obtained from various farms in the Mid-West at monthly intervals over the growing period, June through September 2019. These water samples were collected from the source or at the point of irrigation (drip tape). The sources of water included stream ($N = 2$), pond ($N = 6$) or well ($N = 3$). Water samples were enriched in microaerobic conditions at 37°C for 48 h and colonies were recovered on mCCDA or Anaerobe Basal Agar plates. Colonies were screened for motility and *Campylobacter*-like morphology using a phase contrast microscope; PCR was also performed. Isolates of interest were collected, DNA was extracted, and the Illumina-MiSeq platform was used for Whole Genome Sequencing.

Results: Source water samples from streams and ponds were positive for *Arcobacter* species at different sampling time points; however, none were positive for *Campylobacter*. Further, well water samples from the source were negative for *Arcobacter* and *Campylobacter* species. *Arcobacter* species were recovered from stream (2/2), pond (4/5) and well (2/3) water samples collected at the point of irrigation.

Significance: Further studies are needed to evaluate the potential significance of *Arcobacter* in irrigation water and implications of possible transfer to produce.

P3-87 Factors Associated with the Implementation and Documentation of Risk Management Practices on Strawberry Farms in the Southeastern United States

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Introduction: Strawberries are one of several produce commodities in the US linked to cases of foodborne illness; however, the industry is not well characterized. In the Southeastern US there are approximately 2,091 strawberry farms varying widely in acreage, income, production type. As implementation of risk management practices (RMPs) can be affected by many factors, the growers in the Southeastern region are an ideal sample for study.

Purpose: To determine factors associated with RMPs of Southeastern strawberry growers.

Methods: A 43-question survey was developed to collect information regarding grower's location, business characteristics, farm characteristics, and RMPs. Questions were designed based on the Produce Safety Alliance training as well as feedback from expert reviews. The survey was distributed to individual growers and professional organizations.

Results: Farm size ranged from 1-10,000 acres. Half of all growers harvested strawberries on 3 acres or less with 1 acre being most common (13.7%). Strawberries were not the only commodity for 91.9% of growers with 65.5% and 34.5% producing crops or crops/livestock, respectively. Those growing >1 crop, 50.4% were covered by the PSR; however, 34.5% were unsure. Growers reported using RMP but were less likely to document them. For example, 76% of growers reported their employees had attended food safety training, meanwhile, only 40.7% had documented training. There was a significant difference in documentation between and within RMPs. Growers were most likely to keep documentation related to Employee Health and Hygiene (57.3%) with written procedures being most common overall ($P = 0.001$). The frequency of use and documentation of RMPs was also found to be impacted by acreage, commodity type, and exemption status of the farm.

Significance: Our results indicate that Southeastern strawberry growers have significant differences in RMPs. As this is an integral part of the Produce Safety Rule, strawberry growers may benefit from additional education regarding documentation.

P3-88 Validation of an In-Field Produce Sampling Simulation Using Experimental Field Data

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❖ ***Developing Scientist Entrant***

Introduction: The development of effective pre-harvest sampling strategies in fresh produce is a key challenge facing the produce industry, since current practices yield few positive results because contamination typically occurs at low levels.

Purpose: This study aims to validate a produce sampling simulation by evaluating high-resolution stratified random sampling of Point Source (PS), Systematic (SS) and Sporadic (SP) contamination of experimental plots.

Methods: Four trials were performed in plots of 20 x 30 m dimensions planted with spinach, which was artificially inoculated with Rifampicin-resistant *E. coli.* The field was divided in equal plots and subplots to perform stratified random sampling. The trials simulated PS contamination by spraying 3 rings of 5, 3 and 0 log (CFU/mL) of inoculum within a 2-meter radius, simulated SS widespread contamination by spraying 3 log (CFU/ml) over the field, and SP contamination by waiting 5 additional days for die-off. Sampling was carried out 24 hours and 6 days following inoculation. The collected 228 samples per trial were subject to culture-based testing for r*E. coli* presence/absence and inoculated strains confirmation by performing *cplX* PCR. The sampling strategy and parameters described above are the inputs to test the simulation, which was designed to give an estimate of positive samples using the Monte Carlo simulation for 1,000 iterations.

Results: The trials determined 3 and 7 positive samples for PS, 2 and 18 for SP, and 139 for both SS trials. The simulation estimated a 95% confidence interval of positive samples that varied from 4 to 21 for PS, 0 to 26 for SP, and 228 for SS contamination scenario. Three of the six trials were within the estimated range, while the rest showed lower numbers.

Significance: The simulation is partially valid. Lower numbers in some experiments suggest a need to account for die-off.

P3-89 Reduction of *Escherichia coli* O157:H7 in Finishing Cattle Fed Enogen Feed Corn

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❖ ***Developing Scientist Entrant***

Introduction: Cattle are recognized as the principal reservoir for *Escherichia coli* O157:H7. Enogen® Feed corn (EFC; Syngenta Seeds, LLC) contains an enhanced α-amylase enzyme trait, which maximizes starch availability in feedlot cattle. Research demonstrates improved feed efficiency in cattle fed EFC; however, the potential foodborne pathogen shedding impact has not been investigated.

Purpose: This study explores the effect of finishing diets containing EFC on *E. coli* O157:H7 prevalence in cattle.

Methods: A 2x2 factorial experiment was conducted with steers (n = 960) fed diets consisting of 2 levels of silage (EFC or Control) and corn (EFC or Control), fed daily *ad libitum*. Recto-anal mucosal swabs (RAMS) were collected pre-harvest, incubated in Gram-negative broth at 37°C for 6 hours, subjected to immunomagnetic separation (IMS) using anti-O157 beads, spread-plated onto CHROMagar O157 supplemented with 5 mg/L novobiocin and 2.5 mg/L potassium tellurite, and incubated at 37°C for 18-24 hours. Colonies were identified as *E. coli* O157 via latex agglutination and confirmed as *E. coli* O157:H7 using PCR.

Results: *E. coli* O157:H7 prevalence rates ranged from 0/75 (0.0%) - 8/80 (10.0%) depending on sampling day. Tests for silage x corn interaction and main effect of silage had *P*-values of P = 0.5308 and P = 1.0000, respectively. Test for the main effect of corn had *P*-value of P = 0.0706, with EFC reducing the odds of *E. coli* O157:H7 prevalence by 43.3% compared to a control corn diet.

Significance: Cattle diets containing Enogen® Feed corn reduced the odds of *E. coli* O157:H7 prevalence in feedlot cattle.

P3-90 Evaluation of Bacteriophages to Prevent Attachment of *Escherichia coli* O157:H7 to Intestinal Cell Lines

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Escherichia coli* O157:H7 is a foodborne pathogen prevalent in ruminant intestinal tract, leading to cross contamination during food production. Various approaches against the pathogen have been evaluated to control its prevalence in food animals. However, recolonization of previously infected animals, with the same or different strains of *E. coli* O157:H7, limits effectiveness of these strategies. Targeted use of bacteriophages could be used effectively to control this problem.

Purpose: Evaluate the efficacy of bacteriophages to prevent *E. coli* O157:H7 attachment to intestinal cells.

Methods: Caco-2 monolayers were prepared (1x10^5 viable-cells/mL) in complete growth medium (CGM). Bacteriophages (9 logs PFU/mL) were used individually (P7) and as a cocktail (P3, P5, and P7). Overnight bacterial culture (1,mL; 6 logs CFU/cm^2) was centrifuged (12,000xg; 5 min) and pellet re-suspended in CGM without fetal bovine serum. Bacterial culture was added to the cells and centrifuged (164xg; 5 min) before incubation (2 or 4 h; 37°C; 5% CO_2). Phage-treatments were added to the cells before (preventive) or after (corrective) bacterial attachment. In corrective treatment, cells were further incubated for 3 h after phage-treatment. Sterile distilled water was used as control. After incubation, cells were lysed with 0.25% trypsin (3 mL;10 mins) to remove attached *E. coli* O157:H7, that were enumerated by plating on SMAC. Data were analyzed using one-way ANOVA (P < 0.05).

Results: Phage-treatments were significantly effective in preventing *E. coli* O157:H7 attachment to intestinal cells. As a preventive treatment, individual phage reduced the population of attached *E. coli* O157:H7 cells by 2.7 logs CFU/cm^2 (control - 4.4 logs CFU/cm^2) while the phage-cocktail reduced it to undetectable levels. As a corrective treatment also, phage-cocktail was more effective (2.6-logs CFU/cm^2 reduction) than individual phage treatment (1.4 logs CFU/cm^2).

Significance: Bacteriophages could be used effectively as preventive and corrective treatments to prevent the attachment of *E. coli* O157:H7 to intestinal cells.

Poster

P3-91 Comparative Analysis of Miniaturized Most Probable Number and BAX® System SalQuant to Quantify *Salmonella enterica* in Chicken Ceca

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Introduction: Presence and concentration of *Salmonella* in chicken ceca is often utilized by the poultry industry to evaluate bird or flock contamination levels. Rapid *Salmonella* detection and quantification from ceca could provide risk mitigation insight of flock *Salmonella* loads prior to slaughter or to evaluate efficacy of pre-harvest intervention strategies in a more rapid and economical manner.

Purpose: Paired evaluation of BAX® System SalQuant as an alternative, rapid quantitative assay application compared to a miniaturized Most Probable Number (mMPN) assay for estimating *Salmonella* concentrations in cecal samples.

Methods: Chicken cecal samples (N = 96) were screened for *Salmonella* utilizing the BAX® System RT *Salmonella* assay, comparing two media, and presumptively positive (*n* = 50) ceca were used for the quantitative method comparison study. *Salmonella* in positive samples were enumerated using SalQuant and mMPN methods prepared pairwise from the primary cecal homogenate. Primary samples and mMPN assay wells were confirmed by culture following the USDA FSIS MLG 4.10 isolation procedure with isolate confirmation by latex agglutination and *invA* gene presence. An equivalency test was performed to evaluate the methods capabilities to enumerate *Salmonella* utilizing JMP® v.14.2.0.

Results: The mMPN estimated a *Salmonella* concentration mean of 1.93±0.30 log MPN/mL and SalQuant method estimated the mean to be 2.17±0.35 log CFU/mL. Regression analysis indicated a weakly moderate positive collinearity (R = 0.41) between methods but the coefficient of determination was poor (R^2 = 0.17), which may be explained by the fact that although the methods were paired at the sample level, they utilized separate aliquots for each test. Diagnostic assay performance within media type was equivalent to culture, however, media type greatly impacted detection and isolation of *Salmonella*.

Significance: These data indicate SalQuant may be an effective alternative for rapid detection and quantitation of *Salmonella* in chicken ceca as compared to culture methods (mMPN) with improved time-to-results, reduced expenditure, and accurate results.

P3-92 Drought Stress Shifts the Exometabolome Profile of Leaves in Juvenile Kale and Affects *Salmonella enterica* Growth in Leaf Exudates

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❖ ***Developing Scientist Entrant***

Introduction: Extreme weather events frequently subject crops to periods of drought, a plant stress that may affect leaf surface compounds and interaction with bacterial epiphytes.

Purpose: Evaluate the effect of drought on kale leaf exudate composition and on *Salmonella* growth.

Methods: Kale cultivar 'Improved dwarf' plants were grown for 2 or 8 weeks post-germination in a greenhouse (23°C, 16 h L:8 h D), then subjected to drought for 6 or 3 days, respectively depending on age, or regularly watered (control). Subsequently, leaf exudates from 20-day-old and 59-day-old plants were collected by placing leaves in 5% methanol and shaken at 150 rpm for 24 hours. Aliquots were filter-sterilized and inoculated with ~10^3 *Salmonella* Newport cells for growth analysis. Exudates from each sample were freeze-dried and resuspended in 70% methanol with 0.5% formic acid for Electrospray Ionization-Mass Spectrometry (ESI-MS) measurement. Multivariate data were analyzed using Multidimensional Scaling (MDS) ordination and Analysis of Similarity (ANOSIM) using Primer 6.

Results: *Salmonella* counts after 24 hours were higher in exudates of 20-day-old control plants than drought-subjected plants ($P < 0.05$), but no difference was detected between 59-day-old control and drought-subjected plants. *Salmonella* counts in exudates of 59-day-old control plants were lower than in both control and drought-subjected 20-day-old leaf exudates ($P < 0.05$). Based on both negative and positive mode ESI-MS data, MDS ordination and ANOSIM revealed similarity between exometabolome profiles of 59-day-old control and drought-subjected plants. By contrast, exudate profiles of 20-day-old control and drought-subjected plants clustered separately ($P < 0.05$). Under negative mode, exudate profiles of 20- and 59-day-old plants differed regardless of watering regime ($P < 0.005$). Under positive mode, exudate profiles of 59-day-old control plants diverged from 20-day-old control ($P = 0.01$) and drought-subjected plants ($P < 0.005$).

Significance: Drought in juvenile plants and age impacted the chemical composition of kale leaf surface compounds. Plant abiotic stresses and developmental stage may affect crop safety risk.

P3-93 Inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Soil by Glucosinolate Hydrolysis Products in Mustard Seed Meal

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❖ ***Developing Scientist Entrant***

Introduction: Soil is a potential source of produce contamination and treatments to mitigate this risk while maintaining soil health is lacking. Current biofumigation methods that use glucosinolate hydrolysis products in mustard seed meal to control plant pathogens could also be effective against foodborne pathogens in soil.

Purpose: The purpose of this research is to determine the fate of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in soil treated with mustard seed meal.

Methods: Sterilized soil was inoculated with *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* (~6 log CFU/g). Ten grams of the inoculated soil was combined with 1.0 or 1.5 g of mustard seed meal, flooded with 20 mL water and incubated at 20°C. Treated and untreated samples were enumerated for the target pathogen on tryptic soy agar (TSA) immediately after preparation, and at 24, 48, and 72 hours. The data was log transformed and compared using a fixed effect test, to determine if there was a significant difference in pathogen concentrations at different seed meal application rates over 72 hours.

Results: Significant reductions ($P < 0.05$) of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were observed in soil over 72 hours with the addition of 1.0 and 1.5 g of mustard seed meal. Increasing the seed meal concentration did not significantly ($P > 0.05$) increase the observed log reduction for *L. monocytogenes* or *Salmonella*, reductions ranged from 5.6 – 5.9 log CFU/g. However, for *E. coli* O157:H7 seed meal concentration was significant ($P < 0.05$). A 5.7-log CFU/g decrease was observed when 1.5 g of seed meal was used which was larger than the 3.5-log CFU/g reduction observed with 1.0 g.

Significance: Findings suggests that biofumigation with mustard seed meal could potentially be used to reduce *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* in contaminated soil.

P3-94 Isolation of *Salmonella* spp. from Fresh Produce Sold at Farmers' Markets and Urban Gardens

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Introduction: *Salmonella* is one of the major food borne bacteria responsible for produce related outbreaks. According to CDC, *Salmonella* causes about 1.35 million infections, 26,500 hospitalizations, and 420 deaths every year. However, there is little data on the prevalence of *Salmonella* in fresh produce sold at local farmers' markets and gardens.

Purpose: To study the microbial prevalence of *Salmonella* in fresh produce associated with urban food production and distribution system.

Methods: Fresh produce samples were collected from four farmers' markets and three urban farms in metro Detroit and West Virginia from June to August 2019. A total of 432 leafy greens and root vegetables were collected. Individual vegetables were then pooled into 109 composite samples for analysis. An FDA-BAM method was used for *Salmonella* isolation. The samples were pre-enriched with buffered peptone water (BPW) followed by selective enrichment in tetrathionate (TT) broth. Isolation was done using Hektoen enteric (HE) agar and Xylose lysine desoxycholate (XLD) agar. Confirmation was done by gram staining followed by PCR targeting *Salmonella*-specific internal transcribed spacer (ITS) region.

Results: Out of 109 composite samples, 6 (5.5%) were *Salmonella* positive, including red potatoes, kale, and carrots from an urban farm as well as cantaloupe melons, cucumber, and zucchini from a farmers' market.

Significance: The data suggest that locally-grown fresh produce may carry *Salmonella* and pose a public health concern. The study will act as a footstep for further research to determine the antibiotic resistance and virulence potential in *Salmonella*.

P3-95 Oklahoma Weather Effects on *E. coli* in Surface Water and Produce Safety

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Introduction: Fresh produce poses a food safety risk due to often being eaten raw. One known method of produce contamination is using surface water for irrigation. This route of contamination has been seen recently with multiple pathogenic *Escherichia coli (E. coli)* outbreaks from Romaine lettuce.

Purpose: If correlation is found between weather and the generic *E. coli* loads in surface water, then weather monitoring could allow farmers to determine which days may be safer than other days for irrigation of fresh produce crops.

Methods: The study compared 17 weather variables from the Oklahoma Mesonet from 2001 to 2018 to surface water generic *E. coli* samples from the Oklahoma Water Resources Board and the Oklahoma Water Survey using a Pearson Correlation. The data from the Mesonet station nearest each water sampling site was used for correlation. Only the water tests with 100 CFU of generic *E. coli* or more were used resulting in a total of 2,036 water samples.

Results: There were several statistically significant correlations ($P < 0.05$) in the statewide, regional, and water source type correlations. However those correlation coefficients were all below +/- 0.20. Rainfall on the day of the sample collection was consistently the highest correlated variable. Correlations based on individual sites showed the generic *E. coli* levels at certain sites were highly related to weather with coefficients from +/- 0.50 to +/- 0.90, but those correlated variables differed by each site.

Significance: The results suggest that a statewide or regional recommendation of using weather as a predictor of water contamination may not be advisable due to low correlation coefficients; however, a farmer may have success in limiting the contamination of fresh produce from surface water irrigation by completing a weather correlation study on each individual water source.

Poster

P3-96 A Simulation Model of Fresh Spinach Microbial Spoilage Along a Chinese Supply Chain from Harvest to Consumption

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❖ ***Developing Scientist Entrant***

Introduction: Microbial food spoilage is a significant economic, environmental, and societal problem and consumers increasingly expect food systems to operate with minimal food waste and food spoilage.

Purpose: To develop a Monte Carlo simulation-based model for microbial spoilage of fresh spinach in a Chinese supply chain to identify waste prevention strategies.

Methods: A model was built for predicting aerobic plate counts (APC) on fresh spinach from point-of-harvest through end of shelf life in a Chinese supply chain where spinach is grown in greenhouses and distributed via retail stores and eCommerce. The model has 2 components where component-1 simulates APC on spinach from harvest through distribution and component-2 simulates APC on spinach stored in a home refrigerator from day 0 to day 10 in shelf life. Samples were categorized as "spoiled" when APC was 10^7 CFU/g. Preliminary Monte Carlo simulations and sensitivity analyses were performed for component-2; initial microbial concentrations, isolate frequencies, and isolate growth rates in the model were parameterized with data from a pilot study in China conducted in January 2020 as well as comparable ongoing studies in the US (2019 to present).

Results: Preliminary results indicate that 90% of fresh spinach packages will have an APC level >10^7 CFU/g by day 10 of storage at an average temperature of 4.1°C (standard deviation = 2.4°C). This prediction was most sensitive to initial microbial concentration.

Significance: Predicting spoilage of fresh produce due to bacteria will allow for improved predictions of product shelf life and aid identification of interventions at different stages of the supply chain continuum to maximize return on investment, improve consumer experience, and reduce food waste. Our work thus far has identified a need for additional spoilage data to be collected from primary production to consumption in China as the key data gaps.

P3-97 Application of Sonodynamic Therapy for Foodborne Pathogens Disinfection

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❖ ***Developing Scientist Entrant***

Introduction: Sonodynamic therapy (SDT) is a robust treatment based on a combination of chemical compounds (sonosensitizers) and ultrasound processing that can be applied in food processing to improve the inactivation of a diversity of targets include foodborne pathogens and food spoilage microbes.

Purpose: The objectives of this study are to evaluate the effectiveness of SDT systems that use food-grade sonosensitizers to inactivate selected foodborne pathogens in several liquid systems including aqueous and beverage models.

Methods: Two ultrasound systems with different frequency and intensity coupling with food-grade sonosensitizers are employed in this study. The kinetic inactivation models are constructed to quantify the bacterial reduction of the SDT treatments using the standard plate count method. After the synergistic bacterial inactivation is confirmed, the biochemical changes in the treated cells are characterized to study the potential pathway of the inactivation. The bactericidal mechanisms to be studied focus on cellular apoptosis indicators including membrane damage, oxidative stress and the suppression of metabolic activity.

Results: Results showed a significant synergistic bacterial inactivation over the selected bacterial models in a short period of treatment time. The bacterial models were *E. coli O157:H7* and *L. innocua* strains as representatives of Gram-negative and Gram-positive bacteria. We observed that the SDT treatments achieved 5- to 6-log CFU reduction ($P < 0.01$) for both of the tested bacteria within 15 minutes in aqueous solutions. When applied in beverage models, the SDT treatments showed enhanced decontamination of 5 to 6 logs CFU ($P < 0.01$) within 10 minutes while product quality attributes remain unchanged. The treated bacteria showed signs of cellular apoptosis include significant membrane damage, intracellular oxidative damage, and enzymatic activity suppression.

Significance: The results of this study demonstrate the potential of sonodynamic therapy as a rapid and highly effective food processing method for improving the sanitation of food products without compromising food quality attributes.

P3-98 Biofilm Formation Ability of *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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Introduction: *Escherichia coli* O157:H7 and *Listeria monocytogenes* can attach onto equipment surfaces and subsequently contaminate fresh produce at the processing facility.

Purpose: The purpose of this study was to evaluate the biofilm formation ability of *E. coli* O157:H7 and *L. monocytogenes* on abiotic surface.

Methods: Individual strain of *E. coli* O157:H7 (5 environmental isolates) and *L. monocytogenes* (8 environmental isolates) at 2.5 or 5.5 log CFU/ml were transferred to the MBEC (minimum biofilm eradication concentration) plates with peg lid, followed by incubation at 25°C for 48 h. After incubation, bacterial populations in biofilms were determined by rinsing the pegs (N=156) in phosphate buffer saline (PBS), and removing the pegs with sterile cutter from the lid. Each peg was then sonicated for 15 min in 500 µL of PBS to release bacteria in biofilms. Serial 10-fold dilutions were made in PBS after sonication and appropriate dilutions were spot plated (5 µL/spot) on Tryptic Soy Agar. Colonies from each spot were counted following incubation at 37°C for 24 h.

Results: *E. coli* O157:H7 populations in biofilms recovered from MBEC pegs ranged from 5.1 log to 6.8 log CFU/cm^2 and 5.3 log to 7.8 log CFU/cm^2 with the initial inoculation levels at 2.5 log and 5.5 log CFU/ml, respectively. For *L. monocytogenes,* 5.2 to 6.2 log CFU/cm^2 and 4.3 to 6.5 log CFU/cm^2 bacterial populations in biofilms were recovered from the 2.5 log and 5.5 log CFU/mL inoculum, respectively. Results of this study revealed that biofilm formation of *E. coli* O157:H7 and *L. monocytogenes* varied among bacterial strains and the initial inoculum levels.

Significance: Results of this study provide important information to understand the mechanisms of biofilm formation by *E. coli* O157:H7 and *L. monocytogenes* and will be helpful to evaluate the contamination risks of foods by these pathogens during processing.

P3-99 Detection of *Salmonella* Enteritidis on Fresh Produces Having Different Surface Properties Using Phage-based Surface-scanning System

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Kyungpook National University, Daegu, South Korea

❖ ***Developing Scientist Entrant***

Introduction: Phage-based surface-scanning system has been developed as an in-situ, real-time, and direct detection method. Since fresh produces have diverse surface properties including surface hydrophobicity and surface roughness, the effect of surface properties on performance of surface-scanning system should be investigated for its practical application.

Purpose: The purpose of this study was to investigate the effect of surface properties of fresh produces on the performance of phage-based surface-scanning system.

Methods: The hydrophobicity and roughness of fresh produces (apple, orange, and zucchini) were measured by using contact angle method and CLSM, respectively. After *S*. Enteritidis-specific phage was immobilized on sensors, they were placed on the surface of the fresh produces previously contaminated with various concentrations (2, 4, 6 and 8 log CFU/mL) of *S*. Enteritidis. The resonant frequency of the sensor was measured using a network analyzer and bacteria attachment on the sensors was confirmed by using SEM for the comparison of sensitivity, linearity (R^2) and detection limit.

Results: There were significant differences in surface roughness and contact angle for apple (0.44 ± 0.04 and 56.08 ± 4.18), orange (2.10 ± 0.66 and 33.22 ± 2.59) and zucchini (0.86 ± 0.04 and 60.68 ± 2.23) ($P < 0.05$). As the concentration of *S*. Enteritidis on the fresh produces increased, the measured resonant frequency shifts and bacterial densities on the sensor surface also increased. The sensitivity and linearity (R^2) of apple (327.40 ± 85.44 and 0.85) and orange (343.90 ± 27.60 and 0.80) were greater than that of zucchini (257.00 ± 22.31 and 0.78). However, there were no significant differences in detection limit among apple (2.95 log CFU/mL), orange (2.71 log CFU/mL) and zucchini (2.46 log CFU/mL).

Significance: The surface-scanning system exhibited similar performance in a dose-dependent manner at nearly same detection limit on the fresh produces (apple, orange and zucchini), despite the differences in their surface properties.

P3-100 Utility of Rapid Tests to Assess Populations of Indicator Organisms (Aerobic Plate Count, *Enterobacteria*ceae, Coliforms, *Escherichia coli*) and Detection of *Listeria* spp. in Apple Packinghouses

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❖ ***Developing Scientist Entrant***

Introduction: The 2014 listeriosis caramel apple outbreak, linked to apples cross-contaminated within an apple packing facility, has increased the focus on effective cleaning and sanitation practices that must be validated and monitored during packing. Thus, rapid and reliable testing methods are essential when assessing cleanliness of food contact surfaces in the apple packing industry.

Purpose: To assess the prevalence of common indicator organisms (aerobic plate count (APC), *Enterobacteriaceae,* coliforms*, E. coli*, and *Listeria* spp.) on food contact surfaces (zone 1) in apple packinghouses. To evaluate the utility and accuracy of currently used rapid tests (ATP and glucose/lactose residue swabs).

Methods: Food contact surfaces were sampled over a 100 cm^2 area in five commercial apple packinghouses after sanitation events to evaluate populations of indicator organisms APC, *Enterobacteriaceae,* coliforms*,* and *E. coli* ($n = 741$), and rapid tests ($n = 659$). 3M Petrifilm plates were used for quantification

of APC, *Enterobacteriaceae*, coliforms/*E.coli*. Rapid tests [ATP (UltraSnap) and Glucose/Lactose residue (SpotCheck Plus) swabs] were processed on-site. A 0.92 m^2 area was sampled for the detection of *Listeria* spp. (n = 747), following the FDA's Bacteriological Analytical Manual method, and confirmed with PCR via the *iap* gene.

Results: No significant correlation was found between ATP test with populations of APC, *Enterobacteriaceae*, coliforms/*E.coli* , and positive detection of *Listeria* spp. Regarding glucose/lactose residue swabs, only the recovery of APC (log CFU/100 cm^2) was higher for an indication of failed surface hygiene (3.08) than a pass (2.91) (P = 0.031). Populations of APC, *Enterobacteriaceae,* and coliforms were significantly different at each unit operation during the packing process.

Significance: ATP and Glucose/Lactose residue rapid tests are poorly suited for determining microbial load. This emphasizes the need to utilize both rapid tests, which are good indicators of residual matter on a surface, along with traditional microbiological methods to assess cleaning and sanitation practices in apple packinghouses.

P3-101 Impact of the Colonization of *Lactobacillus curvatus* on the Formation of *Listeria monocytogenes* Biofilm on Stainless Steel

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Introduction: The persistence of *Listeria monocytogenes* (LM) in the food processing environment and the formation of LM biofilms have been serious food safety concerns and need to be better controlled.

Purpose: The goal of this study was to evaluate the impact of the colonization of *Lactobacillus curvatus* (a commercial SafePro® RTE culture) on stainless steel surfaces on the attachment of LM cells and the formation of LM biofilms.

Methods: Two experiment trials were designed. In the first trial, the inhibition of SafePro® RTE (~7 log CFU/mL) on the attachment of LM cells was evaluated by co-inoculating stainless steel coupons with SafePro® RTE and LM cultures. In the second trial, stainless steel coupons were first inoculated with SafePro® RTE cultures and were then contaminated with LM. Two LM inoculation levels were tested (3 and 6 log CFU/mL) and two inoculation media, including brain heart infusion broth (BHI) and fresh produce homogenate (FPH), were used. The interactions were investigated by incubating the inoculated coupons at 4 and 37°C. The attached LM cells and SafePro® RTE cultures were enumerated via plating on modified oxford agar and Lactobacilli MRS Agar, respectively.

Results: The anti-biofilm formation capacity of SafePro® RTE were observed when concentrations of LM were ~3 log CFU/mL and the temperature was at 4°C. This anti-biofilm capacity was observed in BHI and FPH. The presence of background microflora also positively contributed to the anti-biofilm results. When SafePro® RTE and LM were co-inoculated onto the stainless steel surfaces, reduced biofilm formation was observed from Day 3 to Day 7. When surfaces were pre-conditioned with SafePro® RTE, decreased cell attachment and reduced formation of LM biofilms were observed from Day 1.

Significance: Results of this study demonstrate the potential use of SafePro® RTE in the processing environment for the control of LM contamination.

P3-102 Identification of the Genes of *Salmonella enterica* Serotype Tennessee Involved in Biofilm Formation

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❖ ***Developing Scientist Entrant***

Introduction: Biofilm formation is a strategy of *Salmonella* to survive in a hostile environment. Although it is well understood that biofilm offers *Salmonella* increased tolerance to stress and enhanced survival on low moisture food like seeds and nuts, the molecular mechanisms underlying *Salmonella* biofilm formation and colonization on dry food have not been fully elucidated.

Purpose: The purpose of this study is to identify the genes that are involved in colonization and biofilm formation by *Salmonella*.

Methods: Mini-Tn*10* transposon mutagenesis was used in the present study to randomly interrupt the genes of *Salmonella enterica* serotype Tennessee, an isolate from the large, widespread peanut butter outbreak in 2007. The ability of selected *Salmonella* mutants in forming biofilms were compared with their wild type parent in a 24-well polystyrene tissue culture plate. Biofilm mass was quantified using the crystal violet binding assay. Mutants forming significantly less (P < 0.05) biofilm mass in comparison to their wild type parent were selected. Genomic DNA of mutant cells were extracted and subjected to deep DNA sequencing. Specific gene in each mutant that was interrupted by mini-Tn*10* insertion was identified by comparing the obtained DNA sequencing data with those deposited in the Genbank using BLAST search.

Results: A total of 56 colonies of *S*. Tennessee mutants were obtained, and only 5 colonies were selected for further analysis according to the results of biofilm assay. Cells of the 5 mutants formed significantly lower (P < 0.05) biofilm mass than the parent strain. Sequencing analysis revealed that the interrupted genes in collected mutants encode for bacterial cell membrane lipoprotein, DNA topoisomerase III, attachment invasive locus protein, bacteriocin immunity protein, or cell division protein.

Significance: The study identified some of the genes that play a role in the formation of biofilms by *Salmonella*. These genes could be likely targets for control of pathogen colonization on low moisture food.

P3-104 Characterization of the Relationship between Post-harvest Fungal Rot and Indicator Organism Die-off Rates on Gala Apples during Three Months of Storage

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Introduction: *Botrytis cinerea* and *Penicillium expansum* can colonize and rot away the apple tissue at an increasing rate as storage time increases, which may impact the growth of saprophytic foodborne pathogens like *Listeria monocytogenes*. While decayed apples are not consumed, they are exposed to the packing equipment and are an unstudied potential source of foodborne pathogen contamination.

Purpose: To determine population changes of *Listeria innocua* as a surrogate for *L. monocytogenes* on apples co-inoculated with *Penicillium expansum* or *Botrytis cinerea* during three months of modified atmosphere cold storage conditions.

Methods: Gala apples (n = 270) were drenched in pyrimethanil and inoculated with *Listeria innocua* (LI) in two 6.25 cm^2 spots on the equator, one of which was wounded. Apples were assigned to one of three treatments: LI-only control, co-inoculation with *Botrytis cinerea* (BC), or co-inoculation with *Penicillium expansum* (PE). Apples were treated with 1-methylcyclopropene and stored under modified atmosphere conditions (1°C, 1% CO_2, 2% O_2). LI population size was determined at 1 week, 1 month, and 3 months. The inoculation site was excised and cells eluted in 24 mL 0.1% peptone with 0.2% Tween 80. Samples were

serially diluted and plated on Modified Oxford Medium [MOX (incubated 48 h at 35°C)] or Dichloran Rose Bengal Chloramphenicol Agar [DRBC (incubated 5 d at 25°C)] for LI and mold enumeration, respectively.

Results: Populations of LI decreased by up to 4.13 log CFU/cm^2, with significant differences in populations based on wound status and treatment. LI survived in higher populations across the three timepoints on wounded, compared to unwounded, surfaces ($P < 0.0001$). LI populations were higher in the control fruit (4.62 log CFU/cm^2) than on those co-inoculated with BC (4.00) or PE (2.64). Populations of LI were similar in unwounded samples (2.46-2.68 log CFU/cm^2).

Significance: Surface wounding significantly affected the likelihood of survival of LI, with mold species playing a significant factor.

P3-105 Bio-control of *Listeria monocytogenes* on the Surface of Fresh Produce

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Introduction: Produce surfaces can get contaminated by *L. monocytogenes*. Its survival despite post-harvest washing highlights the importance for developing and validating effective and easy-to-use intervention approach.

Purpose: To evaluate the efficacy of lactic acid bacteria as an intervention for the reduction of *L. monocytogenes* on fresh produce.

Methods: Two lactic acid bacterial isolates, C-1-92 (*Lactococcus lactis* subsp. *Lactis*) and #152 (*Enterococcus durans*) with excellent ability to inhibit *L. monocytogenes* at 4-37°C in various conditions were evaluated. A cocktail of three *L. monocytogenes* strains were used to inoculate green peppers and apples at 21 ± 1°C at a concentration of 3 log CFU/cm^2. After 48 h post-inoculation, green peppers and apples were individually treated with 0.1% peptone, 5 log CFU/mL C-1-92 or #152. These contaminated fresh produce were sampled daily for enumeration of *L. monocytogenes* and aerobic bacteria.

Results: After 14 days the population of L. monocytogenes on green pepper treated with control, #152, and C-1-92 studies was 3.4, 2.3, and 1.3 log CFU/cm^2, respectively. The application of C-1-92 treatment reduced the *L. monocytogenes* population by 2.1 log CFU/cm^2 ($P < 0.05$) when compared to the control. For apple studies, *L. monocytogenes* population treated with C-1-92 or #152 were below detection level (0.69 CFU/cm^2, $P < 0.05$) and 50% samples were detected by selective enrichment only at day 14; whereas, *L. monocytogenes* in control group was 2.6 CFU/cm^2. The aerobic bacterial counts on peppers and apples revealed there were no difference among control and lactic acid-treated groups.

Significance: The intervention by *Lactococcus lactis* subsp. *Lactis* (C-1-92) can substantially reduce the existence of *L. monocytogenes* on the surface of fresh produce.

P3-106 Antimicrobial Evaluation of an In-Situ UV Treatment Unit for Fresh Produce Decontamination

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Introduction: In-house treatment strategy for fresh produce decontamination has not been emphasized as much as industrial washing in *Salmonella* outbreaks. The most common treatment is rinsing and/or soaking in the sink. In this study, an in-situ water-assisted UV treatment unit was applied and investigated as a potential hurdle technology for fresh produce decontamination in households.

Purpose: This study aimed to determine the bactericidal power of a water-assisted UV treatment unit during washing spinach and grape tomato samples.

Methods: Fresh-brought spinach and grape tomato samples were dipped- and spot-inoculated in a four-strain *Salmonella* cocktail to reach a final microbial population of 6 – 8 log CFU/g and air-dried. The produce samples were washed in 1 gallon tap water under varying combined conditions such as agitation speed (0 – 190 rpm), sample size (50 – 200 g), UV intensity (0 - 30 mW/cm^2) and treatment time (2, 5 and 10 min). The *Salmonella* counts of samples were determined.

Results: All the factors in this study including agitation speed, sample size, UV intensity, and treatment time affected the decontamination efficacy of the UV treatment unit. Among all treatment conditions studied, the most effective conditions were 10-min 30 mW/cm^2 UV treatment at 190 rpm for dipped- and spot-inoculated 200 g grape tomato while 10-min 30 mW/cm^2 UV treatment at 110 rpm for dipped- and spot-inoculated 100 g spinach. The largest log reductions for dipped- and spot-inoculated samples were 3.46 ± 0.30 log, 3.50 ± 0.12 log CFU/g spinach, and 4.16 ± 0.27 log, 4.22 ± 0.32 log CFU/g grape tomato, respectively. Those results were significantly different ($P < 0.05$) from other treatment results.

Significance: The UV treatment appliance developed in this study could potentially be used in households to effectively decontaminate fresh produce and improve food safety.

P3-107 Wax On! Pathogen Off!

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Introduction: The CDC has categorized "Seeded Vegetables" such as tomatoes, cucumbers and peppers as "high risk" for *Salmonella* contamination. These produce commodities have a cuticle consisting of fatty acids that helps in reducing transpiration rates and serves as a barrier against colonizing pathogens. Post-harvest washing, brushing and the use of sanitizers could erode the cuticle and make the surfaces of produce more liable to contamination by foodborne pathogens. Further the use of waxes to coat vegetables and fruits might contribute to their survival.

Purpose: This research was undertaken to explore the efficacy of pelargonic acid as an additive to carnauba wax in mitigating *Salmonella* populations on tomatoes, peppers and cucumbers.

Methods: Surfaces of cucumbers, tomatoes and peppers were spot inoculated with a 5 log CFU/g cocktail of *Salmonella* serotypes Newport, Typhimurium and Poona. Inoculated fruits were coated with either, commercial carnauba wax (CW) (approximately 200 µL of wax/vegetable) or commercial carnauba wax supplemented with 15 mM pelargonic acid (CWPel). All produce commodities were stored at 20°C and *Salmonella* populations were enumerated on days 1 and 7. Significant differences were determined using Analysis of Variance.

Results: *Salmonella* decreased on tomatoes ($P < 0.05$), cucumbers and peppers coated with CWPel by 4.25, 1.61, 0.68 log CFU/g, respectively, in comparison to ones coated with CW. After storage at 20°C for one week, *Salmonella* population on tomatoes ($P < 0.05$), cucumbers and peppers coated with CWPel decreased by 4.05, 1.54 and 2.29 Log CFU/g respectively in comparison to ones coated with CW. *Salmonella* was not recovered from unwaxed tomatoes on day 1 and day 7. The population of *Salmonella* increased on tomatoes, cucumbers and peppers coated with CW by 2.17, 1.90 and 4.32 Log CFU/g, respectively.

Significance: These results indicate that *Salmonella* can survive on produce coated with certain waxes and that the addition of pelargonic acid could serve as a mitigation strategy.

P3-108 Use of Combined Ultraviolet Light, Ultra-Sonication, and Agitation Treatments to Enhance Fresh Produce Decontamination Efficacy

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❖ ***Developing Scientist Entrant***

Introduction: Fruit and vegetables are common targets in *Salmonella* outbreaks. Several combined treatment strategies have been studied as hurdle technology for fresh produce decontamination. However, the biocidal power of ultraviolet light (UV) in combined ultra-sonication (US) and agitated washing was not examined previously.

Purpose: This study aimed to determine the antimicrobial effects of agitation-assisted UV treatment combined with US, and identify strategies that provide aid to decontaminate leafy green and non-leafy green.

Methods: Fresh spinach and grape tomato were dip- or spot-inoculated with four-strain *Salmonella* cocktail (7 – 8 log CFU/mL) and air-dried. The 100 g spinach and 200 g grape tomato samples were washed in an ultra-sonicator containing 3 L tap water. Agitation, soaking, US, UV, and combined treatments were implemented to inactivate *Salmonella* in produce samples for varying treatment time. The *Salmonella* population was enumerated and analyzed statistically.

Results: The soaking of spinach and grape tomato resulted in 0.89 ± 0.12 and 1.75 ± 0.09 log CFU/g reductions of *Salmonella*, respectively. Three of the combined treatment strategies that had significantly better decontamination efficacy ($P < 0.05$) for spinach were US/agitation, UV/agitation, and US/UV/agitation. After 10-min treatment, those treatment strategies resulted in reductions of 1.77 ± 0.02, 1.55 ± 0.28 and 1.69 ± 0.34 log CFU/g for dip-inoculated samples and 1.74 ± 0.74, 2.14 ± 0.63 and 2.75 ± 0.43 log CFU/g for spot-inoculated samples, respectively.

For grape tomato, three significantly better ($P < 0.05$) strategies were US, UV/agitation and US/UV/agitation. Those treatments resulted in *Salmonella* reductions of 2.87 ± 0.17, 3.98 ± 0.33, and 3.72 ± 0.84 log CFU/g for dip-inoculated samples and 3.44 ± 0.45, 4.01 ± 0.26 and 3.92 ± 0.09 log CFU/g for spot-inoculated samples after 10-min treatment, respectively.

Significance: Combined treatment strategies of US and agitation-assisted UV could improve the microbial decontamination efficacy of fresh produce and enhance food safety.

P3-110 Microbial Quality Assessment of Fresh Produce Sold in Food Desert Areas in Central Virginia

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Introduction: Lack of large chain supermarkets (LCSM) in close proximity to food deserts may result in the over-reliance of low-income residents on small neighborhood convenience store retailers to acquire fresh foods. Studies demonstrate that small independently owned markets (SIOM) incur more critical and non-critical code violations in food safety as compared to LCSM.

Purpose: The purpose of this study was to assess microbiological quality differences of fresh produce sold at select SIOM and LCSM in food desert areas of Central Virginia.

Methods: A total of 122 fresh produce samples were procured from nineteen registered markets, consisting of 10 SIOM and 9 LCSM, located in food desert areas of Petersburg and Colonial Heights in Virginia. Purchases were made in duplicate between September 2018 and April 2019. Samples were transported in pre-chilled coolers, followed by microbial testing within 24 hours of arrival. Microbial testing was performed using AOAC-approved or performance-tested methods.

Results: The highest levels of aerobic mesophiles and coliforms were recovered from turnips from LCSM and SIOM. Higher levels of aerobic mesophile counts were found in all samples acquired from SIOM compared to those procured from LCSM. Regardless of food outlet source, *Campylobacter* spp., *E. coli*, and *Listeria spp*. were detected in 11.5%, 4.9%, and 3.3% of the samples, respectively. The majority of *Listeria* spp. (75%) and *Campylobacter* spp. (71.4%) isolates were recovered from SIOM.

Significance: Findings of our study highlight the food safety risks associated with both SIOM and LCSM food outlets. Due to the presence of high microbial levels, the development and dissemination of future food safety training and educational programs are needed for producers, retailers, and consumers, regardless of food outlet type.

P3-111 Validation of Triple-wash Procedures with Sodium Hypochlorite, Lactic-Citric Acid Blend, and Mixer of Peroxyacetic Acid-hydrogen Peroxide to Inactivate *Salmonella*, *Listeria monocytogenes*, and Surrogate *Enterococcus faecium* on Cucumbers and Tomatoes

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Introduction: Cucumbers and tomatoes from West Virginia farmers market were positive for *Salmonella* and *Listeria*. Triple-wash process is recommended by WV Small Farm Center for inactivating pathogens from produce, however, it lacks validated data information.

Purpose: This study aimed to evaluate the two triple-wash procedures with commercial antimicrobials to inactivate foodborne pathogens and surrogate bacteria on cucumbers and tomatoes.

Methods: Fresh locally grown cucumbers and tomatoes were dip-inoculated with *Salmonella* Typhimurium and Tennessee, *Listeria monocytogenes* (3-strain), and *Enterococcus faecium*. Produce were washed through two triple-wash steps (10 s each) including water dip- antimicrobial dip-water dip (WAW), or water dip-water dip-antimicrobial dip (WWA), followed by draining (2 min) on aluminum foil paper. Tested antimicrobials were 1) sodium hypochlorite (SH-100 ppm); 2) lactic-citric acid blend (LCA-2.5%); and 3) a H_2O_2-peroxyacetic-acid mixer (SaniDate-5.0, SD-0.0064, -0.25 and -0.50%). Surviving bacteria were recovered on XLT-4 (*Salmonella*), MOX (*L. monocytogenes*), and bile esculin agar (*E. faecium*). Data (2 replicates/4 samples/replicate) were analyzed using the Mixed Model Procedure of SAS ($P = 0.05$).

Results: Counts of *Salmonella*, *L. monocytogenes*, and *E. faecium* on unwashed cucumbers and tomatoes were 5.42 to 6.23, 6.31 to 6.92, and 6.05 log CFU/produce, respectively. WWA achieved additional reductions (LsMeans, $P < 0.05$) of 0.38 log CFU/cucumber (*Salmonella*), 0.56 log CFU/cucumber (*E. faecium*), 1.48 log CFU/tomato (*Salmonella*), 1.09 log CFU/tomato (*L. monocytogenes*), and 0.71 log CFU/tomato than the WAW procedure. Applying SD-0.25% and -0.50% solutions in triple-washing cucumbers and tomatoes indicates better ($P < 0.05$) reductions than SH and LCA treatments. *E. faecium* behaved less susceptible ($P < 0.05$) or similarly ($P > 0.05$) to *Salmonella* when exposure to antimicrobials in WAW and WWA for cucumbers and in WWA for tomatoes.

Poster

Significance: Locally, small produce growers should adopt WWA procedure with commercial antimicrobials to control foodborne pathogens during the postharvest processing. Future pilot plant validation studies and cost-effectiveness analysis is needed for small produce growers.

P3-113 Examining the Distribution of *Listeria monocytogenes* in a Hydroponic System from Contaminated Seeds

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Introduction: The controlled environment of the hydroponic system minimizes the potential microbial contamination from agricultural environments. However, contaminated seed often presents a significant threat to food safety because of the moist environment and the circulation of a nutrient-rich solution.

Purpose: This study examined the distribution of *Listeria monocytogenes* from contaminated seeds to fertilizer solution, roots, and seedlings in a hydroponic system.

Methods: Lettuce and tomato seeds were inoculated with *Listeria monocytogenes* to a level of 4 log CFU/g and 2.5 log CFU/g for lettuce and tomato seeds, respectively. The inoculated seeds were inserted in moist rockwools and placed in a laboratory-scale hydroponic seedling system. Fertilizer solutions were adjusted daily to the electrical conductivity of 1600 µs/cm at pH 6. Samples of 100 seeds, 10 leaves, 10 roots, and fertilizer solution were collected at day 0, 5, 10, 15, 20 and 25 for lettuce, and for tomato at day 0, 10, 20, 30, 40, and 50.

Results: *Listeria monocytogenes* from the contaminated seeds were able to spread to the fertilizer solutions and other parts of the seedlings quickly. *Listeria* levels on lettuce fertilizer solution and roots were 1.82 log CFU/mL and 2.39 log CFU/g, respectively (day 10) and on tomato fertilizer solution and roots were 2.29 log CFU/mL and 4.37 log CFU/g, respectively (day 20). After day 20 levels remained similar in tomato roots (4.36 - 5.16 log CFU/g) and fertilizer solution (2.29 - 2.06 log CFU/mL). In tomato leaves levels were significantly increased ($P < 0.05$) from day 10 (4.90 log CFU/g) to 50 days (5.46 log CFU/g). However, levels decreased significantly ($P < 0.05$) in lettuce leaves and fertilizer solution from 4.22 - 3.47 log CFU/g and 1.82 - 0.67 log CFU/ml, respectively, after 25 days.

Significance: The microbial quality of the seeds and environmental sanitation program is important to minimize food safety risks in a hydroponic system.

P3-114 Development of Rapid Molecular Detection Methods for Foodborne Pathogens in Fresh Produce

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Introduction: Foodborne illnesses continue to be a serious concern as a public health issue for the food industry, with bacterial agents being responsible for most of the hospitalizations (63.9%) and deaths (63.7%). With growing public concern in relation to recent outbreaks concerning fresh produce, pre- and post-harvest control of foodborne pathogens is critical to prevent further dissemination. The most notable pathogens identified in fresh produce are *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*.

Purpose: The objective was to develop a multiplex PCR assay to detect multiple potential foodborne pathogens simultaneously and return results within hours versus days.

Methods: *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* were cultivated upon respective media at 37°C for 24 h. Colonies were selected and placed into 5 mL tubes of TSB, followed by incubation in a 200 RPM orbital shaker at 37°C for 18 h. Broths were serially diluted to 10^{-8}, and 1 mL portions (each pathogen separately + all three together) were added to stomacher bags which contained 25 g of romaine or kale and 225 mL of buffered peptone water, before insertion into a stomacher, followed by incubation at 37°C. One mL aliquots were drawn in triplicates at 0, 2, 4, 6, 8, and 18 h. Samples were centrifuged before gDNA isolation via a boiling method. The multiplex PCR assay was optimized and the amplicons were confirmed by agarose gel (3%) electrophoresis.

Results: The developed mPCR assay was able to accurately detect the presence of all three pathogens, together and separately, down to single copies of DNA prior to amplification, and logged subsequent growth throughout the incubation process.

Significance: To date, many detection methods are available for these pathogens, but often require pre-enrichment, which results in a 5- to 7-day turnaround for results. This mPCR assay is capable of returning the same quality of results within a few hours.

P3-115 Evaluation of Produce Safety Training Delivery Methods Using Quantitative On-farm Assessments

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Introduction: Produce growers may gain knowledge from attending produce safety trainings, but monitoring if farmers actually implement good produce safety practices after the training is essential.

Purpose: The purpose of this study was to compare the effectiveness of classroom and on-farm produce safety training on produce growers' food safety practices by conducting on-farm assessments.

Methods: Extension personnel from Kansas State University and the University of Missouri developed a quantitative produce safety on-farm assessment rubric based on the requirements of the FDA Food Safety Modernization Act (FSMA) Produce Safety Rule. We conducted on-farm produce safety assessments on 32 farms in Kansas and Missouri before farmers attended a training. These farmers then attended one of the following trainings: a) farm-based "Introduction to Produce Safety"; b) classroom-based "Introduction to Produce Safety"; or c) FSMA Produce Safety Alliance training. Approximately 3-6 months after their training, we conducted post-training on-farm assessments on 22 farms (10 farms lost to follow-up). We also conducted six-month post-training self-evaluations with all the participants attending the Introduction to Produce Safety trainings.

Results: Overall, post-workshop farm assessment scores improved for all farms. The training format did have a significant effect on the growers' improvement of their farm produce safety assessment ($P = 0.0245$). Assessment scores improved 10.2% ($P = 0.0161$, $n = 5$) for farms attending an on-farm training and 15.13% ($P = 0.0265$, $n = 3$) for farms attending a FSMA PSA training. One farm attending a classroom-based training had an improvement of 57%, but with that outlier removed, the other farms attending a classroom-based training had an average improvement of 8% (n=11), which was not statistically significant.

Significance: This study demonstrates that produce safety training is effective at helping produce growers to improve their on-farm food safety practices, but that further study is needed to determine how to most effectively help growers improve their practices further.

P3-116 Impact of Organic and Conventional Practices in the Microbiological Quality of Fresh Leafy Vegetables Produced in Piracicaba, SP - Brazil

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Introduction: The consumer demand for healthy foods has promoted the production of leafy vegetables from both organic and conventional farming systems. However, surveillance data in several countries point out an increasing association between vegetable consumption and foodborne outbreaks.

Purpose: The aim of this study was to report results on the microbiological quality of organic and conventional leafy vegetables collected in selected farms located in the city of Piracicaba, SP – Brazil.

Methods: A total of 100 samples of leafy vegetables (50 of which were organic and 50 conventional) were collected and submitted to the enumeration of total coliforms and generic *Escherichia coli* using the standard MPN method, in addition to the enumeration of total *Enterobacteriaceae* by plating on VRBG Agar. Moreover, 228 colonies of *Enterobacteriaceae* were randomly selected and submitted to identification on a MALDI-TOF MS Biotyper™.

Results: The mean counts of total coliforms were 2.2 ± 0.8 and 2.8 ± 0.5 log MPN/g in organic and conventional vegetables, respectively. Generic *E. coli* was mainly detected in conventional (40%) rather than organic (6%) samples. Regarding total *Enterobacteriaceae*, the mean counts for organic and conventional vegetables were 5.4 ± 1.4 and 5.1 ± 1.5 log CFU/g, respectively. *Pantoea* and *Enterobacter* were the main genera of bacteria identified by MALDI-TOF in vegetables from both farming systems. The most frequent species in organic samples were *P. agglomerans* (27.8%), *E. cloacae* and *E. ludwigii* (8.9% for both), while *P. ananatis* (24.6%) and *E. cloacae* (15.4%) were the ones most often found in conventional samples.

Significance: These results suggest that the farming system has little, if any, impact on the microbial populations of leafy vegetables in the tested farms, except on the prevalence of *E. coli*, which was higher in conventional samples.

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P3-117 Detection of Culturable Bacteria in Greenhouse-Grown Romaine Lettuce Using the Light Scattering Technology (BEAM)

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❖ ***Developing Scientist Entrant***

Introduction: Romaine lettuce is a widely consumed produce worldwide. This commodity is perishable and susceptible to microbiological contamination during production and distribution increasing the burden of outbreaks of enteric pathogens. To ensure a safe food supply, it is crucial to implement technologies for rapid detection and identification of target groups of bacteria, especially pathogens. Light-scattering technology (BEAM) uses an easier and faster method for detection and identification of bacteria based on the morphological characteristics of the colonies.

Purpose: The aim of this study was to determine parameters for generating the light scatter-patterns from different bacterial genera associated with greenhouse grown romaine lettuce.

Methods: *Bacterial identification:* Lettuce leaves were aseptically harvested, processed in 0.1 M phosphate buffer, plated on plate count agar (PCA) and incubated for 48 h. PCR amplification and sequencing of the region V3-V6 of the 16S rRNA from isolated colonies were preform for further taxonomic assignation by using Blast and RDP databases. *Scatter-pattern parameters standardization:* Single strains were cultured in Luria-Bertani broth and incubated at 30°C for 16 h. Cultures were serially diluted and plated on 20 mL PCA plates and incubated at 30°C. Light-scatter patterns were collected from different colonies.

Results: A total of 24 different bacterial genera were isolated from romaine lettuce leaves. It was found that the genera with higher relative abundance were *Pseudomonas, Erwinia, Bacillus* and *Paenibacillus*, corresponding to 16.38%, 15.25%, 11.86% and 10.17%, respectively. Bacterial growth rates were found to differ among the bacterial genera, allowing to classify the different genera based on incubation time such as fast, intermediate, medium and slow growth rate. Evaluated strains from different genera were suitable for generating scatter patterns under the established conditions.

Significance: Scatter-pattern technology allows for the rapid screening and identification of different bacterial genera from romaine lettuce.

P3-118 Modeling Cross-contamination and Inactivation Dynamics of *Escherichia coli* O157:H7 in Chlorine Wash of Fresh-cut Iceberg Lettuce

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Introduction: Pathogen cross-contamination during fresh produce wash could be reduced with chlorine sanitization. Washing of fresh produce in a sufficient level of chlorine decreases the microbial load, and the presence of sanitizer is essential to prevent the cross-contamination in washing process.

Purpose: Develop a mathematical model based only on free chlorine (FC) levels in the wash water to accurately and rapidly predict pathogen levels in produce wash cycles.

Methods: Five kg of chopped iceberg lettuce was washed per run, during three 10-min runs (n = 3). Sodium hypochlorite (9 mL) was added to a tank containing 20 L of water (pH~6.5, temp~4°C) to achieve FC levels ~12 $mg.L^{-1}$. Uninoculated chopped iceberg lettuce (500 $g.min^{-1}$), along with red leaf lettuce (10 $g.min^{-1}$) inoculated with non-pathogenic *E.coli* O157:H7 (5 log MPN/g), were simultaneously introduced in this tank. Average dwell time was designed to be 30 s for iceberg lettuce. Samples for bacteria levels in red leaf, iceberg lettuce, and wash water were collected every 2 min and FC levels quantified immediately. After completion of first run (10 min), 18 mL of sodium hypochlorite was added to the tank to replenish FC levels (pH~6.5, temp~4°C), and the next run was started after a 5-min delay. A similar procedure was repeated for the third run.

Results: Dislodgement rate of pathogens from inoculated produce to water was found to be independent of chlorine levels. The range of pathogen binding rate from water to non-inoculated produce, and the inactivation rate of *E. coli* by FC were determined. The developed model predicts FC and pathogen levels with >90% accuracy.

Significance: Our mathematical models could help in developing better strategies for maintaining chlorine levels needed to prevent cross-contamination of pathogen in the wash tanks.

P3-119 A Pilot-scale Study of Cold Plasma-activated Hydrogen Peroxide Technology: Effect on Populations of *Salmonella* Typhimurium and *Listeria innocua* and Quality Changes of Apple, Tomato and Cantaloupe During Storage

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Introduction: The occurrence of foodborne illness outbreaks associated with fresh fruits and vegetables continues to be a major concern. Effective technologies that can be applied commercially are urgently needed.

Purpose: The objectives of this study were to investigate the efficacy of cold plasma-ionized hydrogen peroxide (iHP) aerosol, applied on a pilot-scale level, for the inactivation of *Salmonella* Typhimurium and *Listeria innocua* on apple and tomato smooth surfaces, tomato stem scars and cantaloupe rinds, and to evaluate changes in quality attributes of treated fruits during simulated shelf-life study.

Methods: Apple, tomato and cantaloupe fruits with and without inoculated bacteria were treated with aerosolized hydrogen peroxide (17.62 mL m^{-3}) activated with cold plasma for 30 min in a large chamber (4.27 × 2.44 × 2.135 m). Survival bacteria were recovered and enumerated from inoculated fruits while non-inoculated samples were used to assess quality changes during 14 days storage at 17°C.

Results: The inoculated bacteria were significantly reduced to a level below the detection limit (0.70 log CFU/piece) on the smooth surfaces of apples and tomatoes placed as a single layer in crates. Reductions of more than 3 log CFU/piece and approximately 1 log CFU/piece were obtained for cantaloupe rinds and tomato stem scars, respectively. When apples were placed in crates with multiple layers, 22% of fruits exhibited detectable *Salmonella* and 11% of fruits had detectable *Listeria*. Results on quality analysis showed that iHP did not significantly ($P > 0.05$) affect appearance, color, texture, pH, soluble solids content, ascorbic acid, and antioxidants of the fresh produce items.

Significance: The study demonstrates that the technology can be applied to fresh fruits to enhance microbial safety while maintaining quality. Challenges remain as how to facilitate iHP exposure to all fruit surfaces in commercial settings.

P3-120 Sensitivity of Foodborne Pathogens to Chlorine and Peracetic Acid in Sterile Water and Rinse Water of Spinach and Lettuce

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Introduction: Fresh produce harbors large and diverse microbes, which include spoilage and potentially pathogenic bacteria. Contaminated water during flume wash is considered one of the main factors that causes cross-contamination of foodborne pathogens.

Purpose: To investigate the reduction of foodborne pathogens and dynamics of bacterial communities in wash water after free chlorine (FC) and peracetic acid (PAA) treatments.

Methods: A cocktail of foodborne pathogens, including pathogenic *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*, was inoculated in sterile water and spinach and lettuce rinse water and treated with FC (0-50 mg/L) and PAA (0-100 mg/L) for 30 s. Bacterial cells and populations in the 144 inoculated water samples were detected and/or numerated by plate count, fluorescence and transmission electronic microscopes, qPCR, and 16S rDNA high-throughput (HT) sequencing.

Results: After sanitation, inoculated foodborne pathogens were not detected in sterile and rinse water at 10 mg/L FC and 30 mg/L PAA or higher levels by direct plate count. Nevertheless, viable but nonculturable (VBNC) cells of foodborne pathogens were observed after sanitation, especially after high dose of PAA treatment. The sensitivity of foodborne pathogens to sanitizers was significantly higher in sterile water than in rinse water, which might be influenced by the organic matter and contextual microbiomes dislodged from spinach and lettuce. Most of the FC and PAA resistant bacteria in spinach and lettuce rinse water were gram positive, such as *Bacillus* and *Brevibacterium* spp., while several PAA resistant *Pseudomonas* spp. were also isolated from lettuce rinse water.

Significance: This research demonstrated the presence of VBNC cells and provided insight about how produce background phytobiota might contribute to the tolerance of foodborne pathogens to commonly used sanitizers in fresh produce industry.

P3-121 Effect of Lettuce Cultivar and Irrigation Water Source on the Dynamics of Innate Microbiota and Survival of Pathogenic *E. coli* and *Salmonella* spp. on Lettuce

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Introduction: Fresh produce, like lettuce, is a known vehicle for a number of recent foodborne outbreaks. Survival of bacterial pathogens on produce might be influenced by the diverse phytobiota.

Purpose: To investigate the effects of lettuce cultivar and irrigation with different types of water sources, like reclaimed wastewater, on the survival of foodborne pathogens and dynamics of innate microbiota on lettuce.

Methods: Three cultivars of lettuce, including Annapolis, Celinet, and Coastline, were grown in a high tunnel greenhouse and irrigated by ground water, primary-, and secondary-waste water during the growth. The harvested lettuce was inoculated with pathogenic *E. coli* and *Salmonella* (7 log CFU/mL for each) and stored at 5°C. Lettuce samples (54 non-inoculated and 54 inoculated) were tested one and seven days after inoculation to assess the change of microbiome and the potential effect on the pathogens by plate counting, PMA-qPCR, and 16S rDNA gene high throughput sequencing.

Results: Although microbial compositions in the three irrigation water were significantly different, the dominant taxa on lettuce were not significantly altered by irrigation using different types of water. Populations of total bacteria on lettuce remained at the same level or increased after 1-week storage at 5°C, whereas the populations of inoculated pathogenic *E. coli* and *S. enterica* were reduced by about 1 log. Survival of inoculated foodborne pathogens varied on different lettuce cultivars. The composition of most abundant bacterial taxa (top 10) on different lettuce cultivars grown in the same greenhouse showed greater similarity, which were not significantly affected by irrigation, inoculation of foodborne pathogens, and storage at refrigerated temperature.

Significance: Data derived from this study can benefit research on the management of foodborne pathogens and spoilage bacteria on fresh produce.

P3-122 Survival of Planktonic- and Biofilm-grown *Listeria monocytogenes* on Apples as Affected by Apple Variety, Grower Region, and Storage Conditions

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❖ ***Developing Scientist Entrant***

Introduction: The safety of whole apples can be compromised by contamination with *Listeria monocytogenes* (*Lm*) as evidenced from outbreaks of listeriosis in 2014 and 2017.

Purpose: This study assessed the effect of apple variety, growing region and storage conditions on survival of *Lm* grown planktonically or as a biofilm.

Methods: Unwaxed Gala, Granny Smith (GS), and Honeycrisp (HC) apples were received from growers in Michigan, Pennsylvania, and Washington and dip-inoculated in an 8-strain (~6.5 log CFU/ml) *Lm* cocktail of planktonically-grown cell suspensions. Michigan-grown apples were also dip inoculated with 48-h biofilm cultures previously established in 100-mm diameter polystyrene Petri dishes. After inoculation, the apples were dried and stored at 2°C aerobically and under controlled atmosphere (1.5% O_2, 1.5% CO_2). At specific timepoints, duplicate samples (6 apples/sample) were taken, the calyx/stem portions were aseptically removed, combined, stomached in PBS, diluted, plated on Modified Oxford Agar plates, and incubated (37°C/48 h) for *Lm* enumeration. Skins from the middle portion were similarly processed.

Results: Controlled and aerobic storage yielded similar survival of *Lm* on apples regardless of the variety ($P > 0.05$). After two months of storage, *Lm* populations decreased on Gala apples from all three regions (0.7 to 2.3-log reduction) and on GS apples from Michigan and Pennsylvania (0.3-log reduction each), while a non-significant ($P > 0.05$) increase (0.7 log) was noted for GS apples from Washington. On HC apples, *Lm* population reductions were higher ($P \leq 0.05$) for those from Michigan (2.83 log) as compared to those from Pennsylvania or Washington. Previous growth of *Lm* as a biofilm enhanced survival ($P < 0.05$) on apples, regardless of the variety or source.

Significance: The physiological state of *Lm* (planktonic vs biofilm), apple variety, and growing region can all impact overall survival of *Lm* on apples during 2°C storage.

P3-123 Learning from the On-Farm Readiness Review: Farm Preparedness and Educational Needs of New Jersey Farms for FSMA PSR Compliance

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Introduction: The Rutgers On-Farm Food Safety Team partnered with the New Jersey Department of Agriculture to conduct On-Farm Readiness Reviews (OFRRs) to assess farm readiness for the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) inspections.

Purpose: Farm readiness for inspection and priorities for improvement, which are tracked by inputting data into an online survey after each OFRR, inform the food safety team of educational outreach needs.

Methods: Eighty-four voluntary OFRRs were conducted during the 2018 and 2019 growing seasons on New Jersey farms growing produce typically consumed raw. The anonymous online OFRR post-visit survey was used to track general farm information, the walk-around questions used, and the three priority areas for improvement that needed to be made to meet the regulatory requirements. The state survey results indicate needs for improvement overall, by farm produce sales ranges, and by acreage ranges. Survey results were reviewed and used to determine the priority areas for classroom trainings and on-farm workshops.

Results: A total of 62% ($n = 325$) of NJ farms who need to comply with the FSMA PSR have attended the required training, and 16% ($n = 82$) of covered farms participated in an OFRR. Of the OFRR assessed farms ($n = 82$) 33% met minimum FSMA PSR standards, 46% need minor improvements to meet minimum FSMA PSR standards, and 21% need significant improvements to meet minimum FSMA PSR standards. Priority areas for compliance with the FSMA PSR on New Jersey farms are improvements to worker health and hygiene and postharvest sanitation.

Significance: FSMA PSR specific educational opportunities will continue to be offered throughout New Jersey. Outreach has focused on the record keeping requirements of the rule, worker hygiene best practices, and proper steps for surface cleaning and sanitation. On-farm workshops have been developed on packing equipment sanitation and the proper use of sanitizers, additional workshops are planned.

P3-124 Food Safety Needs Assessment for Produce Gleaning Organizations in California

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Introduction: "Gleaning" or harvesting and collecting otherwise unsold produce, usually for donation to those in need, is an increasingly popular practice that reduces food waste and increases food security in communities. It is imperative to determine the unique food safety challenges related to produce gleaning and the gleaning supply chain to support these food recovery efforts.

Purpose: The goal of this project was to conduct a needs assessment coordinating with growers, gleaners, food banks, policy makers and regulatory agencies to assess the areas of concern regarding food safety and food gleaning.

Methods: A questionnaire was developed for each stakeholder group. Focus groups were held in the counties of Los Angeles, Sacramento, Alameda, and Sonoma due to the high density of gleaning organizations in these areas. Focus groups were held with food gleaning organizations and food bank/food pantry staff. Phone interviews were used to gather information for regulatory agencies and growers. Qualitative data analysis was conducted by identifying common themes and coding participant responses within each theme.

Results: Several common themes were identified following the focus groups. First, "gleaning" means different things in different contexts. Depending on the interviewee, different terminology was used such as harvesting or generally food recovery or rescuing. Next, there is a limited understanding of the breadth of "food safety." Many interviewed equated food safety to quality or freshness or food, not identifying food safety risks. Also, food safety practices are often invisible/obscure. Most interviewed identified food safety training as synonymous with general volunteer training. Finally, regulatory challenges are present, but not evenly experienced by all. Most did not directly experience any regulatory hurdles to gleaning, but had second-hand knowledge of issues that had occurred.

Significance: Minimal information exists related to food safety practices and risks associated with produce recovered from farms, farmers' markets, and homes. This study provides insight into the current food safety perspectives and practices of produce gleaning operations.

P3-126 Survival of Inoculated Generic *Escherichia coli* on Almonds at Different Phases of Maturity

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Introduction: The impact of using contaminated water for crop protection sprays applied to developing almonds is unknown.

Purpose: To evaluate the survival of inoculated generic *E. coli* on developing almonds during different phases of almond maturity.

Methods: A cocktail of three non-pathogenic rifampicin-resistant *Escherichia coli* (validated environmental surrogates) was sprayed onto Nonpareil almonds at either high (HI; ~6 log CFU/mL) or low (LI; ~4 log CFU/mL) levels before hull split (May 23, July 10; 2018), during hull split (July 22, 2019) and after hull split (August 12, 2019) in a single orchard on different trees. Single almonds (10–40 per timepoint) were aseptically harvested (over ≤6 weeks); bacteria were washed off into 0.1% peptone water. Diluent was plated onto tryptic soy (TS) agar +rifampicin (+rif). When low populations were anticipated, remaining diluent was filtered and applied to CHROMagar ECC+rif and samples were enriched with TS broth+rif; presence of inoculated *E. coli* was determined by streaking onto CHROMagar ECC+rif.

Results: After inoculation, *E. coli* was recovered at 6.47 ± 0.40 (May 2018), 5.40 ± 0.70 (July 2018), 2.80 ± 0.22 (July 2018), 3.60 ± 0.50 (July 2019), and 3.84 ± 0.38 (August 2019) log CFU/nut. When almonds were inoculated before hull split, *E. coli* populations declined by >4.5 log CFU/nut at HI and were undetectable by enrichment at LI 2 days after inoculation. For almonds inoculated during or after hull split, *E. coli* populations declined by >2.9 log CFU/nut after 2 days and were detected via enrichment for up to 5 weeks. Counts exceeding the inoculum level by >1 log CFU/nut were detected on Days 1 (2/40), 3 (2/40), 4 (1/40), and Week 2 (1/40) for the July 2019 trial.

Significance: Almond hull condition during application impacted survival outcome of *E. coli* and should be considered when assessing the quality of water used for crop-protection sprays.

P3-130 Survival of *Listeria monocytogenes* on McIntosh, Fuji, and Honeycrisp Apples Stored at 22°C

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✦ ***Undergraduate Student Award Entrant***

Introduction: A recent multinational outbreak of listeriosis in the U.S. and Canada was linked to wholesale apples resulting in 35 illnesses and 7 deaths. In October 2019, a Michigan produce company recalled nearly 2,300 cases of fresh apples including McIntosh, Honeycrisp, and Fuji that could be contaminated with *Listeria*.

Purpose: This study aimed to evaluate and model the survival of *Listeria monocytogenes* on apples during storage at room temperature.

Methods: Fresh organic McIntosh, Honeycrisp, and Fuji apples (without waxing coating) obtained from local wholesale market were inoculated with a 4-strain mixture of *L. monocytogenes* followed by storing onto wholesale produce rack at 22.5°C (50.40% RH) for 7 days and periodically (day-0, 0.125, 0.25, 0.5, 1, 2, 3, 5, 7) analyzing microbial populations. Surviving *L. monocytogenes* were spread-plated on Modified-Oxford agar. Data (2 replicates/4 samples/replicate) were analyzed using the mixed-model-procedure of SAS, USDA-Integrated-Predictive-Modeling-Program and GinaFit software.

Results: *L. monocytogenes* on McIntosh, Honeycrisp, and Fuji apples decreased ($P < 0.05$) from 4.72 to <0.89, 4.31 to 1.80, and 4.57 to 1.89 log CFU/g after 3, 7, and 7 days storage, respectively, with the faster ($P < 0.05$) decline rate and greater ($P < 0.05$) reductions showed on McIntosh apples. The *P*-values of Mafart-Weibull model were 0.30-0.45 indicating that no shoulder-time existed among tested apples. The Kmax value of linear-tail and the Kmax1 value of biphasic model showed greater ($P < 0.05$) inactivation rates of *L. monocytogenes* on McIntosh (Kmax=10.98, Kmax1=11.15) than on Fuji (Kmax=6.91, Kmax1=7.97) and Honeycrisp apples (Kmax=6.06, Kamx1=6.46), and the tail time is shorter ($P < 0.05$) on McIntosh (1.16 days) than Fuji (1.80 days) and Honeycrisp (1.88 days).

Significance: Results of this study filled the data gap for understanding of microbiological risks associated with postharvest practices of tree fruit production. Future studies are needed to quantify the natural wax amount on various organic apples.

P3-131 Survey of Agricultural Water Microbial Quality in Kansas and Missouri

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❖ ***Developing Scientist Entrant***

Introduction: Agricultural water represents a significant risk for microbial contamination of fresh produce; therefore, the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) requires growers to develop a microbial water quality profile (MWQP) of their water source(s), helping to minimize this risk.

Purpose: The purpose of this study was to survey the microbial quality of agricultural water used on Kansas and Missouri produce farms to help reduce PSR compliance barriers and strengthen produce safety in the two states.

Methods: A convenience sample ($n = 376$) of agricultural water sources (surface, ground, or other) were collected from the two states, placed on ice, transported, and tested in the laboratory within 30 hours after collection. The 100 mL samples were tested using the Environmental Protection Agency (EPA) method 9223 B, Colilert Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME) to quantify generic *Escherichia coli*, and reported as MPN/100 mL.

Results: Per PSR guidelines, the geometric mean (GM) of the MWQP of an agricultural water source used in pre-harvest applications must not exceed 126 CFU generic *E. coli* /100 mL and the statistical threshold value (STV) must be < 410 CFU/100 mL. Agricultural water sources used post-harvest must have no detectable generic *E. coli*/100 mL sample. Of the total agricultural water samples collected ($n = 376$), 177 (47.97%) had no detectable generic *E. coli*, 49 (13.28%) exceeded the GM level, while 22 (5.96%) surpassed the STV level. Six (1.63%) samples exceeded 2419.6 CFU/100 mL, the maximum reportable value of the Colilert test. Surface water samples ($n = 181$) contained the highest number of positive generic *E. coli* tests (153, 84.53%).

Significance: Overall, microbial water surveillance indicated 91.06% of agricultural water sampled in Kansas and Missouri did not exceed the FSMA PSR generic *E. coli* geometric mean standard for pre-harvest use and 47.97% had no detectable generic *E.coli,* thus appropriate for post-harvest use.

P3-132 Trace Back SCUTELLO *Bacillus thuringiensis* Strain Used for Crop Protection from Field to Fork

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Introduction: *Bacillus thuringiensis* (Bt) is a widespread spore-forming bacteria that produce insecticidal parasporal crystalline inclusions. Bt-based pesticide has become the most successful microbial pest control agent used worldwide for forest and crop protection since the 50's. Most commercial products contain crystal proteins and spores that are applied to foliage, soil, water environments or even food storage facilities. While Bt-based product provides highly efficient crop protection, the persistence of spores on vegetables represents a major issue for Food Business Operator with raw materials showing *B. cereus* presumptive spores contamination overpassing 3 log CFU/g.

Purpose: The aim of this work was to distinguish and trace the *Bacillus thuringiensis* strain used in SCUTELLO from field to fork.

Methods: Based on previous work on a well-characterized collection representative of the biodiversity of *B. cereus* Group, PFGE subtyping was used to acquire molecular fingerprints and trace strain from field to fork. This posters shows the case study on broccoli. Broccoli were grown in the field with and without SCUTELLO pesticide. Samplings were performed to quantify bacterial spore and presumptive B. cereus (ISO7932) on broccoli after harvest (Step1), before (Step2) and after team cooking and production of broccoli purée (Step3), during shelf-life at 4 and 8°C (Step4).

Results: *B. cereus* presumptive counts indicate a contamination of 2 and 3.7 log CFU/g on raw broccoli, respectively without and with exposure to SCUTELLO pesticide during crop. Clustering of PFGE fingerprints enables to trace back the strain used in SCUTELLO but as well to identify a psychrotrophic *B. cereus* population naturally occurring in non treated broccoli.

Significance: The clustering of PFGE fingerprints enables the distinction of strains used as bioinsecticide from foodborne contaminants, highlighting the persistence of Bacillus spores in soil, on raw broccoli and in the purée during shelf life. This case study is part of BtID project, supported by the French ministry CASDAR program.

P3-133 Evaluate the Effectiveness of Air Bubbles during Washing to Dislodge Microorganisms from Cucumber and Bell Peppers

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❖ ***Developing Scientist Entrant***

Introduction: Microorganisms may attach firmly to produce matrices and could affect the efficacy of disinfectant during washing.

Purpose: This study evaluated the effectiveness of force aeration during washing to dislodge microorganisms from produce surfaces.

Methods: Sixteen Bell peppers and sixteen cucumbers purchased from the local market were spot inoculated with *Listeria innocua* (5 Log CFU/g or cm^2). After drying in the biosafety cabinet for 1h, the contaminated bell peppers and cucumbers were washed with water or sanitized with 100 ppm of chlorine for 3 min with or without aeration. Force aeration was performed from a perforated pipe sitting on the bottom of the washing container and connected to an air blower with an airflow rate of 90 CFM. Bell pepper samples were processed from stomacher while cucumber samples were hand massaged with PBS solution and enumerated for *Listeria* using Oxford Agar plates using a detection limit of 0.16 log CFU/g for bell peppers and 0.39 log CFU/g for cucumbers.

Results: Washing with water alone was only able to reduce the *Listeria* level from bell pepper and cucumber by less than 0.5 Log CFU/g or cm^2. Force aeration from the bottom of the washing container increased the reduction by 0.3 Log CFU/g in bell pepper; however, the level remained similar on the cucumber surface. A significantly higher reduction ($P<0.05$) of *Listeria* was achieved while washing in chlorinated water with forced aeration as compared to washing with plain water alone with a reduction of 1.3 Log CFU/g in bell pepper and 1.3 Log CFU/cm^2 on cucumber. *Listeria* levels in wash water with aeration were significantly higher ($P<0.05$) with >1 Log CFU/g (bell pepper) and > 1.5 Log CFU/cm^2(cucumber) as compared to without aeration. *Listeria* levels in chlorine wash solution with or without aeration were below the detectable limit of the test.

Significance: Air bubbles during washing facilitate the dislodge of microorganisms from produce surfaces. The use of chlorine during washing will minimize cross-contamination during washing.

P3-134 Prevalence and Concentration of *Listeria* Species and *Listeria monocytogenes* for Raw Produce Arriving into Frozen Food Manufacturing Facilities

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❖ ***Developing Scientist Entrant***

Introduction: Ubiquity of *Listeria monocytogenes* in the environment impacts the broader food industry and presents concerns for frozen food facilities. Information on prevalence and concentration of *Listeria* associated with incoming materials such as raw produce can complement risk assessment efforts aimed at understanding modes of contamination in processing environments and on finished food products.

Purpose: The study determined prevalence and population of *Listeria* species and prevalence of *Listeria monocytogenes* on raw produce arriving at frozen food facilities

Methods: Raw produce was collected using multi-level blinding protocols to ensure anonymity of participants and avoid traceback. Five raw vegetables were selected: corn, carrots, green beans, peas, and spinach to determine the prevalence and concentration of *Listeria*. Raw products were collected before cleaning or other pre-processing steps. The FDA BAM method for detection of *Listeria* spp. and *L. monocytogenes* was followed, with BAX PCR screening followed by selective plating methods. *Listeria* populations were determined for positive samples using MPN methodology.

Results: A total of 290 samples were collected, with 96 and 17 samples positive for *Listeria* spp. (33.1%) and *L. monocytogenes* (5.9%), respectively. Enumeration data for the 96 *Listeria* spp. samples indicated 82 samples had greater than 100 MPN/g and 14 samples less than 100 MPN/g. The prevalence of *Listeria* spp. varied for each commodity: spinach (66.7%), peas (50%), corn (32.2%), green beans (22.2%), and carrots (13%). *Listeria monocytogenes* prevalence was determined in corn (13.6%), peas (6.3%), and green beans (4.2%) arriving at the processing facility.

Significance: U.S. regulators consider *L. monocytogenes* an adulterant and apply a zero regulatory action limit for the presence of this pathogen in ready-to-eat foods. Prevalence and pathogen concentration data from raw commodities found in this study can provide the industry information to conduct more accurate quantitative risk assessments and provide a baseline to model and target appropriate pathogen reduction steps during processing.

P3-135 Effect of the Attachment Level of *Listeria monocytogenes* on the Efficacy of Chlorine Treatment on Bell Pepper Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Strong bacterial attachment or biofilm formation on produce surfaces may result in a limited penetration ability of sanitizers. Therefore, it is important to understand the dynamics of bacterial attachment on produce surfaces and its effect on the efficacy of sanitizers.

Purpose: This study evaluated the effect of the attachment level of *Listeria monocytogenes* on the efficacy of chlorine treatment on bell pepper surfaces.

Methods: Bell pepper surface was spot inoculated with 50 µL of *L. monocytogenes* and stored at 4°C and 25°C. The attachment strength (S_R) of the pathogen was calculated at 0, 24, 48 and 72 h by the attachment assay. The bacterial colonization on the surfaces over time was observed under Scanning Electronic Microscopy (SEM). The variations on the efficacy of chlorine (100 ppm, 5 mins) with the level of attachment were calculated.

Results: The attachment strength (S_R) of *L. monocytogenes* on bell pepper increased significantly ($P < 0.05$) after 72 h at 25°C. A significant reduction of *Listeria* count was observed only after 72 h at 4°C (1.68 log CFU/g) and after 48 h during storage at 25°C (1.19 log CFU/g). Loosely attached cells decreased with storage period with significant reduction after 48 h at 4°C and after 24 h at 25°C which ultimately increased the S_R value. However, after initial reduction within 48 h at 25°C, the strongly attached cells increased significantly (from 4.68 log CFU/g to 5.27 log CFU/g) after 72 h. Chlorine treatment was much more effective on contaminated samples with the lowest S_R value (stored at 25°C within 24 h) with a reduction of *Listeria* up to 3.6 log CFU/g.

Significance: Even with a reduction in total *Listeria* count, the levels of strongly attached cells increased during storage increasing the S_R value which affected the efficacy of chlorine making treatment more effective on bell pepper with lower S_R value.

P3-136 Role of Biological Soil Amendments in Pathogen Persistence and Transfer to Foliar and Root Crop in a Pre-harvest Environment

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Introduction: Organic produce growers utilize biological soil amendments of animal origin (BSAAO) to improve yield and soil quality. However, BSAAO use presents a risk of pathogen contamination and transfer to foliar and root crops.

Purpose: To evaluate poultry-based BSAAOs for their effect on *Escherichia coli* survival in soils and transfer to spinach and radishes.

Methods: Twenty plots (3 m^2) were amended with composted poultry litter (CPL), or unamended inorganic fertilizer (UN) in quadruplicate. Each plot was spray-inoculated with *E. coli* TVS355 (1 L, 6 log CFU/mL). Ten plots were planted with spinach (Tyee) or radishes (Early Scarlet). Composite soil samples (n = 70) were collected every 20 days until crop maturation (60 dpi; days post-inoculation), along with edible portions of spinach (n = 40) and radishes (n = 30), to determine *E. coli* populations, *Salmonella* spp. presence, following a modified FDA-BAM protocol. Soil extrinsic (temperature, moisture) and intrinsic (conductivity, soluble-carbon, carbon: nitrogen ratio) factors were evaluated. Data were analyzed using one-way ANOVA ($P < 0.05$).

Results: A significant ($P < 0.05$) decrease in *E. coli* populations (~4.7 log CFU/g) was observed in between 0 and 60 dpi in all plots. *E. coli* populations were reduced significantly in CPL-amended (1.29 log CFU/g) and UN-amended (0.89 log CFU/g) soils planted with spinach, respectively, after 40 dpi; and to undetectable levels (<0.4 log CFU/g) by 60 dpi. In radish plots, *E. coli* populations were reduced to undetectable levels in CPL- and UN-amended soils by 40 dpi. More transfer of *E. coli* to crops was observed in CPL compared to UN plots. Greater *E. coli* transfer was observed to the edible portion of radish (1-2 log MPN/g) than to spinach (0.47 log MPN/g) in CPL plots. *Salmonella* spp. was only confirmed in CPL-amended spinach plots. No significant change was observed in soil extrinsic and intrinsic factors.

Significance: Data suggest that root crops may be at higher risk of pathogen transfer compared to foliar crops.

P3-138 Kitchen-scale Treatments for Reduction of Listeria monocytogenes in Prepared Produce for Immunocompromised Populations

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❖ ***Developing Scientist Entrant***

Introduction: Listeriosis, a foodborne illness caused by *Listeria monocytogenes,* has relatively low incidence, but substantial mortality rates, particularly in immunocompromised populations. Because several large listeriosis outbreaks have been attributed to produce, these individuals are often placed on neutropenic diets that exclude fresh produce.

Purpose: The study aimed to evaluate kitchen-scale treatments to reduce the risk of *L. monocytogenes* in prepared produce.

Methods: Cucumbers, apples, and celery were dip inoculated with a three-strain cocktail of *L. monocytogenes* and dried for 24 h. Inoculated products were subjected to several treatments as applicable: commercial antimicrobial produce wash (90 s), tap water rinse (15 s), tap water soak (90 s), flash boiling (25 s), tap water rinse (15 s) followed by peeling, and flash boiling (25 s) followed by peeling. Treated samples were plated and incubated for 48 h at 37°C. *Listeria* populations were then enumerated and compared to the untreated control (log CFU/g).

Results: Flash boiling was the most effective treatment ($P < 0.05$) for celery and cucumbers, with average reductions of 2.3 and 4.9 log CFU/g, respectively. Flash boiling and flash boiling followed by peeling were most effective for apples ($P < 0.05$), with average reductions of 4.9 and 3.5 log CFU/g, respectively. The antimicrobial produce wash, tap water rinse, and tap water soak did not differ in efficacy ($P > 0.05$), which was low for cucumbers (< 1.5 log CFU/g), apples (< 1.2 log CFU/g), and celery (< 0.7 log CFU/g).

Significance: Current food safety guidelines for immunocompromised individuals are vague and do not differ from recommendations for the general population. Effective kitchen-scale antilisterial treatments, such as flash boiling, could potentially allow such individuals to safely consume certain fresh fruits and vegetables that contain dietary elements crucial to fighting disease.

P3-139 A Pilot-Plant Study Evaluating a New Technology to Accelerate *Escherichia coli* Die-Off on Fresh-Cut Lettuce during Cold Storage

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Introduction: Foodborne illness outbreaks associated with consumption of contaminated romaine and iceberg lettuce burden the produce industry and public health. Novel intervention technologies are needed to sanitize fresh-cut produce without compromising product quality and shelf life.

Purpose: The objective of this study was to evaluate the effects of a patent-pending process aid composed of silver dihydrogen citrate, glycerin, and lactic acid (SGL) on the concentration of *E. coli* on fresh-cut tissue immediately after washing fresh-cut lettuce and during cold storage.

Methods: Iceberg lettuce heads inoculated with non-pathogenic *E. coli* were stored at 5°C for 40 hours. During cutting, lettuce heads were sprayed with SGL, rinsed after 30 s of exposure using an over-head water curtain spray, followed by flume wash in chlorinated water, centrifuge-dried and packaging. Five treatments were included: control (unwashed cut dry), SGL only, SGL plus rinse, SGL plus rinse plus flume, and flume. *E. coli* populations and quality for three storage periods, day 0, 7, and 14, were evaluated. Data were analyzed using Analysis of Variance (ANOVA), when effects were statistically significant, means comparison were done with Sidak adjusted *P*-value to maintain experiment-wise error of ≤ 0.05. Washing trials were repeated three times with five samples of fresh-cut lettuce collected per treatment (n = 75).

Results: Immediately after processing, significant difference ($P < 0.05$) was observed between unwashed samples and, SGL-rinse-flume and flume samples. The SGL-rinse-flume samples exhibited the lowest *E. coli* population of 3.94 ± 0.22 log CFU/g. This signifies a 1.35-log reduction at the end of shelf life, in contrast to the 0.61-log reduction for the flume washed samples, in comparison with initial *E. coli* population of 5.30 ± 0.15 log CFU/g. While the *E. coli* population declined significantly ($P < 0.05$) on all samples during storage, a larger decrease in *E. coli* population was observed on samples treated with SGL. Product quality and shelf life of the washed lettuce were not affected by SGL application once the process aid was removed by flume washing.

Significance: These results suggest that SGL treatment may have the potential to accelerate *E. coli* die-off during storage.

P3-140 Use of Lactic Acid Bacteria to Control *Listeria monocytogenes* on Apples during Simulated Storage Conditions

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Introduction: Over the last decade, the inclusion of fresh produce in the American diet has been steadily increasing due to heightened consumer awareness of the associated health benefits. This increase in fresh produce consumption has been associated with a concomitant increase in foodborne outbreaks. More specifically, the recent recall of several popular apple varieties including Honey Crisp and Red Delicious due to potential *Listeria monocytogenes* contamination, indicates that there is a critical need for antimicrobial strategies, which are effective, and sustainable along the production continuum.

Purpose: In this regard, lactic acid bacteria (LAB) can serve as excellent candidates for reducing pathogen levels on fresh and minimally-processed produce, like apples.

Methods: The present study investigated the application of LAB namely, *Lactococcus lactis* B-23802 (LL1), *L. lactis* B-23804 (LL2), *Lactobacillus rhamnosus* B-442 (LR) and *Lactobacillus plantarum* B-4496 (LP) for improving the microbial safety of apples. Specifically, the study evaluated spray application of LAB strains to control *Listeria* on apples during storage at the packing-house (4°C) and retail/ home (22°C) conditions. For this experiment, apple plugs and halved apples were spot inoculated with *Listeria* (6 log CFU/plug or half) and then sprayed with LL1, LL2, LR or LP (8 log CFU/plug or half). The apples were then stored at 4°C or 22°C for 14 days and bacterial populations were enumerated throughout the study.

Results: Spray application of LAB significantly ($P < 0.05$) reduced *Listeria* populations on apple plugs/ halves by ~ 1 - 2 logs, on storage at ambient and refrigeration temperatures. However, ~ 6 log CFU of *L. monocytogenes* was still recovered from the control plugs/halves and ~ 5 log CFU of LAB at the end of the experiment.

Significance: Based on the results, we expect that LAB can be effectively applied to control *Listeria monocytogenes* on apples along the production process in handling and packaging facilities.

P3-141 Growth of *Listeria innocua* on Broccoli Stalk, Beet Greens, Brussels Sprouts, and Kale under Simulated Storage and Distribution Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Produce is exposed to temperature and physical abuse during storage and distribution increasing the potential growth risk of foodborne pathogens like *Listeria*. While the impact of temperature abuse on produce safety has been studied, the impact of physical abuse on the growth of pathogens is limited.

Purpose: The purpose of this research is to determine the impact of physical abuse on the growth of *L. innocua* on shredded broccoli stalk, chopped beet greens, sliced Brussels sprouts, and chopped kale.

Methods: Produce was surface inoculated with *L. innocua* and then exposed to a series of physical abuses (compression, drop, and vibration) typical of storage and distribution. After abuse, product was incubated at 4 and 8°C and sampled post-abuse through 16 and 11 days, respectively. Samples were enumerated for *L. innocua,* aerobic and psychrotrophic microorganisms, and lactic acid bacteria using Modified Oxford Agar (MOX), Tryptic Soy Agar (TSA) and de man Ragosa Sharpe agar (MRS), respectively. Counts were log transformed, graphed, and means were compared using ANOVA and Tukey's HSD.

Results: Abuse had no significant effect on *L. innocua* growth regardless of vegetable or incubation temperature ($P > 0.05$). Significant growth at 4 and 8°C was seen on day 6 and 4 in beet greens, 11 and 6 in Brussels sprouts, 16 and 4 in kale, and 16 and 6 in broccoli stalk ($P < 0.05$). The highest average increase in *L. innocua* populations was observed on beet greens > kale > broccoli stalk > Brussels sprouts at both 4 and 8°C.

Significance: The results indicated that for the tested products, exposure to physical abuse during storage and distribution was not found to significantly increase the risk beyond what was observed as a result of chopping, slicing, or shredding.

P3-142 Ethanol Vapor to Control *Salmonella* on Fresh Produce

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Introduction: Water used in fresh produce conveyance and washing has been suspected as a potential vehicle of foodborne pathogen (e.g. *Salmonella enterica*) contamination in fruits and vegetables. To reduce risk, waterless systems to clean and sanitize fresh produce in packinghouses are of interest.

Purpose: The objective of this work was to assess the efficacy of ethanol mist to reduce *Salmonella* populations on the surfaces of tomatoes and cantaloupes.

Methods: Whole mature red fresh tomatoes and cantaloupe plugs were inoculated with nalidixic acid-resistant *Salmonella enterica* ser. Newport to a concentration of 6-7 log CFU/tomato or cm^2 for cantaloupe. Ethanol (70%) mist was applied to whole tomato and excised cantaloupe surfaces using a commercial sanitation system. Application time (0, 5, 10, and 15 s), wet vs. dry surface, and stem scar were tested on tomatoes while only application time was evaluated on cantaloupe. After treatment, samples were placed in stomacher bags containing 90 mL 0.1% peptone and shaken in a pulsifier for 60 s. Diluent was plated onto TSA containing 50 ppm nalidixic acid and incubated at 37°C for 48 h.

Results: At 15 seconds of ethanol mist application *Salmonella* populations were decreased by approximately 3.3 log CFU/tomato, while reductions for both wet/dry and infiltration evaluations were <1.5 log CFU/tomato. Populations of *Salmonella* decreased by approximately 1.2 log CFU/cm^2 on cantaloupe surfaces after 15 seconds of ethanol application.

Significance: Ethanol mist application to dry tomato surfaces resulted in significant ($P < 0.05$) reductions of *Salmonella enterica* populations. However, ethanol was not as effective on cantaloupes, when the surface of the tomato was wet, or if the *Salmonella* was placed on the stem scar. The application of ethanol mist onto fruit and vegetable surfaces may result in beneficial reductions of *Salmonella*; however, each type and condition of produce should be independently evaluated for efficacy.

P3-143 Survival of *Salmonella* Typhimurium in Hydroponic Lettuce Systems

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❖ ***Developing Scientist Entrant***

Introduction: Globally, there has been an increase in hydroponic production, specifically in leafy greens. However, there is a lack of evidence for the establishment of water management strategies.

Purpose: To determine if *Salmonella* Typhimurium can survive/persist in nutrient flow technology (NFT) systems during regular and flooded contamination events, during the lifecycle of lettuce.

Methods: NFT systems were inoculated with *Salmonella* Typhimurium to simulate flooding (~$1x10^8$ CFU/mL) and non-flooding conditions (~$1x10^4$ CFU/mL). Nutrient solution, rockwool medium, roots, and lettuce leaves were collected at seven time points during the plant life cycle for pathogen detection and/or enumeration.

Results: *S*. Typhimurium persisted in the NFT system throughout the lettuce growth cycle. The pathogen was detectible in high concentration on the roots and rockwool one hour post inoculation (5.71 ± 0.32 CFU/g, 6.48 ± 2.51 CFU/g, in non-flooding and flooding conditions, respectively), and decreased to 4.43 ± 0.68 CFU/g; 3.80 ± 0.61 CFU/g by day 28, suggesting trapping and sequestration of pathogens by the root-rockwool matrix. *Salmonella* was detectible in the nutrient solution under both conditions during the first 21 days. Although it was not detectible in nutrient solution by day 28, it was still present on lettuce leaves. Under flooding conditions, *Salmonella* was detectable at 1 hour, 24 hours, day 7, day 14, day 21 post inoculation, and at 12 hours, day 7, and day 28 days under non-flooding conditions, suggesting that the contamination of lettuce leaves in NFT system was not homogeneously distributed, but consistent during the growth cycle.

Significance: *Salmonella* from inoculated nutrient solution was able to readily transfer to edible portions of lettuce. Pathogen sequestration in the root-rockwool matrix is of consideration for lettuce sold with the root-rockwool matrix attached (living lettuce). These findings will provide knowledge and justification for the development of management practices, such as system sanitation.

P3-145 Risk Factors Associated with *Escherichia coli* Persistence in Soils Amended with Raw Manure in Certified Organic Farming Systems in Four Regions of USA

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Introduction: Animal-manure is used to improve soil health and is particularly important in organic agriculture. Soil amended with untreated manure can become contaminated by fecal bacteria (e.g., generic *E. coli*) and pathogens. Management practices and environmental factors can also influence bacterial presence and survival in the soil.

Purpose: To identify predictors of generic *E. coli* (gEc) prevalence in manured-soil considering different variables (management practices, farm, regions, weather, and soil health) in soils amended with raw manure in certified organic farms.

Methods: Nineteen USDA-NOP certified organic farms in different USA regions (CA, ME, MD, MN) were enrolled in a 2-year longitudinal study. Samples (manure, soil, produce, and water) were collected during two growing seasons (2017-2018) and were quantified for gEc populations by mini-Most Probable Number (MPN) assay and confirmed by PCR. Mixed effect univariate and multivariate linear regressions were used to analyze potential risk factors for high levels of gEc using log (MPN) as the outcome.

Results: Using univariate models and accounting for clustering in time and space, we found a significant increase in soil gEc concentrations immediately after manure application (coef=1.27, 95% CI=[1.12, 1.43]), followed by significant decrease from 30 days post-application and onwards (coef from 0.54, 95% CI=[0.39, 0.70] to 0.20, 95% CI=[0.04, 0.36], *P*-values < 0.15). Soil samples amended with cattle or horse manure had lower concentrations of gEc compared to poultry manure (coef=-0.72, 95% CI=[-0.92, -0.53] and coef=-0.57, 95% CI=[-0.90, -0.24]). Increased soil moisture and precipitation events as well as increased wind speeds were all associated with higher counts of gEc. Higher soil temperatures were associated with lower concentrations of gEc, however other weather-related variables showed no significant associations.

Significance: Results of this study provide science-based information to identify potential risk factors influencing fecal bacteria survival in pre-harvest produce production environments for soils amended with raw manure on NOP-certified organic production systems.

Poster

P3-146 Evaluating the Efficacy of Peroxyacetic Acid at Lower Than Recommended Levels As a Sanitizer for Tomato Fluming Operations

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❖ ***Developing Scientist Entrant***

Introduction: Salmonella has been reported to be involved in several foodborne illness outbreaks associated with the consumption of raw tomatoes. Sanitizers are essential for the prevention of cross-contamination when fluming tomatoes. Peroxyacetic acid (PAA) has been considered as an alternative to chlorine-based sanitizers. A better understanding of the efficacy of peroxyacetic acid (PAA) will aid in setting concentrations necessary to prevent cross-contamination.

Purpose: Various PAA concentrations were tested to better understand its efficacy at lower concentrations (<80 ppm), in the prevention of *Salmonella* cross-contamination in a model flume system.

Methods: For each trial, tomatoes (3) were inoculated with a cocktail of five rifampin-resistant *Salmonella* (10^8 log CFU/tomato). The inoculated tomatoes were flumed concurrently with uninoculated tomatoes (12) in a model system containing 0, 20, 40 or 80 ppm PAA with no organic matter. Three randomly chosen tomatoes were removed at time intervals of 30, 60, and 120 seconds. The water of the model flume system was also tested for contamination immediately after each simulation was run.

Results: All 27 tomatoes tested positive for *Salmonella* in absence of PAA, whereas there were 16 and 15 positives with 20 and 40 ppm PAA in water, respectively. With 80 ppm of PAA in water, 7 out of 27 tomatoes became contaminated. None of the tomatoes sampled at 120s in treatment with 80 ppm PAA were positive for *Salmonella*. There was no *Salmonella* detected in water samples containing PAA at any of the concentration levels. Experiments with lower inoculation levels (10^6 and 10^8 log CFU/tomato) and different levels of organic matter (300 ppm of COD) are still ongoing.

Significance: This study provides insights on *Salmonella* survival and potential of cross contamination of tomatoes when lower than recommended amounts of PAA are used in tomato fluming operations.

P3-147 Validation of the BAX® System Real-time PCR Assays for *Salmonella*, *E. coli* O157:H7 and Non-O157 STEC in Cruciferous Vegetables

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Introduction: Vegetables within the *Cruciferae* family have shown a greater susceptibility for pathogen contamination compared to vegetables like lettuce or tomatoes. Consequently, in 2018, there was a cauliflower recall in connection with a large multistate outbreak of *E. coli* O157:H7 in romaine lettuce after a sample of sediment from a nearby water reservoir tested positive.

Purpose: The purpose of this study was to evaluate the performance of a real-time PCR method to detect *Salmonella, E. coli* O157:H7 and non-O157 STEC in various cruciferous vegetables.

Methods: Three cruciferous vegetables; broccoli, green cabbage and green kale, were divided into 25-g and 375-g samples for the test methods and 25-g samples for *Salmonella* and *E. coli* reference methods. For each vegetable and method, samples were co-inoculated with various strains of *Salmonella* and *E. coli.* All samples were stored at 4°C for 48 hours. Twenty-five-gram and 375-g test method samples were homogenized with 225 and 1,500 mL of pre-warmed (42°C) MP media, respectively, and incubated at 42°C for 10-20 hours. Sample were analyzed using real-time PCR. The reference method samples were enriched and plated according to the procedures in the FDA-BAM.

Results: For the low inoculation level, each real-time PCR assay returned fractional positive results for both sample sizes of every vegetable tested. All presumptive results were identical to culture. When compared to the reference methods for *Salmonella* and *E. coli*, POD analysis indicated no statistical difference.

Significance: The results of this study demonstrates the ability of the BAX® System to accurately detect *Salmonella, E. coli* O157:H7 and non-O157 STEC in 25-g and 375-g samples of various cruciferous vegetables equivalent to the reference method.

P3-148 Establishing a Scientific Basis for Buffer Zones Following Animal Intrusion in Florida Tomato Fields

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Introduction: Wild animals can carry human pathogens in their feces and can spread that contamination by depositing feces as they move through a field.

Purpose: The objective of this study was to determine microbial dispersal due to wild animal fecal deposits on or near tomato plants in commercial tomato fields.

Methods: Over the 2018-2019 Florida tomato season, 40 animal intrusion events were investigated in commercial tomato fields. Samples collected from bird dropping events (n = 38) included fruit or leaves with visible droppings; fruit from the contaminated plant sampled < 30 cm below, < 30 cm above, > 30 cm below, or > 30 cm above the point of visible contamination; and fruit from adjacent plants. The remaining events (n = 2) were found in the row (> 30 cm from tomatoes) and assumed to be mammalian; the fecal samples were collected along with fruit from adjacent plants. All samples were evaluated for aerobic plate count (APC), coliforms, generic *E. coli*, and *Salmonella*. Control fruit were collected from a plant ca. 25 feet away that had no visible contamination.

Results: *Salmonella* was not isolated from any sample collected in the study (n = 341). Populations of APC (4.75 – 6.66 log CFU/g) and coliforms (3.40 – 4.84 log MPN/g) were not significantly different between sample groups and the controls (P > 0.05). Populations of generic *E. coli* were below the limit of detection (< 1 MPN/sample) in 92.3% of samples where there was no visible contamination (n = 274). There was no evidence in the two observed events that contamination from mammalian droppings in rows was transported to tomatoes on adjacent plants in the field.

Significance: The requirements of FSMA's Produce Safety Rule, not harvesting visibly contaminated fruit, should adequately minimize risk from potential dispersal of pathogens while at the same time minimizing potential economic impacts resulting from excessive no-harvest buffer zones around an instance of animal intrusion and droppings.

P3-150 Transfer of Indicator *Escherichia coli* to Spinach, Carrots and Tomatoes Grown in Organic Soil Amended with Raw Animal Manure in California, 2018–2019

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Introduction: Untreated biological soil amendments of animal origin (BSAAOs) are potential sources of fresh produce contamination by zoonotic enteric pathogens. National Organic Program (USDA-NOP) wait period standards for use of untreated BSAAOs have not been validated as a food safety practice.

Purpose: In this study we investigated the persistence in soil amended with different animal manure types of indicator *E. coli*, and its potential transfer to fresh organic vegetables during two growing seasons in Northern California 2018-19.

Methods: Horse manure (HM), dairy manure solids (DMS) and chicken litter (CL) were tilled separately into four replicate soil plots (4×1 m) at a UC-Davis NOP certified research field in October 2018. Plots were inoculated with rifampicin-resistant indicator *Escherichia coli* (*E. coli*^{rif}) at 10^8 MPN/g. Controls included un-amended (UA) and un-inoculated (UI) plots. Organic spinach and carrot seeds were planted 7 days post application (dpa), and tomato seedlings transplanted 14 dpa. *E. coli*^{rif} was quantified from soil and plant samples by direct plating and MPN.

Results: *E. coli*^{rif} persisted in all HM, DMA, CL, and UA plot soils growing carrot, spinach and tomato through 180 dpa (means, 2.9×10^3, 3.2×10^2 and 1.7×10^0 MPN/g respectively). *E. coli*^{rif} enumerated from spinach leaves (mean 2.4×10^3 MPN/g) as well as carrots (mean 8.3×10^1 MPN/g) grown in manure-amended and UA plots were not significantly different ($P > 0.05$). *E. coli*^{rif} was isolated once from tomatoes (133 dpa) on an HM plot (2×10^{-2} MPN/g).

Significance: The long-term survival of *E. coli* in manure-amended soil and transfer to spinach and carrots under certified organic cultivation was observed, similar to findings the previous crop year; however, transfer to tomatoes was not previously documented. Our findings suggest longer USDA-NOP (>120 days) wait periods or composting may be needed to protect organic vegetable crops from fecal pathogen contamination via raw animal manure-amended soil.

P3-151 Determination of the Presence of Foodborne Pathogens, Total Coliforms, and Escherichia coli in Ready-to-Eat Leafy Greens Sold at Retail

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Introduction: Leafy greens are important components of a healthy diet and are available as ready-to-eat at retail. Unfortunately, they are frequently linked to foodborne disease outbreaks.

Purpose: This study compared the microbiological profiles of ready-to-eat, washed and unwashed samples of a variety of leafy greens, including romaine, spinach, kale, and mixes.

Methods: Samples of packaged (washed and unwashed) and loose (unwashed) leafy greens were collected from grocery stores in New York, New Jersey, and Connecticut ($n = 283$). Total coliform counts (TCC) and generic *E. coli* counts (ECC) were performed by AOAC method 991.14 (~50 g sample). Samples were screened for the presence of *E. coli* O157:H7 and non-O157 STEC, *Salmonella* spp., and *L. monocytogenes* (*Lm*) (~150 g sample each) using multiplex PCR and confirmed using FDA BAM methodology. Confirmed positives underwent whole genome sequencing.

Results: Of the 283 samples analyzed, 6 were confirmed positive for *Lm*. Two of the 6 samples were packaged, pre-washed greens (spinach and organic spinach-spring mix); and 4 were loose heads or bunches of green kale, red leaf lettuce, green leaf lettuce and spinach. TCC averages ranged from <10 CFU/gram to 520,000 CFU/gram, with no wash indicated samples having lower average log concentration of TCC ($P \leq 0.05$) than wash indicated. Only two samples had ECC above <10 CFU/gram. No samples were confirmed for *Salmonella* spp., *E. coli* O157:H7, or non-O157 STEC.

Significance: Leafy greens continue to be associated with foodborne disease outbreaks. Certain populations are at high risk for acquiring a severe form of listeriosis; these populations should consider the risk involved in eating raw produce and consider consuming cooked leafy greens.

P3-152 Leafy Greens As Source of Ceftazidime- and/or Ciprofloxacin-resistant *Enterobacteriaceae*

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Introduction: Antibiotic use in humans and animals has contributed to the dissemination of resistant bacteria into the environment including soil and into water used for crop irrigation. Leafy green and other vegetables are consumed fresh, and the presence of resistant microorganisms can cause foodborne diseases and may contribute to the acquisition and dissemination of resistant bacteria.

Purpose: The purpose of this study was to study the presence of ceftazidime- and ciprofloxacin-resistant enterobacteria in leafy greens in an agricultural town in central Chile.

Methods: Thirty parsleys, 30 lettuces, 30 beets and 30 chards were collected from 5 different farmer markets on May 2019 in a town in central Chile. Samples of 25 g of each vegetable were pre-enriched with buffered peptone water buffered at 37°C for 18 to 24 h. Next day, 100 µl from each one was streaked onto 2 MacConkey plates, one supplemented with 2 µg/mL ceftazidime (CAZ) and the other with ciprofloxacin (CIP). For this study only lactose-positive isolates were included. The isolates were identified using MALDI-TOF. Each bacterial isolate was tested against a panel of 15 antibiotics using the Kirby-Bauer disk diffusion method following CLSI guidelines.

Results: A total of 40 isolates were obtained. From these, two isolates obtained from parsley that were selected on CAZ were identified as *Citrobacter freundii.* The other 38 isolates were selected in CIP and were classified as *E. coli.* These were obtained from parsleys, beets, chards, and lettuce. On these isolates, 14 showed resistance to amoxicillin, two showed resistance to cephalosporins, and 38 were ciprofloxacin resistant. Nine isolates presented a multidrug resistance phenotype.

Significance: Antibiotic-resistant enterobacteria in vegetables may contribute to the human dissemination of resistant isolates through consumption, particularly in ready-to-eat food.

P3-153 Whole-Genome Analysis of Shiga Toxin-producing *Escherichia coli* and *Salmonella* spp. Isolates from Untreated Cattle and Poultry Manures in California and Arizona

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Introduction: Untreated cattle and poultry manures are used as biological soil amendments for growing produce, but have been identified to be the potential reservoirs of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp.

Purpose: The objective of this study was to apply whole-genome sequencing (WGS) to characterize STEC and *Salmonella* spp. isolates from untreated cattle and poultry manures in California and Arizona.

Methods: STEC (*n* = 316) and *Salmonella* spp. (*n* = 177) were isolated from cattle (*n* = 158) and poultry (*n* = 66) manure samples, respectively, from livestock farms and composting facilities. Isolates were submitted to FDA's Genome Trakr for Illumina MiSeq sequencing. WGS data were submitted to the National Center for Biotechnology Information's (NCBI) pathogen database. Based on the WGS data, serotype, virulence genes, antimicrobial resistance genes (ARGs), and plasmids of each isolate were predicted. Whole-genome phylogeny was analyzed to compare these isolates using the alignment of single nucleotide polymorphisms (SNPs).

Results: STEC isolates belonged to 29 serotypes, such as O157:H7, O26:H11, O103:H11, and O111:H8, while *Salmonella* spp. isolates were grouped into 21 serotypes, such as Mbandaka, Newport, Anatum, Altona, and Hadar. Our genome annotation revealed a number of major virulence genes in all isolates. ARGs were identified in 16% (*n* = 49) of STEC and 30% (*n* = 53) of *Salmonella* spp. isolates, out of which 53% (*n* = 26) of STEC and 92% (*n* = 49) of *Salmonella* spp. isolates displayed multidrug resistance. Plasmids were present in 75% (*n* = 237) of STEC and 32% (*n* = 57) of *Salmonella* spp. isolates. Isolates generally clustered by location, but some farms and facilities had isolates belonging to multiple clusters.

Significance: This study highlights genomic diversity among STEC and *Salmonella* spp. isolates from untreated animal manures, which provides critical genome-based data for risk assessment relevant to the Food Safety Modernization Act Produce Safety Rule.

P3-154 Experimental Field Trial to Assess *Escherichia coli* Presence and Concentration in Organic Soil and Tomatoes Following Sheep Rotational Grazing on Cover Crop

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Introduction: Diversified farms may integrate livestock into human and animal feed crop production (e.g., alfalfa, orchards, vegetables) to promote sustainable agricultural systems. However, the microbial food safety risks associated with fresh produce grown in soil exposed to sheep manure are unclear.

Purpose: To conduct an experimental field trial to assess persistence and transfer of indicator and Shiga toxin-producing *Escherichia coli* (STEC) from sheep feces to organic soil and tomatoes following grazing on cover crop prior to planting.

Methods: Cover crop (vetch, oat, pea mix) was planted in fall 2016 at a USDA-NOP certified organic research farm at UC Davis. The field was divided into exposed (grazed A and B) and unexposed (non-grazed C and D) plots using portable livestock fencing. Sheep (25 per field) were rotationally grazed in May 2017. Organic tomato transplants were grown (4 replicates each) on plastic mulch using standard agronomic practices. Indicator *E. coli* from sheep feces, soil, and tomatoes was quantified using direct plating and MPN through harvest. Plot variation among samples (log MPN/g +1) were evaluated using ANOVA ($\alpha = 0.05$); Tukey's multiple comparison test was performed to compare each pair of means ($\alpha = 0.05$).

Results: Overall prevalence of *E. coli* in exposed and unexposed soil was 91% and 86%, respectively. *E. coli* concentration was significantly higher in soil from exposed plot A (mean: 2.04 log MPN/g) and B (1.62 log MPN/g) compared with unexposed plot C (mean: 1.10 log MPN/g) and D (0.95 log MPN/g). *E. coli* concentration in both exposed and unexposed plots increased until 21 dpa, followed by a decrease until 119 dpa. *E. coli* concentration on tomatoes (mean: 0.155 log MPN/g) was significantly lower compared with soil samples.

Significance: This study is important in defining research-based waiting periods between grazing and harvest, which could inform FDA guidance for growers under the Produce Safety Rule.

P3-155 Environmental Screening of a Cannabis Production and Processing Facility Using Multiplexed DNA Microarrays: A Method to Enhance Growth and Prevent the Spread of Contamination

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Introduction: As the cannabis industry expands and becomes more heavily regulated, the need for screening tools to detect microbial contamination at the source increases. To date, screening has focused on the raw product and there has been little emphasis on the actual facilities in which that product is processed, which generates the potential to be a source for "follow-on" contamination prior to sale.

Purpose: The following case study was performed to demonstrate the utility of high-resolution environmental screening in a cannabis production and processing facility.

Methods: In this 3-month study, 56 swabs were collected across 11 locations throughout the cultivation, processing, and manufacturing facility. Samples were collected using cotton swabs for assessment of microbial contamination. To compare the effectiveness of screening methods, each sample was assessed by traditional microbiological plating and by the PathogenDx "EnviroX" DNA microarray, which interrogates for the presence of a set of 50 bacteria and fungi.

Results: The study was based on 56 swab samples in which each tested positive for bacterial and fungal contamination when analyzed by both methods. Throughout the three-month study, the most prevalent species detected were *Pseudomonas* spp., *Golovinomyces* spp., and *Cladosporium* spp. While there were variations in the microbial species present within each of the rooms tested, EnviroX was found to detect contamination with higher sensitivity, and with much higher level of speciation, than traditional plating.

Significance: It was seen that EnviroX microarray testing revealed temporal and spatial relationships not seen with plating. The data suggest that standardized monitoring deployed before, during and between grows may be key to preventing the spread of contamination, thus providing a higher yield of clean, safe product for the consumer.

P3-156 Hygiene Status of Fresh Peach Packing Lines in Georgia

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❖ ***Developing Scientist Entrant***

Introduction: Current Produce Safety Rules in the U.S. requires fresh produce packing equipment be clean and maintain sanitary conditions.

Purpose: This study surveyed the hygiene status of selected fresh peach packing lines in Georgia.

Methods: Surface swabbing samples (n = 464) were collected from 14 selected sites on each of the 4 fresh peach packing lines at 3 times a day, before packing in the morning (AM), at lunchtime (NOON), and the end of the day (PM), in 3 repeated visits per line during the harvest seasons of 2018 and 2019. Each swab sample was collected from a 100 cm^2 area using a sterile sponge pre-moistened with Dey-Engley neutralizing broth. Collected samples were transported to our laboratory under refrigeration condition and analyzed within 16 - 24 hours. The levels of three hygiene indicators, total aerobes (TA), total yeasts and molds (YM), and total coliforms (TC), as well as the incidences of thermo-tolerant coliforms (TTC) and enterococci (EC) in collected samples were determined. Results were analyzed using the split-plot ANOVA tests fitted in a general linear regression model.

Results: Counts of the three hygiene indicators in the AM samples were significantly lower (P < 0.05) than those in the NOON and PM samples. The incidence of TTC increased from 13.50% at AM to 45.83% at PM, while that of EC from 6.75% to 15.11%. Higher levels of TA, YM, and TC counts and higher incidences of TTC and EC were observed on the brushes/rollers inside the washer/waxer and optical sizer compared to other sites sampled. In comparison, harvest bins were only high in TA and YM counts. Samples from manual sorting area had the highest TTC incidence among samples from all surveyed sites.

Significance: Results suggest that microbial build-up on fresh peach lines is time-dependent. The study pinpointed critical sanitation control points on fresh peach packing lines.

P3-157 Detection of *Listeria monocytogenes* from Plastic Surfaces Using the Insite *L. mono* Glo Swab with Confirmation on the BAX® System Standard and Real-time PCR Assays

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Introduction: The InSite *L. mono* Glo environmental swab is an all-in-one test used to sample, enrich and detect the presence of *Listeria* from environmental surfaces. Test results can easily be interpreted from the device without additional lab equipment to monitor and evaluate preventive controls.

Purpose: The purpose of this validation is to demonstrate the compatibility of a PCR-based method to be used in conjunction with the device to confirm the presence of *L. monocytogenes* from plastic surfaces.

Methods: Unpaired plastic surfaces were inoculated with *L. monocytogenes* and a competitive microorganism and dried overnight. Surfaces were sampled by swabbing 4" x 4" test areas with *L. mono* Glo sampling devices. Swabs were held at room temperature for 2 hours prior to activation and incubated at 37°C for 24-48 hours. All swabs were inspected for color change and analyzed by PCR. Reference method sponges were enriched and confirmed following the USDA MLG Chapter 8.11.

Results: A positive color change to grey/black was visible for 16 low-spiked and 5 high-spiked samples at 48 hours. These presumptive results also fluoresced green when exposed to UV light verifying the presence of *L. mono*. When tested by PCR, 8 assays were in 100% agreement and identical to culture. When compared to the reference method, POD analysis indicated no significant statistical difference.

Significance: The utility of this method can be used to reduce testing costs by screening environmental surfaces with the test swab first, and then confirming all presumptive positives that exhibit a chromogenic color change with any of the BAX® System PCR assays for Genus *Listeria* and *L. monocytogenes.*

P3-159 Quantitative and Qualitative Comparison of Commercially Available Indicator Organism Methods

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Introduction: Mapping hygienic conditions of food facilities requires indicator organisms to be identified and quantified efficiently and accurately. Current methods have extended incubation periods coupled with extensive labor, causing a shortfall to the demands of rapid, actionable data throughout the food industry.

Purpose: Quantitative and qualitative variables were evaluated for 3M Petrifilm™, Biomerieux Tempo®, and Hygiena MicroSnap™ to objectively compare indicator testing methods.

Methods: Various microorganisms were grown under optimum conditions and combined equally into 3 cocktails for analysis of Aerobic Plate Count (APC), *Enterobacteriaceae* (EB), and *Escherichia coli* and Coliforms (EC/CC). Utilizing each method and their dynamic countable ranges, Petrifilm (n = 156), Tempo (n = 120), and MicroSnap (n = 32), were evaluated quantitatively for accuracy and precision and qualitatively for time per technician, sample touch points, incubation time, and results processing time. Data were collected as CFU/mL and transformed into log CFU/mL for statistical comparison between methods with significant differences at $P \leq 0.05$.

Results: Quantitatively, all methods were comparable for APC, EB, and EC/CC counts ($P \leq 0.05$). However, differences in precision for each method were shown in the log Root Mean Square Error across the dilution series. Log RMSE ranged from 0.06-0.14 for Petrifilm and MicroSnap, whereas, Tempo consistently had decreased precision with log RMSE ranging from 0.25-0.39. Qualitatively, technician time was 0.26 min/sample for Petrifilm, 0.38 min/sample for MicroSnap, and 1.5 min/sample for Tempo. Both Petrifilm and MicroSnap had 1 touch point, whereas, Tempo had 3 touch points for sample preparation. MicroSnap had the shortest enrichment time of 6-7 h, Petrifilm enriched for 24-48 h, and Tempo for 22-27 h. Following incubation, all methods had comparable results processing time (30 min).

Significance: Quantitative and qualitative comparisons of indicator methods prove that shortened enrichment, decreased labor intensiveness, and improved accuracy provides the industry with a solution for rapid, actionable indicator organism mapping.

P3-160 Novel Hygiene Assessment Technology Exhibits Higher Frequency of Soil Contamination Detection Than ATP Assessment

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Introduction: Adenosine triphosphate (ATP)-based hygiene assessments are commonly used in applications for rapid determinations of surface contamination. Previous work has demonstrated that ATP concentrations are transient and susceptible to dephosphorylation based on the metabolic state of the organisms involved. Therefore, a novel technology (AXP) was developed wherein ATP and its dephosphorylated homologues (ADP and AMP) are simultaneously surveilled. We hypothesized an AXP-based technology is more sensitive to contamination events than an ATP-only based technology.

Purpose: This work was conducted to determine and compare the frequencies of process control deviations (considered contamination events) using ATP- and AXP-based hygiene assessment technologies.

Methods: For a practical feasibility assessment, all data were collected from inspected dairy foods and meat processing facilities. ATP and AXP monitoring devices were obtained and operated per the manufacturers' instructions. Both technologies were employed as a part of routine hygiene verification procedures on a variety of pre-identified contact and non-contact surfaces wherein repeated, parallel assessments or swabs were taken within each facility over early Spring and Summer seasons yielding 1,920 total assessments. Statistical process control using bootstrapping methodology was used to create a 90% upper confidence limit (UCL) and to then determine the number of control deviations, thus contamination events, beyond this UCL.

Results: Including data points for both dairy and meat facilities, the AXP-based technology detected contamination events that exceeded the 90% UCL 1.91 times more often than the ATP-only based technology. The increased frequency was less dependent upon season, facility or other factors such as sanitizer type and was more pronounced in the meat facility.

Significance: These data support the study hypothesis wherein AXP is more sensitive to hygiene or contamination events than an ATP-only based technology. This result is likely explained by improved detection sensitivity when ATP homologues impacted by ATP depletion are included in assessment technologies.

P3-161 AMP, ADP, and ATP Concentrations Differentially Affected by Common Manufacturing Steps in Meat Processing

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Introduction: Adenosine triphosphate (ATP) is used as a target for assessments of surface hygiene in meat processing venues. There are evidences that concentrations of ATP are depleted through dephosphorylation into adenosine di- (ADP) and monophosphate (AMP) homologues. These depletions occur as a function of cellular metabolism, yet, there is little evidence describing how common meat processing steps will affect ATP concentrations, e.g. tissue disruption, chemical additives, thermal treatment.

Purpose: This work determined concentrations of ATP, ADP, AMP, and AXP (sum concentration of all homologues) in a lab setting and *in situ* meat processing venues as a function of common meat processing steps.

Methods: Using a luminometer/luciferin-luciferase based technique, concentrations of adenylate homologues were calculated for all samples using standard curves of target adenylates. All study samples were either collected from homogenized beef tissue treated with the chemical additives (sodium chloride, sodium nitrite, sodium erythorbate, natural smoke condensate, and sodium acid pyrophosphate), during sausage manufacture at pre-determined steps and from retail meat products. ANOVA was conducted to compare adenylate concentrations.

Results: No significant differences in AXP were observed during homogenization steps regardless of chemical addition. ADP generally comprised about 90% as a mole fraction of the AXP homologues across all treatments with the exception the final cook step where AMP predominated. Across all conditions, ATP concentrations averaged 2 log values lower than ADP and AMP. AXP profiles in retail samples followed general trends of most having minimal ATP concentrations with ADP predominant in uncooked samples and AMP predominant in cooked samples.

Significance: AXP homologue concentrations change up to several orders of magnitude based on steps common to meat processing, especially thermal treatments. These changes should be considered when ATP-based hygiene assessments are applied as a means of hygiene assessment.

P3-162 Differential Biofilm Formation of *Listeria monocytogenes* Strains under Single- and Dual-species (with *Lactobacillus* spp.) Conditions

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Introduction: Previous studies on *Listeria monocytogenes* biofilm formation have characterized the difference among strains on their ability to form biofilms. However, in food processing areas, biofilms consist of multiple microorganisms that occupy the same niche and may be introduced into the facility together i.e., *L. monocytogenes* and *Lactobacillus*.

Purpose: This study was undertaken to characterize the biofilm architecture of three *L. monocytogenes* strains and the biofilms in dual-species combination with three *Lactobacillus* species.

Methods: *L. monocytogenes* strains and *Lact. fermentum*, *Lact. bavaricus*, and *Lact. plantarum* were selected by their ability to form robust biofilms using a crystal violet assay. Static biofilms were developed in $MnSO_4$-supplemented BHI in microscopy chamber slides for 24 h at 34°C. A cell fluorescent marker, SYTO®9, and fluorescent markers targeting matrix polysaccharides and proteins, FITC-WGA and SYPRO® Ruby, and confocal laser scanning microscopy (CLSM) with image analysis program (COMSTAT2) were used to obtain qualitative and quantitative data. Statistical analyses were performed using ANOVA.

Results: Biofilms formed as dual-species had a greater tower-like architecture with significantly greater surface-to-volume ratio (SVR) and thickness than those formed individually ($P < 0.05$). As single-species, SVRs were 0.78 ± 0.52 and 0.21 ± 0.10 for *L. monocytogenes* ATCC 19115 and *L. plantarum*, respectively, whereas in dual-species conditions, the SVR was 2.08 ± 0.70. Considering thickness parameter, 13.77 ± 3.47 and 10.44 ± 1.43 (single-species), and 26.63 ± 6.87 (dual-species) were obtained. We also found that biomass parameters of polysaccharides and proteins were 6 and 4 times higher in dual-species conditions ($P < 0.05$). Results revealed that interspecies interactions had a significant effect on the structural features of biofilms.

Significance: These data suggest detailed biofilm characterization is crucial in understanding biofilm structure-function relationships and important in deciphering the persistence of microorganisms in the food industry.

This study was supported by the Fulbright Senior Award 2019/20.

Poster

P3-163 Tolerance of *Pseudomonas aeruginosa* and *Listeria monocytogenes* in Co-culture Biofilms after Successive Quaternary Ammonium Compound Exposure

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❖ ***Developing Scientist Entrant***

Introduction: Biofilm formation on difficult-to-clean surfaces facilitates microbial persistence in food production environments.

Purpose: Investigate how the sensitivities of *Pseudomonas aeruginosa* (*Pa*) and *Listeria monocytogenes* (*Lm*) to a commercial quaternary ammonium compound (QAC) sanitizer change over time in a co-culture biofilm.

Methods: Co-culture biofilms were generated in the CDC Biofilm Reactor on stainless steel coupons at 21°C and treated daily for a period of 7 days with a commercially available QAC sanitizer (1,000 ppm, 10 minutes) under agitation (n = 3 replicate experiments). Biofilm populations were regenerated to initial levels by culturing for 24 hours in between sanitizer treatments. *P. aeruginosa* and *Lm* levels were enumerated using standard cultural methods by plating on TSA and MOX, respectively.

Results: Co-culture biofilms had initial *Pa* and *Lm* populations of 9.6 ± 0.5 and 8.7 ± 0.5 log CFU/coupon, respectively. *P. aeruginosa* sensitivity to QAC inactivation diminished with time, evidenced by log CFU/coupon reductions of 1.8 ± 0.5, 1.1 ± 0.2, and 0.5 ± 0.2 after 1, 4, and 7 successive exposures, respectively ($P < 0.0044$). A simple linear regression revealed that changes in *Pa* sensitivity to QAC could be predicted from the number of successive QAC exposures (R^2=.55, $F(1,51) = 62.88$, $P < 0.0001$); (b = 0.75, $P < 0.0001$). *L. monocytogenes* was inactivated by 1.6 ± 0.7 log CFU/coupon after QAC exposure one. *L. monocytogenes* populations remained highly variable with relative ratios of *Lm* to *Pa* ranging from 0.42 ± 0.50 after exposure one to 0.02 ± 0.05 after exposure seven ($P > 0.05$).

Significance: Prolonged use of the same chemical sanitizer may result in reduced sensitivity of some microorganisms in the co-culture biofilm state, which may impact overall the balance between the organisms. The significance of this continues to be investigated.

P3-164 Development of Dual Functional Superhydrophobic Coatings with Bacterial Antimicrobial and Anticontact Characteristics

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial pathogens are responsible for millions of cases of illnesses and deaths each year throughout the world. The development of novel surfaces and coatings that effectively inhibit and prevent bacterial attachment, proliferation, and growth is one of the crucial steps for tackling this global challenge. Therefore, the use of a dual-functional coating for aluminum surfaces in food industry is a very promising application for reducing the number of pathogens and preventing their attachment and transfer of pathogens from one surface to another surface.

Purpose: The purpose of this study was to coat the aluminum surfaces with dual-functional coatings to prevent the bacterial attachment and reduce the number of bacterial pathogens.

Methods: Aluminum surfaces were coated with lysozyme and fluorinated silica nanoparticles (Ly/FSNP) by simple one-step dip coating method. Ly/FSNP-coated and bare surfaces were dip-inoculated for 4 h with Gram-negative *Salmonella* Typhimurium LT2 and Gram-positive *Listeria innocua* at bacterial concentrations of 8.8 ± 0.2 log CFU/mL. Bacterial antiadhesion and antimicrobial assays were performed by plate count method.

Results: In this study, we report the successful preparation of dual-functional coating with antimicrobial and anticontact properties achieved by immobilizing the antimicrobial protein lysozyme within a superhydrophobic coating made from the functionalization of sintered silica nanoparticles. The contact angle of water on bare aluminum surfaces, which was θ = 73.0 ± 1.7° (hydrophilic). On the other hand, the contact angle on the Ly/FSNP-coated aluminum surfaces were much higher: θ = 159 ± 0.7°, indicating a successful transformation from hydrophilic to superhydrophobic surface. The activity of the coating against surface colonization was exceptionally high with 6.5 ± 0.1 log-cycle reduction (>99.99997%) and 4.0 ± 0.1 log-cycle reduction (>99.99%) for *Salmonella* Typhimurium LT2 and *Listeria innocua*, respectively, compared to bare aluminum surfaces.

Significance: The coating technique used in this study can also be used in the hygienic design of healthcare environment and surfaces and food contact surfaces to reduce or eliminate potential risks associated with various contamination and cross-contamination scenarios.

P3-165 A Scalable and Rechargeable Antimicrobial Coating for Food Equipment

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Introduction: Since it has been an increasing challenge for food manufacturing plants to control environmental pathogens (e.g., *Listeria monocytogenes*, Lm) using existing sanitation tools, we recently developed a novel *N*-halamine-based antimicrobial coating system (HaloFilm) that can be easily applied onsite and *in situ* on food equipment surfaces and provides continuous protection for surfaces against persisting pathogens and biofilms.

Purpose: The aim of this USDA Small Business Innovation Research (SBIR) grant funded project is to further validate the efficacy and durability of our newly developed HaloFilm system for preventing *Listeria* biofilms on stainless-steel food equipment surfaces.

Methods: Antimicrobial efficacy of HaloFilm was tested on stainless-steel coupon using Lm cocktail strains that are isolated from real food processing environment. Also, Lm biofilm prevention effect of HaloFilm was validated using both live/dead staining and bacteria count methods. In addition, HaloFilm was also applied on a real food equipment and surface Lm controlling efficacy was validated using a standard environmental sampling protocol. Besides, the stability and robustness of HaloFilm were also challenged under multiple manufacturing and sanitation conditions through a chlorine titration method.

Results: HaloFilm-coated stainless-steel coupons achieved more than three-log reduction of inoculated Lm (~ 10^6 cells/sample) within 2 h of contact. HaloFilm also significantly mitigated Lm biofilm formation in a five-day biofilm formation test based on both live/dead staining and bacterial count results. On the real stainless-steel food equipment, HaloFilm-coated part significantly reduced Lm contaminates from 580-780 CFU/cm^2 to 0-38 CFU/cm^2. HaloFilm was stable and robust enough to support real application in food processing establishments: HaloFilm maintained sufficient chlorine (>10^{15} atoms/cm^2) after one-month storage, sanitation abrasion (80 cycles of sponge scrubbing), hot water treatment (141°F), and chemical attacking (Quats, degreaser, acid, alkaline, alcohol).

Significance: HaloFilm has great potential as high-performance, low-cost, and easy-to-apply coating on food equipment surfaces for Lm biofilm preventive-control.

P3-166 Combined Effects of Essential Oil Vapors in Inactivating *Shigella flexneri* and *Staphylococcus aureus*

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✦ ***Undergraduate Student Award Entrant***

Introduction: Recently, essential oil (EO) vapors as natural antimicrobial agents have received attention, but antimicrobial activities of individual and/or combinations of EO vapors against *Shigella flexneri* and *Staphylococcus aureus* have not been studied intensively.

Purpose: This study was done to measure the minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) of EO vapors and to determine the combinations of EO vapors causing synergistic antimicrobial activities against *S. flexneri* and *S. aureus* on a laboratory medium.

Methods: Firstly, among 97 EO vapors, EO vapors with relatively strong inhibitory activities against *S. flexneri* and *S. aureus* were screened by a vapor diffusion assay. Next, the MICs and MLCs of screened EO vapors against *S. flexneri* and *S. aureus* were measured. Finally, the combinations of two EO vapors showing synergistic effects against *S. flexneri* and *S. aureus* were determined using a modified checkerboard assay.

Results: Nine EO and 10 EO vapors with strong antimicrobial activities against *S. flexneri* and *S. aureus*, respectively, were screened. Cinnamon bark EO vapor showed the lowest MIC (0.0781 mL/mL) and MLC (0.1563 mL/mL) against *S. flexneri,* and citronella, lemongrass, and oregano EO vapors showed the lowest MIC (0.0781 mL/mL) and MLC (0.1563 mL/mL) against *S. aureus.* The combination of oregano and thyme thymol EO vapors showed synergistic antimicrobial effects (fractional inhibitory concentration [FICI]=0.5) against *S. flexneri,* and the combination of citronella and lemongrass showed partial synergism (FICI=0.6250) against *S. aureus.*

Significance: This is the first study which measured the MICs and MLCs of EO vapors and determined the combinations of EO vapors with synergistic antimicrobial activities against *S. flexneri* and *S. aureus*. These results may provide useful information in developing antimicrobial methods using EO vapors.

P3-167 A Comprehensive Approach to Evaluating Product Performance of Dry Wiper Systems Used for Electronic Touch Screens

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Introduction: Electronic touch screens are prevalent in the food service and grocery industries. Because microbial contamination of screens is common and can contribute to disease transmission and instances of foodborne illness, an effective sanitization strategy that does not compromise screen integrity after repeated use is needed.

Purpose: Standard sanitizing/disinfecting wipes often leave undesired residue on screens and can damage surfaces. We investigated the performance of a novel dry wiper system saturated with an ethanol-based sanitizer/disinfectant. Testing was completed to evaluate antimicrobial efficacy, product residue and surface compatibility.

Methods: Antimicrobial efficacy was confirmed using a modified EPA food contact sanitizer test method using *Escherichia coli* and *Staphylococcus aureus* as challenge organisms. Organisms were applied to the surfaces of Presto A3 touchscreens at a concentration of approximately 1x10 10^6 CFU/mL and allowed to dry. Sanitizer was applied using a saturated dry wiper and held for the contact time specified on the product label. Swab samples were collected from the screens to enumerate surviving organisms. Material compatibility testing was performed by applying the sanitizing solution directly to the screens, wiping and allowing to dry for 5 minutes over 1,000 applications to simulate 3 years of actual use. Computer image analysis of residue on screens was also performed.

Results: The results of the antimicrobial efficacy evaluation demonstrated that SMS wipes saturated with sanitizer at the point of use achieved a greater than 3-log reduction for both *E. coli* and *S. aureus* on touch screens. Material compatibility testing demonstrated that no permanent screen damage was sustained. Some residue was observed, however, it was easily remediated with a dry wiping step.

Significance: Factors such as antimicrobial efficacy and material compatibility should be carefully considered prior to the selection of an electronic screen wipe. Proper screening can confirm sanitizer performance and prevent screen damage.

P3-168 Performance Evaluation of Commercially Available Dry Wiper Systems Used in Foodservice and Implications for Long-term Use

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Introduction: EPA allows application of some food contact sanitizer/disinfectants with a mop, cloth or sponge. Dry wipers systems are a popular option for product application in food service due to ease of use.

Purpose: Testing was done to compare the antimicrobial performance and maximum hold times post saturation of three common dry wiper systems saturated with an EPA approved food contact sanitizer/disinfectant.

Methods: The maximum hold time for wipers was evaluated by extracting solution from wipes at 0, 3 and 10 days post saturation. Solution was tested for pH and percent active ingredient. Antimicrobial performance was measured for two different wipe saturation levels. Testing was performed using a modified EPA food contact sanitizer test method with *Escherichia coli* as a challenge test organism.

Results: Wipes from the test systems containing synthetic/natural blend wipes and MSM polypropylene wipes were evaluated and within the label and SDS claims for active concentration and pH for at least 10 days post saturation. The active concentration in the third system, containing a hydro knit substrate, was acceptable for the duration of the study however, the pH levels dropped below the acceptance criteria by day three. The results of the antimicrobial efficacy evaluation demonstrated that log reductions varied based on substrate type and saturation volume. However, both saturation volumes of 32 oz. and 0.5 gallon of solution per container of wipes were sufficient to achieve reductions of greater than 5 log for *E. coli*.

Significance: Differences in antimicrobial performance were observed for the three systems based on the substrate composition and saturation volume. Wipe stability was also affected by the type of substrate. Based on these results, it is recommended that the optimal saturation volume, hold time and substrate type be evaluated prior to the use of a selected sanitizing/disinfecting solution with a dry wiper system.

P3-169 Validation of Sanitizer Effectiveness against *Staphylococcus* and *Pseudomonas* Biofilms, Natural Biofilms from Worker's Boots, and Selective Correlation of Biofilm Bacteria to Sanitizer Chemistry

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne pathogens are known to adhere strongly to surfaces and can form biofilms in food processing facilities whereby the potential to contaminate manufactured foods underscores the importance of sanitation, but all too often they are applied with little or no validation.

Purpose: The objectives of our study were to 1) confirm sanitizer effectiveness on biofilms of *Staphylococcus* and *Pseudomonas*, 2) validate sanitizer effectiveness on real-life samples of workers' boots from a slaughter floor environment, 3) identify biofilm bacteria from old boots in relation to sanitizer chemistry, and 4) evaluate enzymatic treatment to breakdown biofilms prior to sanitizer application.

Methods: A sanitizer that demonstrated superior effectiveness against *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* was applied at 2 concentrations against enhanced biofilms of 5 strains of *Staphylococcus* spp. and *Pseudomonas* spp. (as required by EPA) in 96-well microplates. Additionally, worker boots were swabbed with trypsin solution and then treated with the sanitizer spray solution. Bacteria isolated (before treatment) were identified by 16S rRNA PCR and DNA sequencing.

Results: All treatments were carried out in triplicate replication and analyzed by RM-ANOVA using the Holm-Sidak test for pairwise multiple comparisons to determine significant differences ($P < 0.05$). The data show a significant difference between sanitizer treatment and control groups. There was a ~4 to 5-log reduction of bacterial strains (microplate assay) within the first 1 min of treatment and also greater > 3-log reduction in bacterial population from encrusted biofilms from workers' boots.

Significance: The data show that new, next generation QAC (quad) sanitizers may be more effective than prior single/dual-QAC sanitizers and enzyme pre-treatment can facilitate biofilm sanitizer penetration on any food contact surface. Rotation of sanitizer chemistries may prevent selective retention of chemistry-tolerant microorganisms if they may occur.

P3-170 Systematic Evaluation of Commercial Disinfectants against Human Norovirus Surrogates and *Clostridium difficile* in Suspension Test

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Introduction: Human noroviruses and *Clostridium difficile* are frequently associated with outbreaks of acute gastroenteritis among residents in long-term care facilities. Among the many commercial disinfectants, none are labeled as effective against both pathogens due to the lack of systematic evaluation of those products.

Purpose: To evaluate the efficacy of EPA-registered disinfectants against two human norovirus surrogate viruses and *C. difficile* spores.

Methods: Nine disinfectants were chosen from list G, EPA's Registered Antimicrobial Products Effective Against Norovirus, based on reported effectiveness against feline calicivirus (FCV) and low occupational health hazard. Tulane virus (TuV) and FCV, surrogates of human noroviruses, and *C. difficile* endospores were subjected to suspension tests with selected disinfectants, consistent with product labels. After mixing with each product for 1 min or 10 min, reduction of endospore concentration and remaining virus titer was determined. Pure hydrogen peroxide (H_2O_2) at concentrations of 0.5-5% was tested as the active ingredient control.

Results: Two (product 1 and 4) of 9 tested products achieved >3.0-log reduction of FCV, TuV, and *C. difficile* spores after exposure for 1 and 10 min, respectively. Products #1 and #4 reduced >5.16 logs and 4.47 logs FCV in 1 min, 3.85 logs and 3.80 logs TuV in 10 min, and >5.62 logs and >5.62 logs *C. difficile* spores in 10 min, respectively. Although H_2O_2 was the main active ingredient listed for most products, 5% pure H_2O_2 achieved lower reduction (1.93 log) of *C. difficile* spores after 10 min than product #1 (3.13% H_2O_2) and product #4 (0.5% H_2O_2), which reduced *C. difficile* spores by >5.62 logs.

Significance: We identified two H_2O_2-containing disinfectants with strong activities against both *C. difficile* spores and two human norovirus surrogates. Both are believed to be effective and safe for use in long-term care facilities as an alternative to chlorine-based products.

P3-171 Implementation of Targeted Cleaning and Sanitation Directed by ATP Swabbing to Improve the Microbiological Quality of Finished Food Products

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Introduction: Cleaning and sanitation can control for environmental spoilage microorganisms, but verifying its efficacy and impact remains a challenge.

Purpose: This study evaluated the impact of *targeted* cleaning and sanitation in a tofu production facility, directed by ATP swabbing, on the microbiological environment and product quality.

Methods: Evaluation occurred over two phases: pre-intervention and post-intervention of targeted cleaning and sanitation practices. During the pre-intervention phase (t = 3 weeks), cleaning and sanitation proceeded normally, without targeted efforts. ATP was quantified using 3M™ Clean-Trace™ Surface ATP Swabs at 30 high-impact sites in the production room immediately following cleaning and sanitation to determine the facility's baseline hygiene level. After this period, the cleaning and sanitation crew targeted sites requiring additional attention, as determined by the ATP swab results. An intermediary period (t = 6 weeks) was given to allow for adjustment to the program before the post-intervention study phase (t = 16 weeks). ATP, yeasts and molds, lactic acid bacteria, and aerobic microorganisms were quantified from swabs of the 30 high-impact sites and products produced on the line (25 g subsamples) to determine impact.

Results: The failure rates of ATP (RLU/swab > 500, n_{pre} = 399, n_{post} = 920), lactic acid bacteria (log CFU/swab > 2.30, n_{pre}= 390, n_{post} = 691), and aerobic microorganism (log CFU/swab > 2.30, n_{pre} = 390, n_{post} = 691) swabs were significantly lower post-intervention of targeted cleaning and sanitation practices than pre-intervention ($P < 0.05$, Fisher's exact test); whereas, there was no significant difference in the failure rate of yeast and mold swabs (log CFU/swab > 1.30, n_{pre} = 390, n_{post} = 691). The mean rank log CFU/g of post-intervention products ($n = 68$) was significantly lower than pre-intervention products ($n = 19$) for yeasts and mold, lactic acid bacteria, and aerobic microorganisms ($P < 0.05$, Mann-Whitney U test).

Significance: These data suggest that targeted cleaning and sanitation, directed by ATP swabbing, improves both microbiological environment and product quality.

Poster

P3-172 Determination of the Perceived Threshold for Dirtiness of Food-soiled Surfaces by Panelist Visual Detection

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Introduction: Pre-operational visual inspection is widely used in sanitation program monitoring. However, the degree of residual food soil on equipment surfaces that is below the limit of visual detection has not been determined. Moreover, the sensitivity of visual inspections may be influenced by a variety of environmental conditions and inspector attributes.

Purpose: To evaluate the sensitivity of visual inspection of food-soiled surfaces.

Methods: Panelists (n = 25) completed a vision and height test and were shown examples of flour-soiled stainless steel and white HDPE 12 x 12 inch sheets (1 and 1.25 g/ft^2, respectively). Panelists then viewed 15 stainless steel and 15 HDPE sheets, clean or soiled, in a randomly generated order and were asked if they could visually detect soil on each surface. Stainless steel surfaces were soiled from 0 to 0.08 g flour/ft^2 while white HDPE was soiled up to 1.0 g flour/ft^2. Soiling level was also measured with profilometry.

Results: Panelist's height and vision did not significantly affect their soil detection ability. Panelists were better able to detect flour residue on stainless steel compared to white HDPE, although no significant difference in detection was observed among the soiling levels on stainless steel evaluated here. Significant differences (P < 0.05) were found between soiling levels on HDPE surfaces with an average visual threshold of 0.25 g flour/ft^2.

Significance: Pre-operational visual inspections may fail to recognize residual food soil up to 0.25 g/ft^2 depending on food soil and surface combinations, suggesting the importance of additional monitoring and verification strategies to ensure effective cleaning and allergen residue removal.

P3-173 Evaluation of Food Delivery Bag/Box Cleanability

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Introduction: Globally, an increasing number of consumers are ordering food online for delivery at a higher frequency.

Purpose: This study investigated the cleanability of selected food delivery bags and boxes and recommended an optimal cleaning method.

Methods: Ten delivery bags from the US and one delivery box from China were used. Evaluations were done of the bag/box design, their cleanability, and how to optimally clean them. Bags were visually inspected against five design parameters: insulation, interior lining material, exterior lining material, interior seams and opening area.

Cleanability testing: Five milliliters of an *E. coli* suspension (10^6 CFU/mL) were spread onto the interior surface of each bag/box using sterile sponges. After 30 minutes, each side of the interior surface of the bag/box was cleaned using a consistent cleaning process. Two cleaning methods were evaluated:

1. Cleaning/sanitizing method using a microfiber cloth saturated with a cleaner/sanitizer.
2. Cleaning/disinfection method using a microfiber cloth saturated with a cleaner/disinfectant.

A control was run by cleaning the bag/box using a microfiber cloth saturated with non-antimicrobial detergent. The interior surfaces were swabbed before and after each cleaning process to determine remaining *E. coli* populations as an indication of achieved cleanliness. Statistical analysis methods are Paired T-Test and Mann-Whitney Test.

Results: Cleaning of delivery bags without internal seams achieved a 2-log-greater reduction in *E. coli* than cleaning bags with internal and side-seams, regardless of the cleaning method. Cleaning using a cleaner/sanitizer, or cleaner/disinfectant achieved a higher microbial reduction than the control, P = 0.02 and P = 0.03, respectively.

Significance: Delivery bags without seams were easier to clean. A method using a combination cleaner with sanitizer or disinfectant will likely reduce cross contamination risk.

P3-174 Microbial Contamination Levels in Disposable Tableware in Korea

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Introduction: Since frequency of eating out increases, use of disposable tableware also increases in Korea. However, although they are contacted with mouth, microbial contaminations of them have not been studied in Korea, yet.

Purpose: The objective of this study was to analyze the microbial contamination of disposable tableware in Korea.

Methods: Disposable forks (n = 300), knives (n = 280), spoons (n = 310), chopsticks (n = 300), and cups (n = 280) were collected from retail stores and online stores. Disposable forks, knives, spoons, and chopsticks were placed into filter bags containing buffered peptone water. Disposable cups (n = 280) were swabbed inside of the sample surfaces with sterilized cotton swabs dampened with 10 mL buffered peptone water. The samples were homogenized, and the homogenates were plated on plate count agar, *Escherichia coli*/coliform count plates, Baird Parker agar, cetrimide agar, and potato dextrose agar to enumerate aerobic bacteria, coliform, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and fungi in the samples, respectively.

Results: Aerobic bacteria were detected in 32 samples (5.0 – 9.3×10^2 CFU/g) of disposable forks, in 56 disposable knives (5.0 – 6.8×10^4 CFU/g), in 41 disposable spoons (5.0 – 1.6×10^3 CFU/g), in 53 disposable chopsticks (5.0 – 3.3×10^3 CFU/g), and in disposable cups (5.0 – 2.6×10^2 CFU/cm^2). Fungi were detected in 17 samples (5.0 – 3.8×10^2 CFU/g) of disposable forks, in 13 disposable knives (5.0 – 1.3×10^3 CFU/g), in 11 disposable spoons (5.0 – 1.3×10^3 CFU/g), in 33 disposable chopsticks (5.0 – 2.1×10^3 CFU/g), and in 9 disposable cups (1.0 – 2.5×10 CFU/cm^2). Coliform, *E. coli*, *S. aureus*, and *P. aeruginosa* were not detected in all samples.

Significance: Although pathogenic bacteria were not contaminated with disposable tableware, hygiene management needs to be established to control aerobic bacteria and fungi.

P3-175 Environmental Conditions Impact the Recovery of Microorganisms from Non-porous Surfaces

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Introduction: Environmental monitoring (EM) is used to determine harborage sites of microorganisms, assess sanitation program effectiveness, and prevent transmission of microorganisms. However, there is little known about the impact of environmental conditions on recovery and how EM tools perform under these varying conditions.

Purpose: To determine if EM recoveries are impacted by environmental conditions and surface composition.

Methods: One milliliter of 10^9 CFU/mL bacterial cocktail (*Listeria monocytogenes*, *Salmonella* Typhimurium) was directly inoculated onto two environmental surfaces (stainless steel, neoprene) and allowed to dry. The surfaces were held in an environmental chamber for 24 or 72 h. The environmental conditions

were 30°C or 6°C at 85% relative humidity (RH). After the designated timeframe, the surfaces were swabbed with a polyurethane foam sponge pre-hydrated with 10 mL of 1×PBS. Samples were plated onto selective agar. Experiments were replicated and analyzed in duplicate.

Results: The recovery of microorganisms varied significantly ($P < 0.05$) from each other under similar conditions. The mean recovery of *L. monocytogenes* was 77.8% compared to *S.* Typhimurium which was 56.5%. Data indicate that temperature, at the same RH, significantly impacts the recovery of microorganisms from the surfaces with a mean recovery of 76.7% and 57.6% for 6°C and 30°C, respectively. Specifically, the highest mean recovery was 87.3% of *L. monocytogenes* from the neoprene surface at 6°C/85% RH after 72 h where the lowest recovery was 32.3% of *S.* Typhimurium from the neoprene surface at 30°C/85% RH after 72 h. However, overall, the mean recovery did not vary significantly between surface types or sampling times.

Significance: Results indicate that the recovery of microorganisms from environmental surfaces significantly varies by microbial type and ambient temperature. This variation emphasizes the need for further understanding how EM and its results are impacted by environmental conditions in food processing facilities.

P3-176 Difficulties of Spiral Freezer Decontamination: Eradicating *Listeria* spp. Using Chlorine Dioxide Gas

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Introduction: Spiral freezers are notorious for microbial contamination and being very difficult to clean because of the complex and crowded interiors. Current methods of cleaning spiral freezers (i.e., spraying, foaming and wiping) are not completely effective because they are liquid-based methods and have difficulty getting into tight spaces and hard to reach areas. A new thought is to fill the space with a sterilant gas achieving complete coverage, resulting in more effective decontamination and shorter downtimes.

Purpose: A study was initiated to validate the efficacy of chlorine dioxide gas (CD) on the inactivation of *Listeria monocytogenes* at low concentration levels in short periods of time.

Methods: Traditional exposure for CD to achieve a 6-log sporicidal kill is 720 ppm-hours. Glass coupons inoculated with *Listeria monocytogenes* were introduced into a 0.5 m³ chamber and exposed to 50 to 720 ppm.h decontamination cycles at 1 mg/L. The inoculated and exposed coupons were then enumerated and/or enriched along with proper controls to determine the total log reduction. Concentrations can be monitored in real time with a spectrophotometer to guarantee the correct exposure was achieved throughout the target area.

Results: Chlorine dioxide has achieved a greater than 5-log reduction with a 400 ppm.h exposure on glass coupons. A case study involving a 560 m³ spiral freezer that was contaminated with *Listeria* spp. was successfully decontaminated using chlorine dioxide gas resulting in no positive swabs for up to 6 weeks after exposure.

Significance: Spiral freezers are very difficult to clean and therefore make it hard to prevent microbial contamination. Traditional methods for cleaning spiral freezers (i.e., spraying, foaming and wiping) are not completely effective because they are liquid-based methods and have difficulty getting everywhere. Filling the space with a sterilant gas and achieving complete coverage results in a more effective decontamination.

P3-177 Recovery and Detection of *Cyclospora cayetanensis* from Agricultural Water: A Multi-laboratory Validation Study

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Introduction: *Cyclospora cayetanensis* is a protozoan parasite that causes foodborne diarrheal illness outbreaks associated with consumption of various types of fresh produce. Agricultural water may represent an important vehicle in contamination of crops.

Purpose: We designed and conducted a multi-laboratory collaborative study to validate a new analytical method that relies on dead-end ultrafiltration to concentrate *Cyclospora* oocysts from agricultural water samples combined with a robust molecular detection.

Methods: Eight laboratories from FDA ORA and CFSAN, state public health departments, and private laboratories, contributed data to the study. The study consisted of four rounds of analysis by each laboratory of eight 10 L water samples seeded with 6 or 100 oocysts of *C. cayetanensis*. Unseeded samples were also included to verify specificity of the method. Method performance was characterized by Sensitivity (SE) and Specificity (SP) rates which were calculated following the principles of ISO 16140, 2016.

Results: Seven out of the eight participating laboratories provided satisfactory results. Data from one laboratory were excluded from the study due to false positive results derived from systematic errors attributable to the laboratory. None of the other 7 laboratories obtained positive results for unseeded samples. Positive *C. cayetanensis* samples were detected as expected for seeding levels of 6 and 100 with rates of 89.2% (100/112) and 100% (72/72), respectively. Specificity was 100% for all laboratories and sensitivity ranged from 71.43% to 100% among samples at the seeding level of 6 oocysts in 10 L water.

Significance: Through the validation study, it was demonstrated that the method was sufficiently sensitive and specific and could detect at least 6 *C. cayetanensis* oocysts in 10 L of agricultural water samples. After the completion of the study, the validated method became available through the FDA Foods Program Compendium of Analytical Laboratory Methods Bacteriological Analytical Manual as a reference method.

P3-178 Evaluation of BAM Chapter 19b Method for Detection of *Cyclospora cayetanensis* in Mixed Bagged Pre-cut Salads

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Cyclospora cayetanensis* is a major cause of diarrheal illness and outbreaks. Recently, a large outbreak occurred in the US linked to consumption of a variety of salads containing romaine lettuce and carrots in restaurants in the Midwest (CDC, 2018). The FDA Bacteriological Analytical Manual (BAM) Chapter 19b method has been validated in carrots and romaine lettuce by matrix extension studies but has not been previously evaluated in mixed pre-cut salads containing these ingredients.

Purpose: This study evaluated BAM Chapter 19b method for the detection of *C. cayetanensis* in two different commercial bagged pre-cut mixed salads.

Methods: Twenty-five-gram samples of pre-cut mixed salad 1 (containing romaine and iceberg lettuce, carrots and red cabbage) and mixed salad 2 (containing romaine and iceberg lettuce, carrots, red cabbage, radish and pea pods) were seeded with 5 and 200 *C. cayetanensis* oocysts. Unseeded produce was

used as negative control. The method included washing of the produce, extraction of *C. cayetanensis* DNA, and molecular detection using a Taqman assay targeting the 18S rRNA gene with and internal amplification control (IAC).

Results: As few as five oocysts were detected in both mixed salads (n = 10 in each type) with positive detection rates of 30% and 60%, respectively, for mixed salad 1 and mixed salad 2. All unseeded salad samples were negative, and all salad samples seeded with 200 oocysts (n = 7 in each type) were positive. Statistically significant differences were observed in 18S rRNA *C. cayetanensis* C_T values in samples seeded with 200 oocysts between both salads ($P < 0.05$).

Significance: The method was consistent and able to detect as few as 5 oocysts in bagged mix salads following BAM Chapter 19b method. Differences in detection among mixed salads highlights the importance of evaluating the performance of the *C. cayetanensis* detection method in different food matrices.

P3-179 Assessment of Commercial DNA Clean-up Kits for Elimination of PCR Inhibitors in the Detection of *Cyclospora cayetanensis* in Cilantro

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Introduction: *Cyclospora cayetanensis* is a major cause of diarrheal illness and outbreaks. A method for the detection of *C. cayetanensis* DNA in cilantro was validated by the U.S. Food and Drug Administration (FDA).

Purpose: The presence of soil in cilantro samples may create an inhibitory effect on qPCR reactions, thus producing false-negative results. This study evaluated the performance of five commercial DNA clean-up systems for the ability to reduce qPCR inhibition in cilantro samples contaminated with soil.

Methods: Portions of 25 g of cilantro samples contaminated with soil were seeded with 200 oocysts (n = 9). DNA was extracted according to the FDA BAM Chapter 19b method. The DNA extracts were pooled and three 25 µL samples of the pooled DNA were processed in triplicate using five commercial DNA clean-up kits. Performance of the clean-up kits was assessed after molecular detection using the Chapter 19b duplex TaqMan assay, targeting the 18S rRNA gene of *C. cayetanensis* and an internal amplification control (IAC).

Results: Inhibitory cilantro samples seeded with 200 oocysts had average C_T values of 33.3 ± 0.4 for the 18sRNA target with average IAC C_T values of 33.2 ± 0.1, which is more than three C_T values higher compared to the non-template control reactions. After clean-up, average IAC C_T values returned to values considered normal for non-inhibited samples; i.e., IAC C_T from 23.9 to 24.9. However, significant differences in the 18S rRNA gene target C_T values were observed ($P < 0.05$), ranging from 33.05 ± 0.2 to 35.5 ± 0.3 among the kits.

Significance: DNA clean-up kits can be used to reduce inhibition in cilantro samples. Each of the five DNA clean-up kits was able to reduce inhibition based on IAC C_T values. Although all samples were positive, some differences in *C. cayetanensis* detection were observed among kits. This highlights the importance of evaluating each kit before use.

P3-180 Independent Laboratory Validation Study of Detecting *Cyclospora cayetanensis* in Agricultural Water

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Introduction: *Cyclospora cayetanensis* is an emerging parasitic protozoan pathogen causing cyclosporiasis. Some of the 2,299 cases in 2018 were associated with domestically grown fresh produce. Agricultural water used for the irrigation process could be a contamination source.

Purpose: An improved method for recovery and detection of *C. cayetanensis* in agricultural water was developed by the FDA. According to the FDA Guideline for Microbial Method Validation, independent laboratory validation (ILV) study is the prerequisite of any collaborative validation. In this ILV study, the method was validated for its sensitivity with samples from different water sources.

Methods: Water samples (10 L each) collected from pond, river, lake and well were inoculated with oocysts at five inoculation levels, including one blank, two fractional and two positive control levels. The water was filtered through Dead-End Ultrafiltration system. The oocysts in each filter were recovered by backflushing and concentrated by centrifugation. DNA was extracted from the pellets and purified. *C. cayetanensis* 18S rRNA gene was detected by using real-time PCR according to the BAM Chapter 19B with some modifications.

Results: The results from seven trials showed that the detection rates for inoculated pond, lake and well water were similar (over 90%) while the percentage of positives were much lower (38.5%) for inoculated river water. Overall, the sensitivity of detecting the *C. cayetanensis* gene was 100% at the inoculation levels of 100 and 200 oocysts/10 L. At the levels of 6 and 12 oocysts/10 L, the detection rates were 74.3% and 91.7%, respectively.

Significance: The ILV study suggested that the method was sensitive enough to detect 6 oocysts from surface water and well water samples with turbidity between 3 to 10 NTU. The multi-laboratory validation of this method would be conducted upon successful completion of the current study.

P3-181 The Relationship between Season, Weather, Physicochemical Properties and the Presence of *Cryptosporidium* spp., *Toxoplasma gondii,* and *Giardia intestinalis* in Potential Alternative Sources of Agricultural Water: A Conserve Project

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Introduction: There is a public health concern surrounding parasitic protozoa in agricultural irrigation water and their contamination of fresh produce. Given recent epidemiological findings with foodborne outbreaks, data gaps must be addressed.

Purpose: To examine the impact of season, weather, and physicochemical properties of water on the presence of protozoan parasites in potential alternative sources of agricultural water.

Methods: Water samples (n = 72) were collected (6/2017 to 10/2018) from surface water (river, pond), and recycled water sites in the Mid-Atlantic region. Envirochek HV sampling capsules were used for sampling (10 to 20 L) and elution of (oo)cysts according to U.S. EPA method 1623.1. Water concentrates were used for DNA extraction. Weather conditions and physicochemical properties of the water sources were measured during sampling. Data were analyzed using binary logistic-regression and chi-square tests with XLSTAT.

Results: For *Cryptosporidium* spp., 36.10% (26/72) of samples tested positive. For *Giardia intestinalis*, 9.72% (7/72) of samples tested positive. For *Toxoplasma gondii*, 9.72% (7/72) of samples tested positive. Ambient temperature was found to have a potential impact on the detection of *Cryptosporidium* and *Giardia* across water types ($P < 0.05$). When examining surface water and recycled water separately, *Cryptosporidium* detection may be impacted by surface water temperature ($P < 0.05$) and *Giardia* by reclaimed water temperature ($P < 0.001$). Precipitation 24 h and 14 d prior to sampling (PTS) was associated with detection of *Toxoplasma* across water types ($P < 0.05$). By water source, *Cryptosporidium* detection in ponds was linked with precipitation 14 d PTS and in river and reclaimed waters at 24 h PTS ($P < 0.05$). Salinity was linked with *Cryptosporidium* detection in river and reclaimed waters ($P < 0.05$), while turbidity was found to relate to presence in reclaimed water ($P < 0.05$). Season and presence of protozoa were found to be independent ($P > 0.133$).

Significance: Protozoan parasites were detected across all seasons and relationships were observed between presence of specific protozoa and environmental conditions.

P3-182 Verification and Implementation of the US-FDA BAM Chapter 19b Method for Routine Detection of *Cyclospora cayetanensis* in Leafy Greens and Berries by a Canadian Food Inspection Agency Laboratory

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Introduction: Foodborne outbreaks of *C. cayetanensis* infection in humans have occurred regularly in the US and Canada over the past decade; however, the implicated food vehicle is rarely definitively identified. Reliable, validated detection tools are critical to improve disease outbreak investigations and routine surveillance of produce for *C. cayetanensis.*

Purpose: To facilitate harmonized and effective surveillance and investigation of cyclosporiasis outbreaks in the US and Canada, we verified additional matrices and adapted the BAM 19b method for routine diagnostic use in our laboratory.

Methods: Performance characteristics of the method were verified by spiking 200, 10, 5 or 0 *C. cayetanensis* oocysts onto three berry types (50 ± 5 g, $n = 85$), and 200, 10 or 0 oocysts onto green onions (25 ± 3 g, $n = 24$) and five leafy green types (25 ± 1 g, $n = 120$), recovering oocysts by washing with 0.1% Alconox, extracting DNA by bead-beating, and amplifying a 100 bp 18S rDNA fragment using our laboratory's qPCR platform. The robustness of the method was assessed by subjecting the produce and washes to aging (0 or 7 days) and freezing conditions prior to testing.

Results: For berries spiked with 200, 10, 5 or 0 oocysts, 31/31, 14/31, 3/11, and 0/12 samples were positive, respectively. For leafy greens and green onions spiked with 200, 10 or 0 oocysts, 56/60, 18/60 and 0/24 were positive, respectively. The positive detection rate was not affected ($P = 0.22$) by age or condition of produce (7 d, fresh, frozen) or wash concentrate (3 d, fresh, frozen), however Cq values were higher ($P = 0.009$) for raspberries aged 7 d (37.46 ± 0.29) compared to fresh (35.36 ± 0.29).

Significance: These results independently verify the reported performance characteristics and robustness of the US-FDA BAM 19b method for detection of *C. cayetanensis* in a variety of matrices, under adverse sample conditions, using a unique detection platform, and support its employment for routine diagnostic use in our CFIA laboratory.

P3-183 Validation of Loop-mediated Isothermal Amplification (LAMP) Assay for Rapid and Reliable Detection of *Giardia duodenalis* Cysts in Leafy Greens

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Introduction: A rapid and reliable diagnostic test is essential to mitigate food safety risks for leafy greens contaminated with *Giardia.*

Purpose: We optimized washing procedures for recovery of *Giardia* cysts from leafy greens and validated an EF1α LAMP assay for detection of *Giardia* DNA to support routine diagnostic surveillance and disease outbreak investigations.

Methods: Four leafy green types (35 ± 1g) were spiked with 100 *Giardia* cysts and washed by shaking with 1M glycine ($n = 20$) or 0.1% Alconox® ($n = 20$). DNA was extracted from washes, tested by LAMP and melt curve analysis, and time to positive (TTP) values compared. Detection limit was determined by spiking 10 ($n = 40$) *Giardia* cysts onto these same types of leafy greens and processing as above. Method robustness was assessed by subjecting spring mix ($n = 45$ total) to aging (1, 3 or 7d) and washes to aging and freezing conditions prior to testing. Assay repeatability and specificity were evaluated, and an artificial positive control (APC) distinguishable by melt temperature (Tm) from *Giardia* gDNA was designed to rule out cross-contamination from the control.

Results: *Giardia* detection rates were higher and TTP was lower for 0.1% Alconox (19/20, 8.85 ± 0.3 min) compared with 1M glycine (15/20, 14.53 ± 7.2 min). The LAMP assay detected 10 *Giardia* cysts spiked on leafy greens in 13 - 34 min in 14/40 samples tested. Robustness assessment showed that TTP was higher ($P < 0.05$) when spiked produce was stored for 7 d (13.09 ± 1.14 min) compared to fresh (9.72 ± 0.43 min). No un-spiked samples were positive by LAMP, and the Tm of *Giardia* gDNA was higher ($P < 0.001$, 87.43 ± 0.05ºC) than the APC (86.43 ± 0.12ºC). Within-assay repeatability CV for TTP was 0.074, and no cross-contamination occurred when spiked and un-spiked samples were processed in alternate order.

Significance: The optimized EF1α LAMP assay is a sensitive, specific, labor-saving, and rapid method for the detection of *Giardia* cysts in leafy greens.

P3-184 Inactivation of Encysted Muscle Larvae of *Trichinella spiralis* in Pigs after Antihelminthic Drug Treatment

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Introduction: The presence of microorganisms in meat and products containing meat can result in a range of human health problems, as well as economic losses to producers of these products. Trichinellosis is caused by the parasite *Trichinella*. Infections of this disease occur worldwide, but are most prevalent in regions where raw of undercooked pork or wild game is consumed.

Purpose: Therefore, it is necessary to evaluate new therapeutic and/or chemoprophylactic strategies to treat or prevent infection in animals maintained in management systems that do not preclude infection. The purpose of this study was to evaluate the effect of 4 anthelmintic treatments on the viability of *T. spiralis*-encysted muscle larvae (ML) in pigs.

Methods: Pigs were infected at 8 weeks of age with *T. spiralis* (ISS 44) first stage muscle larvae (ML) obtained by artificial digestion of infected rat muscle. At 55 days post-infection (PI), pigs were randomly assigned into 5 groups: control (sterile saline), levamisole, mebendazole, doramectin, or moxidectin. Pigs were humanely euthanized on day 66 PI and ML were then isolated from pig tissues and counted to determine worm burdens. These ML were then inoculated into mice for 30 days to assess viability.

Results: Mebendazole treatment group had the lowest muscle larvae count in pig tissues. After inoculating mice with ML to assess viability, mebendazole treatment had the most significant effect in lowering *T. spiralis* viability in mice.

Significance: In conclusion, this experiment provides a way to eliminate *T. spiralis* and its potential to cause illness from infected pork.

P3-185 Imported Raspberries Linked to Norovirus Cruise Ship Outbreak

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Introduction: Several norovirus and hepatitis A virus (HAV) outbreaks have occurred in recent years from the consumption of soft fruit in the U.S. and abroad. In 2017 and 2019, outbreaks of norovirus on cruise ships occurred and, in both instances, the implicated product was imported frozen raspberries from a single country of origin.

Purpose: The objective of this analysis was to detect, enumerate, and characterize norovirus to confirm imported raspberries as a causative agent in the 2019 norovirus associated cruise ship outbreak.

Methods: Frozen raspberries were analyzed for the presence of norovirus using FDA's validated methods for concentration, extraction and detection of enteric viruses from soft fruit. Norovirus levels were enumerated utilizing standard curves. Gel electrophoresis, conventional RT-PCR, and big-dye terminal sequencing of the polymerase region of the norovirus genome was used for characterization.

Results: Norovirus was detected at approximately 2.5 copies per 50-gram frozen raspberry sample. Sequence characterization of the amplicons demonstrated that the strain was GII.P16. A different strain of norovirus GII.P16 was detected in the clinical samples. While the strains were not a match, this is likely an indication of the presence of multiple strains of norovirus in the raspberry product. Multiple strains have been observed in previous foodborne norovirus outbreaks.

Significance: Norovirus was detected and characterized from imported frozen raspberries and, therefore, confirmed as the source of illnesses this outbreak. These findings emphasize the importance of routine surveillance of berry products and the potential occurrence of multiple norovirus strains in foods, which is indicative of production under insanitary conditions.

P3-186 Ultra Low Temperature High-pressure Processing Inactivation of Foodborne Viruses

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Introduction: Both norovirus and hepatitis A are significant health risks in products such as frozen berries. High pressure processing at ultralow temperatures (HPP_{ULT}) has been demonstrated to be an effective means of reducing bacteria pathogens such as *Listeria*. Pressurization at temperatures below -20°C can cause ice phase changes under pressurization. Therefore, the potential effects on inactivation of viruses at ultralow temperature have been evaluated as a prelude to evaluating HPP inactivation within a frozen food matrix.

Purpose: In this study, inactivation studies were conducted on both Tulane virus (norovirus surrogate) and hepatitis A to determine efficacy of HPP_{ULT} on virus inactivation.

Methods: Each virus was placed in 1 mL aliquots in double-sealed pouches and stored at -70°C prior to pressurization. One pouch of both viruses was placed in HPP chamber that was subsequently filled with -40°C pressurization fluid. Samples were pressurized at 200, 300, 400, or 500 MPa and held at pressure for 5 min. Tulane virus plaque assay and hepatitis A plaque assay were utilized to measure virus survival.

Results: The untreated control Tulane virus titer was 3.3×10^5 PFU/mL. Two hundred MPa treated virus titer was 2.67×10^1, representing > 4-log reduction. At 300 MPa, only 1 PFU was detected, a drop of > 5 logs. At 400 and 500 MPa viable Tulane virus was not observed. Thus, the pressure required to inactivate Tulane at very low temperatures is substantially reduced. In contrast, for hepatitis A limited inactivation was observed at 500 MPa (about 1 log) with no observed inactivation at 400 MPa or below.

Significance: These data suggest that high pressure processing at ultralow freezing temperatures may be more effective than refrigeration or room temperature as a means of inactivating noroviruses but perhaps not hepatitis A.

Poster

P3-187 Enhanced Inactivation of Foodborne Viruses by Cinnamaldehyde Nanoemulsions Require a Lipid Envelope

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❖ ***Developing Scientist Entrant***

Introduction: Human noroviruses are the leading cause of foodborne illness globally. Many challenges exist in control of these viruses as many disinfectants show modest inactivation. Previous work has demonstrated that restructuring disinfectants into charged nanoemulsions can enhance inactivation of bacteria and fungi, but their effect on viruses is unknown.

Purpose: The purpose of this study was to conduct comparative inactivation studies of cationic cinnamaldehyde nanoemulsions on norovirus surrogate phage MS2 and *Escherichia coli* to see if cationic nanoemulsions could enhance efficacy of cinnamaldehyde.

Methods: MS2 bacteriophage, a norovirus surrogate, and *E. coli* strain C3000 were treated with different concentrations of cationic cinnamaldehyde nanoemulsion and the cinnamaldehyde essential oil (5.55 µg/mL - 27.7 µg/mL cinnamaldehyde) for 5-60 minutes at 37°C by suspension assay.

Results: Overall, significantly more reduction for MS2 treated for 1 hour with cinnamaldehyde alone was observed compared to cationic cinnamaldehyde nanoemulsion. For instance, 4.02 ± 0.102 PFU/mL and 2.78 ± 0.34 PFU/mL log reductions were observed when treated with 27.7 µg/mL and 5.55 µg/mL of cinnamaldehyde alone, respectively. Whereas, 1.54 ± 0.08 PFU/mL log reduction with 27.7 µg/mL and no reduction with 5.55µg/mL of cinnamaldehyde in nanoemulsion was observed. Alternatively, significant reduction of *E. coli* was observed with treatment of cinnamaldehyde nanoemulsions. For instance, >7-log reduction was observed with 16.6 µg/mL cinnamaldehyde nanoemulsion for 5 mins. Shelf-life study of nanoemulsion showed stability over 2 weeks when stored at 20°C and 4°C.

Significance: These data suggest the hydrophobic protein contacts that maintain non-enveloped virus structure are not easily targeted by cationic nanoemulsions generated with low energy, high surfactant formulation, and suggests such a formulation requires targets with a lipid envelope for efficacy. This work informs future formulation to improve disinfectant efficacy against foodborne pathogens.

P3-188 Evaluation of Non-traditional Irrigation Water Sources for Atmospheric, Physicochemical, and Viral Indicators of Viral Enteric Pathogens: A Conserve Study

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Introduction: Surface and reclaimed waters can supplement groundwater use for irrigation but have increased susceptibility to contamination. Monitoring these non-traditional irrigation water sources for viral enteric pathogens (VEPs), including norovirus and hepatitis A virus, is complex due to the inherent variability, increased microbial and particulate levels. Using indicators for VEPs, like pepper mild mottle virus (PMMoV) or easily monitored atmospheric and physicochemical variables, may improve costly and labor-intensive testing of irrigation water.

Purpose: This study analyzes non-traditional irrigation water sources and investigates potential atmospheric, physicochemical, and viral indicators of VEPs.

Methods: Samples (n = 71) were collected from surface and reclaimed water sources June 2017-October 2018. Atmospheric data - cloud cover, precipitation - 24 hours, 7 days, and 14 days prior to collection, ambient and water temperatures - were obtained from local agencies. Physicochemical variables - %DO, pH, salinity (PSU), and turbidity (FNU) - were recorded. Indicator virus, PMMoV, and VEPs were evaluated using an adsorption-elution method followed by RT-qPCR. Viral data were analyzed using binary logistic regression and Chi-square tests of independence.

Results: VEPs were detected in 4.1% of surface (n = 49) and 31.8% of reclaimed (n = 22) samples. PMMoV was detected in 33.3% of surface (n = 42) and 66.7% of reclaimed (n = 21) samples. PMMoV and VEP detection were significantly correlated in reclaimed (R^2=0.63; P < 0.05), not surface (R^2=0.48; P = 0.78) water samples. Of the atmospheric variables, water temperature significantly impacted VEP detection (P < 0.05) and precipitation (14 days) impacted PMMoV detection (P < 0.05). Of the physicochemical variables, salinity significantly impacted detection of both VEPs and PMMoV (P < 0.05).

Significance: Irrigation water provides a potential route of microbial contamination to produce in pre-harvest environments. Correlations between atmospheric, physicochemical, and viral indicators may reduce the testing required to reliably gauge possible viral enteric pathogen contamination of irrigation water. This research closes the knowledge gaps regarding those variables effecting the quality of non-traditional irrigation water sources.

P3-189 Evaluation of Concentrating Methods for Enteric Viral Detection in Water

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Introduction: Testing for bacterial pathogens that cause foodborne illness is standard procedure in today's food industry. Enteric viruses (Hepatitis A, Norovirus GI, and Norovirus GII) represent a significant threat to consumer health in the food industry, but testing for these viruses in food is much less common. This is in part due to the difficulty of virus testing and sampling. Enteric viruses are highly transmissible spread through the fecal-oral route and are often associated with foods that are eaten after limited or no processing. Viruses can be spread in food handling environments, by person-to-person contact or through environmental contamination. A potential environmental source of contamination is the water used in growth, harvesting, or processing of raw food materials.

Purpose: As viral particles are naturally sparse in water, one approach to aid virus testing and detection is to increase sample volumes. As such, large water volumes require a method to concentrate the viral particles before testing can be performed. The commonly used concentration methods are time intensive and cost prohibitive.

Methods: Here we compare a commonly used tangential flow cassette against two concentrating pipettes to aid in viral detection. We spiked in the following levels of known starting viral copies into 1 liter of unfiltered water: none (0), low (250), medium (2,500), and high (25,000) and concentrated the water using the various devices. After which we extracted the viral RNA and performed RT-PCR according to the standard method.

Results: Overall, the concentrating pipettes performed comparably to the tangential flow cassette, but with reduced sample preparation time of 30 minutes versus 3 hours.

Significance: This reduced sample processing time may allow for earlier viral detection, enabling appropriate measures to be taken to prevent the spread of contamination through downstream processing.

P3-190 Comparing the Efficacies of Alcohol-based Hand Sanitizers against Human Norovirus Using Two American Society for Testing and Materials (ASTM) Finger Pad Methods (E1838-10 and E1838-17)

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Introduction: Fifty-three percent of human norovirus (HNV) foodborne outbreaks are associated with poor personal hygiene of infected food handlers. The efficacy of hand hygiene products for reducing or inactivating HNV on contaminated hands is poorly characterized.

Purpose: To evaluate the efficacy of candidate alcohol-based hand sanitizers against HNV using two different fingerpad protocols, i.e., ASTM E1830-10 (traditional method) and ASTM E1838-17, the latter of which includes a rubbing step.

Methods: Deidentified stool specimens testing positive for HNV GII.4 Sydney were suspended 20% in PBS and used as inoculum. Fingerpad studies using human volunteers (n = 10 for each protocol) were done with exposure times of 30 s for Product A (benchmark, 60% ethanol) and Products B and C (commercial products containing 70% and 85% ethanol, respectively). Virus concentrations before and after exposure to the sanitizers, and including controls, were evaluated by RT-qPCR preceded by RNase treatment.

Results: Using ASTM E1838-10 (traditional fingerpad method), log reduction in genome equivalent copies (GEC) was 1.5 ± 0.4, 1.7 ± 0.4 and 3.4 ± 0.2 for Products A, B and C, respectively. For E1838-17, which included a rubbing step, reductions of 1.5 ± 0.5, 2.4 ± 0.4 and 3.2 ± 0.3 log GEC were observed for Products A, B and C, respectively. Product C was the most efficacious by both methods. Only for Product B could statistically significant differences be observed when comparing the two methods, with ASTM E1838-17 showing greater log GEC reductions ($P \leq 0.05$).

Significance: The two ASTM finger pad methods (E1838-10 and E1838-17) were comparable but did not always produce identical results. The inclusion of the rubbing action in method E1838-17 may enhance virus inactivation; alternatively, differences in elution protocols may contribute to differing results.

Poster

P3-191 Investigating the Role of Lettuce Leaf Surface Exudates on the Persistence of Human Norovirus Surrogates

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❖ ***Developing Scientist Entrant***

Introduction: Human norovirus (hNoV) is one of the major causes of outbreaks linked to leafy greens. Understanding virus persistence on pre-harvest leafy greens will help to develop effective prevention and control strategies.

Purpose: This study investigated the persistence of hNoV surrogate Tulane viruses (TV) on 40- day lettuce leaves.

Methods: Six oakleaf lettuce heads were cultivated in lab hydroponically under controlled growth conditions. At age of 40 days, 5×10^5 PFU of TV were inoculated on one leaf of each lettuce by pipetting droplets then allowing to air dry. At post-inoculation days (PID) 0, 1, 2, 3 and 4, one lettuce head was taken out and the inoculated lettuce leaf was detached. The leaf was placed in the tube containing 10 mL elution buffer (1×MEM supplemented with 2% FBS and 1% penicillium). The tubes were then shaken by hand vigorously followed by vortexing for 1 min. Afterwards, the 0.45mm filter was applied to remove bacteria and leaf tissue debris in solution. The samples were then ultra-filtered at 5000×g for 7 min to concentrate the recovered virus and detected by plaque assay.

Results: Tulane virus on 40 days lettuce leaves survived for more than 4 days. At PID 0, the recovered TV after air drying was 1.7×10^5 PFU/leaf. At PID 1 and 2 the TV reduced by 1.15 and 2 log PFU/leaf, respectively. At PID 3 and 4, the TV concentration remained stable at approximately 2 log PFU/leaf.

Significance: The persistence of TV during pre-harvest can possibly pose a risk on the harvest and even post-harvest stages. These data contribute to the understanding of virus persistence on lettuces and will ultimately inform the development of effective preventive control strategies.

P3-192 Effects of Pasteurization, Freezing and Preserving Agents on Survival of Bacteriophage MS2, a Norovirus Surrogate, in Acerola-Cherry Pulp

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Introduction: Consumption of frozen berries has been linked to norovirus illness. Acerola-cherry (*Malphigia glabra* L.) is a Brazilian fruit primarily consumed after processing. Little is known about effects of physical or chemical treatments used to preserve acerola-cherry pulp on virus survival.

Purpose: This research assessed the effects of freezing and juice preserving agents on survival of the norovirus surrogate bacteriophage MS2 in acerola-cherry pulp at conditions currently used by the Brazilian fruit processing industry.

Methods: Acerola-cherry pulp (7° Brix; pH 2.8, 5mL) was inoculated with 8.4 log PFU/mL of MS2. Samples were submitted to fast (94°C/30 s) or slow (65°C/30 min) pasteurization, freezing (-20°C, 24 h), or the addition of ascorbic acid (1 mg/mL), or citric acid (0.3 mg/mL) and stored at 5°C for 24 h. MS2 was enumerated using *Escherichia coli* C3000 as a host on tryptic soy agar (TSA) plates using the double agar overlay method. TSA plates were incubated overnight at 37°C and MS2 plaques were enumerated. Unprocessed inoculated samples were used as controls.

Results: Fast pasteurization caused a slight reduction ($P < 0.05$) of ~0.8 log PFU/mL, while addition of ascorbic or citric acid at concentrations allowed by Brazilian legislation did not affect MS2 concentration after 24 h refrigerated storage. Freezing for 24 h at -20 °C also had little effect on MS2 concentration. Slow pasteurization gave an apparent increase ~0.9 ($P < 0.05$) log PFU/mL in MS2 titer. Since MS2 does not reproduce outside its *E. coli* host, this apparent increase may be due to disaggregation of the virus particles from the initial inoculum.

Significance: These findings show that the currently used treatments applied in the processing of acerola-cherry pulp have little to no effect on MS2 concentration and suggest that norovirus might be similarly unaffected.

P3-193 Investigation of Novelty and Practicability of Pathogenic *Salmonella*-specific Phage

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❖ ***Developing Scientist Entrant***

Introduction: There has been an enormous revival of interest in searching novel phages for use as biocontrol agents. The practicability of phage is dependent on its concentration (MOIs), stability, selectivity, as well as lytic property. Since *Salmonella* is considered as one of the major foodborne pathogens, we isolated and purified *Salmonella*-specific (SM) phage from a pig for using as a biocontrol agent.

Purpose: The purpose of this study was to investigate the novelty and practicability of SM phage used as a biocontrol agent.

Methods: The gDNA of SM phage was sequenced using MGISEQ platform. Its ORFs were predicted and annotated using BLAST+. Selectivity of SM phage was determined against 10 pathogenic *Salmonella* strains and other major 31 foodborne pathogens using a dot assay. For investigation of lytic property of SM phage, overnight culture of *Salmonella* was mixed with phage at various MOIs of 0.01, 0.1, 1, 10, 100 for incubation at 37°C. At every 2-h interval, the sample was collected for bacterial enumeration. The pH and temperature stabilities of SM phage were investigated by exposing it to wide range of pHs (1 to 12) and temperatures (-70 to 70°C) for 1 h.

Results: Whole genome of SM phage consisted of 112,230 bp with 40.00% GC contents. SM phage had 121 ORFs encoding holin, host receptor binding proteins, and structural proteins. Notably, SM phage genome doesn't have genes encoding lysogenic activity, virulence, antibiotics resistance and potential allergens. SM phage exhibited lytic activity against pathogenic *Salmonella* strains only. SM phage showed significant bactericidal effect against *Salmonella* spp. in comparison with control group which was sustained up to 8 h at 37°C, even at the lowest MOI ($P < 0.05$). Furthermore, SM phage was stable under wide ranges of pHs (3 to 11) and temperatures (-10 to 60°C).

Significance: This study demonstrated the novelty and practicability of SM phage as a biocontrol agent against pathogenic *Salmonella.*

P3-196 Validation of Bench and Commercial-scale Dry Roasting Process to Reduce *Salmonella* on Hazelnuts

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Introduction: Tree nuts have been linked to multiple *Salmonella* outbreaks; therefore, processing treatments must be validated to achieve a 5-log reduction of this pathogen. A dry roasting procedure has been previously validated for almonds; however, other tree nuts have not been evaluated. Similarly, the efficacy of dry roasting at multiple production scales has not been published.

Purpose: The purpose of this study was to validate that a dry roasting process for hazelnuts could achieve a 5-log reduction of *Salmonella* at bench and commercial scales using *Enterococcus faecium* ATCC 8459 as a surrogate.

Methods: For bench-scale trials, hazelnut samples (25 g) were inoculated with a *Salmonella* cocktail and *E. faecium* (>7 log CFU/g) and then roasted (166°C, up to 8 minutes) in an air fryer toaster oven (Cuisinart Model #TOA-60). Samples for the commercial-scale trial were inoculated with *E. faecium* and roasted in the processing facility's dry roaster (maximum temperature: 166°C, up to 8 minutes). Samples were enumerated using standard dilutions and plated on Tryptic Soy Agar overlaid with Hektoen Enteric Agar or m-*Enterococcus* Agar and incubated at 37°C, 72 hours prior to enumeration.

Results: Bench-scale trials achieved a 4.26-log reduction for *Salmonella* and a 3.89-log reduction for *E. faecium* when roasted for 8 minutes. The commercial-scale roaster achieved an average of a 5.65-log reduction for *E. faecium* under typical operating conditions (8 minutes).

Significance: Bench-scale trials demonstrated that *E. faecium* ATCC 8459 was a suitable surrogate for *Salmonella* in dry roasting hazelnuts, with the *E. faecium* being consistently more resistant. Dry roasting hazelnuts at 166°C for 8 minutes achieved a 5-log reduction of *Salmonella* in a commercial-scale roaster.

P3-197 Impact of Air Velocity on the Reduction of *Salmonella* and *Enterococcus faecium* during the Dehydration of Sugar-Infused Apples

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Introduction: The majority of published thermal process studies have focused on time-temperature combinations as the primary driver for microbial inactivation. However, other processing parameters (i.e., air velocity, relative humidity) are often ignored or unreported. As companies make processing changes, such as reducing fan speed to reduce energy consumption, companies need to verify their process remains effective to achieve their food safety goals.

Purpose: Quantify the impact of air velocity on the inactivation of *Salmonella* during the dehydration of sugar-infused apples and verify the suitability of *E. faecium* ATCC 8459 as a surrogate for *Salmonella* in this matrix-process combination.

Methods: Diced apples (25 g) were inoculated with a *Salmonella* cocktail and *E. faecium* and dried in bench-scale dehydrators (43°C, 20 hours) at two different fan speeds (<0.40 m/s and 3.6 m/s). Samples were taken throughout the dehydrating process (n = 6) and spread-plated on Tryptic Soy Agar overlaid with either Hektoen Enteric Agar or m-Enterococcus Agar and incubated at 37°C, 48 to 72 hours prior to enumeration. Product and chamber temperature, relative humidity, water activity, moisture content, and pH were monitored throughout processing.

Results: The low fan speed (<0.40 m/s) achieved a 5.86-log reduction of *Salmonella* and a 5.12-log reduction of *E. faecium*, whereas the higher fan speed (3.59 m/s) only achieved a 4.22-log reduction of *Salmonella* and a 2.79-log reduction of *E. faecium*. *E. faecium* demonstrated a similar inactivation profile and was more resistant than the *Salmonella* cocktail, *E. faecium* was demonstrated to be a suitable surrogate for *Salmonella*.

Significance: This study demonstrated air velocity as a significant variable in driving the inactivation of foodborne pathogens in dehydration processes. The validation of this lower-energy process for dried, infused fruits supports sustainability efforts in the industry.

P3-198 Comparative Genomic Analysis of *Salmonella enterica* Subsp. *enterica* Serovars Montevideo and Senftenberg Isolates Associated with Pistachios

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Introduction: Pistachios have been linked to multistate salmonellosis outbreaks caused by *Salmonella* serovar Senftenberg (2013) and serovars Montevideo and Senftenberg (2016). Both serovars have been involved with multiple product recalls dating back to 2009.

Purpose: In this study, whole-genome-sequence (WGS) data from *Salmonella* Senftenberg and *Salmonella* Montevideo isolates associated with pistachio outbreaks, recalls, and investigations over a nine-year period (2009-2018) were analyzed to provide insight into evolutionary relationships and persistence among these isolates.

Methods: The data set consisted of 196 isolates (107 *Salmonella* Senftenberg and 89 *Salmonella* Montevideo) obtained from clinical, food, and environmental sources. The raw sequence data were downloaded from the Sequence Read Archive on NCBI. *In silico* multi-locus sequence typing (MLST) was performed. For each sequence type (ST), a single nucleotide polymorphism (SNP) matrix was produced using the CFSAN SNP Pipeline and maximum likelihood trees were generated.

Results: A total of four STs (Senftenberg – ST14 and ST185, Montevideo – ST316 and ST138) were identified. The SNP comparison identified 266 variants within ST14, 108 within ST185, 107 within ST316 and 48 within ST138. The trees for ST14 and ST316 show 0-31 SNPs within the pistachio clusters from 2009 to 2017. A copper homeostasis and silver resistance island (CHASRI, 32.4kb) was present in all isolates associated with these STs, suggesting an adaptation in response to extrinsic pressures, such as in the farm environment, and persistence. The trees for ST185 and ST138 have less variability (0-8 SNPs) over a five-year period (2013 to 2018), suggesting clonal resident strains from a common source for these STs.

Significance: There is evidence of persistent *Salmonella* Senftenberg and *Salmonella* Montevideo strains in pistachio environments, some of which may be clonal resident strains. Determining the mechanisms of persistence of these strains is of high importance to public health.

P3-199 The Use of a Novel Selective Supplement for the Rapid Recovery and Detection of Pathogenic Gram Negative Organisms from Challenging Food Matrices

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Introduction: Greater food safety, particularly identifying contamination of challenging spices, cocoa-based or raw beef matrices, is required. Through development of a novel supplement, selective recovery of pathogenic gram negative organisms and in turn shortened presumptive result times are achieved in these difficult matrices without impacting specificity, sensitivity and precision.

Purpose: Preferential recovery of *Salmonella* spp. and *Escherichia coli* O157:H7 from multiple enrichment broths following addition of a novel selective supplement. Detection occurs from 25 to 375 g samples through a single enrichment step with a negative or presumptive positive result within 12 to 14 or 24 h for raw beef or challenging matrices, respectively.

Methods: Un-paired matrix studies comparing test to reference protocols were carried out. Samples (25 to 375 g), either un-inoculated or artificially inoculated (1 to 158 CFU/portion) with 13 different *Salmonella* strains and an *E. coli* O157:H7 strain, were enriched with 3 to 9 parts supplemented broth for 10 to 12 or 20 to 22 hours at 41.5°C, respectively, for raw beef and challenging matrix samples. All enriched test and reference method samples were confirmed according to BAM Chapter 5 and USDA MLG 5.09. protocols.

Results: A total of 321 spices/cocoa-based and 83 raw beef samples were tested with 176/321 and 69/83 samples containing *Salmonella* and *E. coli* O157:H7 by the BAM Chapter 5 and MLG 5.09. reference methods, respectively. A total of 181/321 and 67/83 samples tested were ELISA positive with subsequent cultural confirmation and no false positive results. Probability of detection (POD) analysis between respective test and reference methods indicated no significant difference at $P < 0.05$ (-0.06 to 0.09 and -0.14 to 0.09 confidence interval of respective difference of PODs).

Significance: Development of a novel supplement that selectively recovers pathogenic gram negative organisms and shortens time to a presumptive result from challenging matrices.

P3-200 Evaluation of Oxygen Availability and Different Structured Dairy Model Systems on Growth and Inter-Strain Interactions of *L. monocytogenes*

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Introduction: Growth and interactions of different *Listeria monocytogenes* strains, present simultaneously in the same food product, are affected by the matrix.

Purpose: Evaluate the effect of oxygen availability at different dairy media on growth and inter-strain interactions of *L. monocytogenes*.

Methods: Antibiotic-resistant (for selective enumeration) *L. monocytogenes* strains of serotypes 4b(C5,ScottA), 1/2a(6179) and 1/2b(PL25), were inoculated (2.5 log CFU/ml) in single or two-strain cultures (1:1 strain-ratio), on/in Ricotta (RBr) and Camembert broth (CBr) (1 cheese:2 Ringer solution) and structured broth media (0.6% and 1.4% agar) and stored at 7°C (n = 3x2). Aerobic conditions (AC) were achieved with constant shaking or surface inoculation for liquid and solid media, respectively, while static incubation or pour plated media corresponded to hypoxic environment (HC). Anoxic conditions (AnC) were attained by adding 0.1 % w/V sodium thioglycolate and a paraffin overlay (for structured media).

Results: Inter-strain interactions, as manifested by differences in the final population of singly and co-cultured strains, seemed to be strain-dependent, with C5 and PL25 suppressing 6179 and ScottA. A significant observation was the difference in growth of the pathogen under the AC/HC (7.7-9.5 log CFU/mL) compared with growth under AnC (up to 5.5 logCFU/mL). During culture under AnC, growth was noted mainly at the Ricotta media and no interactions were observed. The extent of suppression decreased with the addition of agar, suggesting that inter-strain interactions were significant in dairy broths. Under AC/HC, in RBr, 6179 was suppressed by C5, at least 3 log CFU/mL (HC), compared to the corresponding single culture, which attained population of *ca*. 7.5 log CFU/mL. In CBr, the suppression during co-culture was up to 2.1 (AC), compared to the singly-cultured 6179 (8.8 log CFU/mL) ($P < 0.05$). The growth of ScottA was inhibited both by C5 and PL25. The inhibition of ScottA by PL25, ranged from 1.1 (RBr) to 2.1 log CFU/mL (CBr), regardless of oxygen availability. Contrarily, the inhibition of ScottA, during co-cultivation with C5, was more significant in the RBr than in the CBr.

Significance: Investigating growth interactions in different environments could assist in explaining the dominance of certain serotypes in foods of safety concern for *L. monocytogenes*.

P3-201 Novel Assay for *Staphylococcus aureus* in Nutraceuticals Using Rapid Automated Detection System

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Introduction: Microbiological procedures to evaluate nutritional and dietary supplements <2022> for objectional microorganisms of concern was established by the United States Pharmacopeia (USP). An improved rapid method has been developed to detect the presence of *Staphylococcus aureus* based upon real-time monitoring of color changes due to microbial metabolism in a novel broth/agar combination vial and accompanying reader.

Purpose: Validate the performance of the Soleris vial method against the USP (Chapter <1223>) reference method for rapid detection of *S. aureus* in nutraceuticals.

Methods: The *S. aureus* vials (S2-SA) were assessed as paired samples by Soleris and USP methods for specificity and matrix equivalency. Specificity tested 25 inclusive and 25 exclusive organisms. Twenty-six nutraceutical products were evaluated for the detection of *S. aureus* in a set of uninoculated products and with a low-level inoculum of 5-10 CFU/g. Repeatability was evaluated with 4 matrices, 20 replicates each. Robustness was evaluated on effect of instrument temperature, sample size, and media volume.

Results: Specificity was 100% for inclusive and 92% for exclusive organisms. Equivalency tests showed all 26 uninoculated samples were negative for *S. aureus* by both methods (Kappa index = 1). For low-level inoculated samples, 26/26 samples detected positive with new rapid method and 20/26 were positive by the reference method, indicating better or equivalent assay performance. Repeatability results showed <8% coefficient of variation. Robustness test results obtained $P > 0.05$ for all categories assayed. The study demonstrates that Soleris S2-SA offers a rapid and effective method to detect *S. aureus* in a variety of nutraceutical products.

Significance: A novel Soleris method for the detection of *S. aureus* in nutraceuticals was validated and showed statistically comparable performance against the relevant USP reference method. The rapid method provided results in 22 hours following primary enrichment, compared to 5-7 days by the USP reference method.

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