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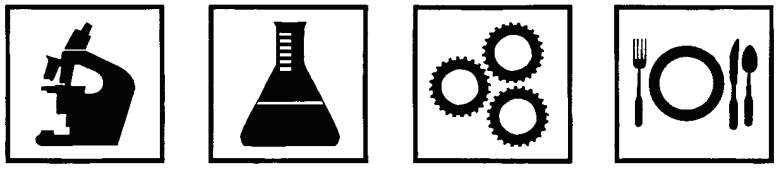

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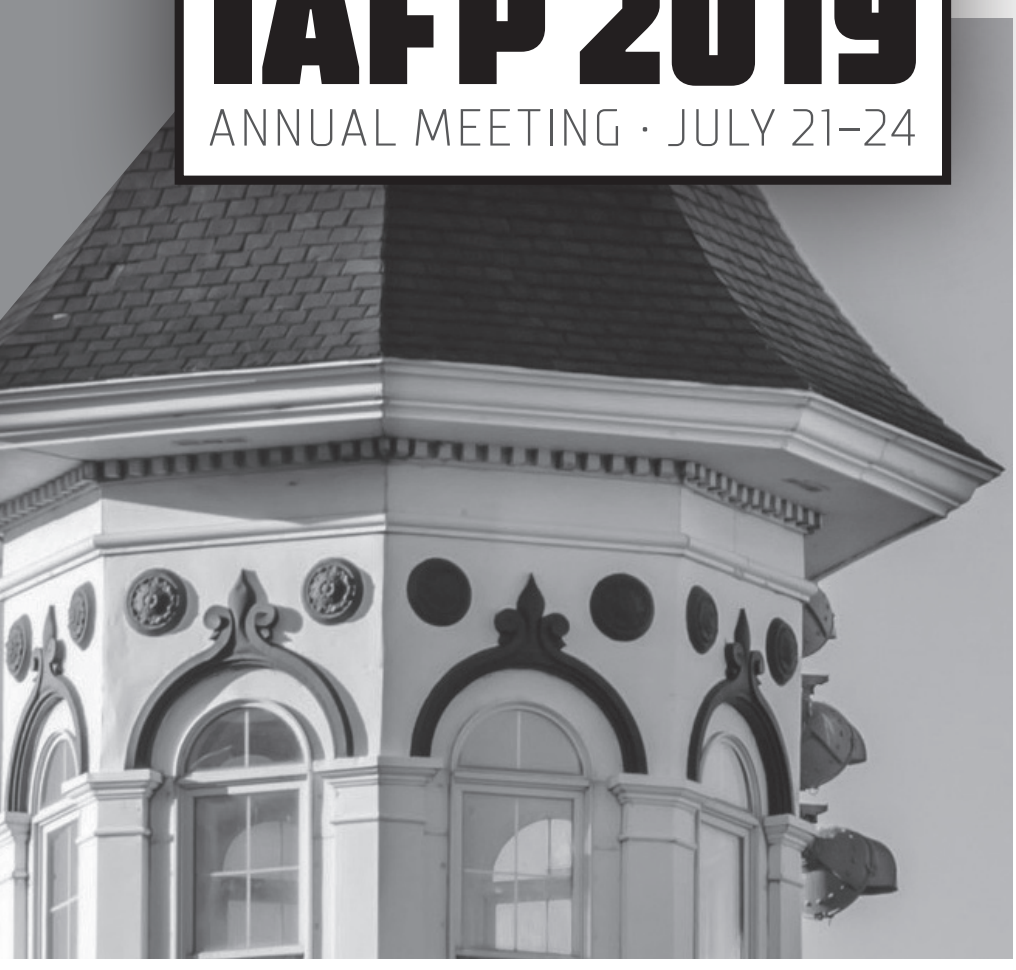

ABSTRACTS

This is a collection of the abstracts from IAFP 2019, held in Louisville, Kentucky

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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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IVAN PARKIN LECTURE ABSTRACT

The Power of Play: Using Media to Educate Our Stakeholders

Barbara Chamberlin, Ph.D.
Professor
New Mexico State University
Las Cruces, New Mexico, USA

Through our research, discoveries and sharing of knowledge, our ultimate goal is usually to change the behavior of consumers, growers, and other educators. The Learning Games Lab at New Mexico State University works with content experts at universities and organizations throughout the nation to develop educational games, animations, videos and interactive labs. Their tools, almost all of which are available free of charge, are developed through a specific development process which is designed to change our clientele.

Dr. Chamberlin will share highlights of the different projects they've created, including:

Ninja Kitchen (game)
ninjakitchengame.org
Speed is important, but in this kitchen, safety comes first. Stop for lunch at a cafe staffed entirely by ninjas.
Sophisticated gameplay reveals principles of food safety in this diner game created for kids.

Potluck Panic (game)
potluckpanic.nmsu.edu
Correct unsafe food preparations before the food is consumed by your friends! Potluck Panic is an interactive game for college students that educates players on safe food-handling procedures, from the factory to the kitchen.

Virtual Labs
myfoodsciencelab.org
A collection of eight interactive web modules (Adobe Flash), and eight iPad apps. Students perform common food science lab procedures step by step in a virtual laboratory.

Don't Wash Your Chicken!
dontwashyourchicken.org
Videos, animation, recipes, and printable fotonovelas reinforce the importance of not washing raw poultry.

Don't Be Gross
dontbegross.org
These short, shareable animations convey the importance of hand washing and other health issues.

Produce Safety Matters
producesafetymatters.org
Designed for extension training and outreach, growers, packers, and retailers learn tips to prevent contamination from farmer's field to consumer's fork in these crisp animations.

The Transformational Design Model is an educational design model based on five key ways to change people: their knowledge (what they know), skill (what they can do), behavior (how they act), emotion, (how they feel) and physiology (how they are). When educational designers start the specific ways in which they want a learner to change, the next step is to design the activities that will lead to that change.

Activity design is more complex, as there are hundreds of ways to learn, experience, develop and grow; such as, receiving information, failing, observing, planning, communicating, thinking, and solving problems. This range of activities includes moving a learner from activities that provide simple exposition, through different types of activities to more inquiry-based learning. This range of activities is helpful in guiding designers through a learning experience. The range provided doesn't offer a continuum of good through bad; rather, it is designed to help developers think through the needs of the player. Sometimes simple exposure to knowledge is useful, when other kinds of learning and change demands reflection, creative activity and building, or learner-centered project development.

Additional resources developed by the Learning Games Lab are available at learninggameslab.org.

JOHN H. SILLIKER LECTURE ABSTRACT

From Outbreak Catastrophes to Clades of Concern, How Whole Genome Sequencing Can Change the Food Safety Landscape

Robert V. Tauxe, MD, MPH
Director
Division of Foodborne, Waterborne and Environmental Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia, USA

Public health plays an important role in food safety. In the changing landscape of foods, tastes and processes, pathogens can find a niche, persist and emerge. Public health surveillance and investigations can identify problems and help target solutions to prevent foodborne illnesses. The tools public health uses for surveillance have also been evolving. Better microbiological methods improve definition of individual strains, separating "signal" from "noise." These improvements mean finding more outbreaks, helping to drive immediate control efforts and longer term prevention policies.

The transition to whole genome sequencing is now underway in our public health surveillance network PulseNet. These new tools already provide better strain resolution and new ways of looking at food safety problems. Whole genome sequencing differs in several important ways from the standard PFGE subtyping PulseNet used for the past 23 years. Resolving differences down to single nucleotides provides a scale of similarity that can be as precise as needed. From sequence, other strain char- acteristics can be predicted including serotype, antibiotic-resistance profile, and virulence. This is changing the workflow in our public health labs, so more characteristics are known when a cluster of related infections is detected. Unlike the previous closed PFGE database of PulseNet, the sequence database is open access. As public health scientists and partners at FDA and USDA will add ~60,000 bacterial sequences a year, the database will be a rich source for future research.

We anticipate that as sequencing is applied to surveillance, investigation of the many smaller outbreaks detected should find more specific control points and guide prevention, including harborage in processing, reservoirs in production and new sources from other countries. As we find even smaller outbreaks, the line blurs between traditional outbreaks and the background of individual "sporadic" cases.

Beyond the traditional role of helping public health find and stop outbreaks, this new surveillance system can do much more to prevent illnesses.

- We can more easily track "clades of concern," investigating them even in the absence of an outbreak. For example, we can track strains with greater confidence that caused major repeated outbreaks in the past, are still present at lower incidence now, and could yet cause future outbreaks. We can see other strains that emerge, increase over time, and may be investigated and controlled before they cause a large traditional outbreak, preventing more foodborne infections.
- Other countries are rapidly adopting similar surveillance strategies. Canada, the European Union, and Australia are in the vanguard with the U.S., and many more are starting soon. By comparing sequences across borders, all can better understand the spread of pathogens through travel and trade.
- As tools for interpreting sequences become more accessible, many in food science will find tracking specific strains useful to examine the ecology of bacterial pathogens in food production and processing. By comparing them with strains in the public database, internal control efforts can be focused.
- It will be possible to use more genetic markers for virulence, persistence, or adaptation to specific reservoirs and hosts. The potential to understand better the biology of these bacteria is growing rapidly.
- The next transition, building on sequencing experience, will someday bypass traditional culture and go directly to meta- genomic analyses to construct genomes directly from specimens. We stand at a threshold in microbial food safety, with the opportunity to accelerate research, investigation and prevention. I hope to learn and relearn much more, together with all of you.

Symposium Abstracts

S1 Tracking FSMA Quantitative and Qualitative Impacts on the Food Industry Under Full FDA Enforcement – Stats, Trends, Challenges and Lessons Learned

GLENN BASS: *U.S. Food & Drug Administration, White Oak, MD, USA*
KARLEIGH BACON: *The Kraft Heinz Company, Chicago, IL, USA*
GREGORY PRITCHARD: *Nestlé USA, Glendale, CA, USA*
ALLEN SAYLER: *EAS Consulting Group, Alexandria, VA, USA*
PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
ELIZABETH FAWELL: *Hogan Lovells, Washington, DC, USA*

The FDA Food Safety Modernization Act (FSMA) was designed to significantly expand the enforcement tools available to the U.S. Food & Drug Administration. The primary intent was to shift both FDA and the food manufacturing and processing focus from reactionary after a contamination event occurred to preventive by emphasizing known approaches, systems and technology that together will likely reduce the incidents of food contamination during manufacturing, packaging and distribution. The two most impactful of the FSMA regulations for most food manufacturers were the "Preventive Controls for Human Foods (PCHF)" and the "Foreign Supplier Verification Program (FSVP)" which were published in final form in September of 2015 and late November of 2016. The PCHF has reached full implementation and enforcement by the FDA, with FSVP not far behind.

This full symposium will review published and unpublished data on the number of FDA FSMA enforcement inspections, the number of 483s, U.S. Customs food rejection and hold data, identify the data for each major food sector and then compare it to FDA recall data as well as CDC illness outbreak data, both historically as well as current to see if there are any trends to identify if FSMA is having a positive impact on food safety in the U.S.

In addition to the quantitative data, speakers will be providing qualitative date case studies on the actual content of the FDA FSMA-based 483s as well as private food manufacturer's own internal auditing summaries to share specific issues that are being identified by FDA and internally by food companies as needed more attention in this the "FSMA Era" of food safety.

Finally, there will be an assessment of whether the efforts of the FDA-supported Food Safety Preventive Controls Alliance (FSPCA) and the Produce Safety Alliance (PSA) to improve the knowledge and skills at all levels in the food processing industry has been effective.

S2 Seek and You Shall Find: The Intricacies of a Robust *Listeria* Environmental Monitoring Plan

JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
LINDSAY WARD-GOKHALE: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*
JOHN DONAGHY: *Nestec Ltd., Vevey, Switzerland*

Listeria is a ubiquitous microorganism and for many food establishments, it is an inescapable food safety matter. This symposium will highlight the components of a strong and effective EMP with special considerations for *Listeria*. Focus topics will include FDA and USDA guidance documents, pathogen harborage points, sampling devices and methodology, detection methods, implementation of preventive controls, and corrective actions. The symposium will begin with an introduction and overview of the current FDA guidance for ready-to-eat foods and prerequisite programs necessary for *Listeria* control. The USDA will then provide insights into the FSIS and their experiences with risk assessment of *Listeria*. An industry expert will subsequently share their perspectives and engage the audience with case studies, lessons learned, and personal insights. Novel technologies for monitoring *Listeria*, including whole genome sequencing and agent-based methods, will be provided. The symposium will conclude with a presentation on current data gaps and research needs for *Listeria*. The overall goal of this symposium is to outline the current FDA and USDA guidance and examine the ideology of a robust EMP for *Listeria* with viewpoints from these government agencies and industry.

S3 Tracing Produce: Where We are and What's Next?

ED TREACY: *PMA, Newark, DE, USA*
KATHERINE VIERK: *U.S. Food and Drug Administration, College Park, MD, USA*
TEJAS BHATT: *Walmart, Bentonville, AR, USA*

After nationwide outbreaks of *E. coli* O157: H7 and *Salmonella* spp. linked to produce items, many questions exist on how to improve traceability of produce from farm to fork and fork to farm. This session will provide information on current industry initiatives underway to address produce traceability, discuss the status of regulations impacting traceability, and introduce technological advances that are making an impact.

S4 Water Management in Food Manufacturing: Be Prepared for Problems

MIEKE UYTTENDAELE: *Laboratory of Food Microbiology and Food Preservation, Ghent University, Ghent, Belgium*
PHYLLIS POSY: *Strategic Services & Regulatory Affairs Atlantium Technologies, Har Tuv Industrial Park, Israel*
ANETT WINKLER: *Cargill, Inc., Munich, Germany*

Food and Beverage industries around the Globe differ enormously in their size, products and capabilities. However, there is a common dependence on water, especially on the water that is safe and suitable for use in a particular operation. Also in common are many challenges in water management, partially driven by climate/weather changes (e.g., hotter summers in moderate climates). The symposium will look at choosing/managing incoming water supplies, how to match water sourcing with treatments that are suitable for which hazards, as well as following the water stream in the manufacturing to ensure adequate quality at the point of use. The symposium would start setting the scene by discussing recent food safety issues where water was involved and how to identify water hazards in overall hazard analysis. Different Water treatment technologies will be outlined and practical approaches to delivering water suitable for use presented. The last segment focuses on maintaining microbial integrity within a plant providing practical insights into storage and distribution challenges and water testing regimes.

S5 Does Zero Risk Really Exist: How to Communicate Variability and Uncertainty to Government and Industry Managers

LAURENT GUILLIER: *ANSES, Laboratory for Food Safety, University of Paris-Est, Maisons-Alfort, France*
LEON GORRIS: *Food Safety Expert, Nijmegen, Netherlands*
KRIS DE SMET: *European Commission, Gent, Belgium*

Science-based risk assessments are the standard tool to ensure food safety. The sources of uncertainty and variability in the available information and parameters used in the risk assessment should be fully characterized and documented in order to provide a more complete account for risk. Hence, communicating the output of such assessments to decision makers from the government and the industry can be challenging especially in the absence of a pre-defined acceptable risk level. The development of suitable approaches and guidelines is crucial to facilitate risk managers to incorporate properly uncertainty in the making decision process thus enhancing the choice of more effective mitigation strategies.

The purpose of this symposium is to give the audience:

• an introduction to the concepts of variability and uncertainty, how to differentiate and use them to ensure food safety

• a presentation on the industry challenges to take a decision in a variable and an uncertain world

• a presentation on how variability and uncertainty are communicated by risk managers and how they are taken into account in regulation

Intended audience: Risk managers and assessors in food safety authorities and food industry

S6 Impact of Robotics and Artificial Intelligence on Food Safety

ALAN CHAN: *Alibaba Inc., Hangzhou, China*
MIKE HARPER: *Soft Robotics, Bedford, MA, USA*
IAN JENSON: *Meat & Livestock Australia, North Sydney, Australia*

Robotization, artificial intelligence (AI) and Machine Learning (ML) are rapidly developing and revolutionizing the agriculture and food industry, from optimizing production and product innovation to tailoring product design and addressing consumer preference. In September 2017, the world's first entirely machine-operated crop was harvested without a human ever entering the field where it was produced. However, while robots typically do what they are told, the intelligence, learning capacity and informed decision making are coming from the computers running robots and those that program the computers. While speed, efficiency and non-stop working may be benefits, the question is whether there are any new governance or operational risks to consider. So, what is happening in the food industry at this point, do we gain experience and do we learn about managing possible risks?

Food and beverage companies are typically among the least prepared to exploit the power of AI and ML, essentially because they lack relevant data. Nevertheless, a few amongst the industry peers are starting to move. In the coming years, new machines making independent decisions will increasingly produce and process our food. In order to avoid having machines contributing to food safety risk as humans are regularly doing now, it will be critical that food safety professionals contribute and participate in the development of these intelligent machines and robots to ensure that food hygiene and food safety concepts are built into the algorithms of these machines. Robotization has the potential to dramatically improving food safety if properly designed. Otherwise, we may find ourselves years from now, having to train robots and machines in food hygiene and food safety.

The short symposium or RT will bring together a few experts in this field to 1) give an update of how far AI and robotics have developed in primary food production and in food processing; 2) the existing (or absence of) consideration of food safety concepts in the design of these machines and 3) current or planned initiatives on the regulatory and standard-setting scene to accompany this revolution. Speakers will be asked to contribute on topics such as: optimizing safe food designs using artificial intelligence; Artificial intelligence impact on food manufacturing operations and food safety assurance and; challenges in embracing artificial intelligence for food safety.

S7 New Methods in Analytical and Bioanalytical Sensing for Food Safety and Quality

LILI HE: *University of Massachusetts, Amherst, MA, USA*
EMMA FARQUHARSON: *Cornell University, Ithaca, NY, USA*
JOEY TALBERT: *Iowa State University, Ames, IA, USA*

The growing demand for new bioanalytical methods to rapidly detect food contaminants has led to several promising techniques. These methods could soon allow portable sensing for point-of-use analysis. The *International Association of Environmental Analytical Chemistry* (IAEAC) is co-sponsoring a symposium to discuss some of the innovative research designed toward improving food safety. We will hear from several early stage investigators who will cover emerging methods toward the early detection of microbial and chemical contaminants. These methods include Surface Enhanced Raman Spectroscopy (SERS), synthetic biology, enzyme engineering, and phage engineering. Audience members will gain a background as well as specific knowledge of what lies on the horizon for rapid methods.

S8 Ensuring Safety by Design: Connecting the Dots of Food Protection throughout the Farm-to-Fork Continuum – A Poultry Case Study

WILLIAM CHANEY: *Diamond V, Cedar Rapids, IA, USA*
JERRI LYNN PICKETT: *WBA Analytical Laboratories, Springdale, AR, USA*
STEPHANIE POLLARD: *Clear Labs Inc., Menlo Park, CA, USA*

Food systems are comprised of many interconnecting and moving parts, which can quickly become very complex and overwhelming when considering that food producers are ultimately responsible for ensuring the safety of their products. With the shift from a reactive approach to food safety issues to more of a preventative approach sparked by the new Food Safety Modernization Act, companies are being challenged to ensure food safety by design from farm to fork. This symposium seeks to connect the food safety dots and considerations between suppliers, food producers, pathogen diagnostic developers, and regulators with an emphasis on the poultry industry. Experts in these respective areas will share their knowledge on topics such as purposeful design of food safety in the live bird pre-harvest environment, product formulation, integrating food safety hurdles in manufacturing, product sampling and testing strategies, challenges and considerations for pathogen detection in challenging matrices, and a regulatory perspective on pathogen reduction in the poultry industry.

S9 Making Sense of Food Allergen Analysis

RAKHI PANDA: *U.S. Food and Drug Administration, College Park, MD, USA*
JUPITER YEUNG: *Nestlé, Fremont, MI, USA*
JOSEPH BAUMERT: *University of Nebraska-Lincoln, Lincoln, NE, USA*

Food allergens are critical food safety hazards and must be considered in the development of a food safety plan. Robust allergen control programs frequently include the implementation of cross-functional allergen controls to prevent the presence of undeclared allergens in products. Many manufacturers and auditors look to data from allergen testing to provide evidence of allergen control effectiveness. Analytical methods are also important tools for the evaluation of the quantity of undeclared allergens present in cases of control failure. Numerous methods, including commercial kits and proprietary assays, are available for the detection and quantification of food allergens, but a substantial amount of confusion remains about choosing the right method for a particular application and interpreting the results of different types of methods. The objective of this symposium is to give attendees a fundamental understanding of how food allergen methods work, why different methods and laboratories may deliver different results, how to incorporate analytical methods into an allergen control program, and what to do with positive quantitative results.

S10 *Listeria monocytogenes* and the Produce Industry: Best Practices for Sanitary Design, Control and Monitoring

JENNIFER MCENTIRE: *United Fresh, Washington, DC, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*
ROBERT DONOFRIO: *Neogen Corporation, Lansing, MI, USA*

This session will focus on the impact of *Listeria monocytogenes* on the produce industry and the associated control measures and monitoring approaches that are currently being applied or are in development. A review of the current regulatory requirements will be covered. Key industry associations will discuss how they are collaborating to leverage industry/academic expertise to translate this science into tools people can use, and have a positive impact on pathogen control. The efficacy of various sanitation approaches and sanitary design on *L. monocytogenes* control will be discussed. Approaches for validating and verifying the performance and claims of methods for monitoring and detection of *L. monocytogenes* and *Listeria spp* will be presented.

S11 Why are We Still Having Food Safety Failures If We All Have Food Safety Systems?

GALE PRINCE: *Sage Food Consulting, Cincinnati, OH, USA*
SALLY CROWLEY: *Cargill, Inc., Hopkins, MN, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

Despite the availability and adoption of preventive food safety systems there continue to be failures resulting in food recalls and disease outbreaks. While there is a tremendous diversity of human food in the global food supply, history teaches that there are root cause themes that emerge from a retrospective view of these food safety failures. These root causes include failure to adequately identify potential hazards and implement the preventive controls, failure to maintain hygienic conditions within the manufacturing facility and failure to identify and track food allergens including ingredient suppliers, manufacturing and labeling. The advances in hazard detection and disease attribution remind us that there are some foods in which there are no definitive preventive controls that will eliminate consumer risk illustrating the importance of investments in research that will help food safety professionals better understand the hazard ecology of our food supply and technologies to reduce or eliminate these hazards.

S12 Water Re-use in the Food Processing Industry: Risk-based Approaches in Practice

KANG ZHOU: *FAO, Rome, Italy*
LEON GORRIS: *Food Safety Expert, Nijmegen, Netherlands*
PHYLLIS POSY: *Strategic Services & Regulatory Affairs Atlantium Technologies, Har Tuv Industrial Park, Israel*
ANA ALLENDE: *CEBAS-CSIC, Murcia, Spain*

While access to reliable sources of potable water varies dramatically around the globe and such sources are often scarce already, global trends including food security and global warming increasingly exacerbate water supply shortages to the extent that water security is under threat. Among the possible other sources of water available to the food industry could be water that is recovered from food or from 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 effectively. There is of yet little science and operational best practices for responsible water re-use in key segments of the food industry. When deciding whether a re-use water source can be utilized for a specific food application and whether a treatment or other type of reconditioning is required to make this water fit-for-purpose, the key criterion is that the re-use water does not pose a health risk to consumers. A risk-based approach needs to drive matching re-use water sources/treatments to possible applications, but what does it look like operationally? Risks need to be carefully assessed and managed in the context of the specific food facility, and risk-mitigation measures must be managed within that facility's food safety management system (e.g., GHP/HACCP). As more food industry sectors in more countries develop or improve water re-use solutions, and more data become available, there is an opportunity to reduce the learning curve by sharing practical experiences. This symposium brings together regulatory and industry expertise from around the world to share practical lessons learned in developing and implementing risk-based operational guidelines.

S13 Artificial Intelligence and Machine Learning: What They are and Their Potential Applications for Food Safety

HENK DEN BAKKER: *Center for Food Safety, University of Georgia, Griffin, GA, USA*
WENDY WHITE: *Georgia Tech, Greensboro, GA, USA*
ABIGAIL HORN: *Center for Applied Network Analysis, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*

While the term was first coined by a computer scientist in 1956, the field of artificial intelligence (AI) has been expanding in the last few years due to improvements in processing power and data storage capacity, increasing data availability, and rapid progress of analytical techniques. AI is a broad term and encompasses many different subfields (e.g., machine learning, natural language processing, and robotics). There is a great deal of excitement surrounding AI across public and private sectors and it is has already been transforming a range of industries such as healthcare, finance, and retail. AI is just starting to be adopted by some organizations in the food industry (in areas such as automation of processing and supply chain optimization) but is not widely utilized. Recently, there have been efforts to adopt AI technologies in the area of food safety, yet there is a great deal of unlocked potential. For example, in the last year many organizations have been exploring blockchain technology (e.g., IBM Food Trust), digitally tracking products from farm to consumer – this presents an opportunity for companies to then apply AI technologies to improve food safety. It is time to discuss how AI can be used

to improve food safety. The overall goal of this symposium is to make AI accessible across audiences, in order to foster discussion and encourage the application of AI to drive the food industry towards a better future. Thus, this symposium seeks to (i) define artificial intelligence and provide a basic understanding and framework for discussion, (ii) discuss case-studies representing diverse applications, and (iii) present opportunities and challenges of AI in the food industry.

S14 Food Microbiome Transfer Dynamics from Farm to Processing – What Can Metagenomics Add to the Picture?

CHRISTOPHER GRIM: *U.S. Food and Drug Administration – CFSAN, Laurel, MD, USA*
STEVEN RICKE: *University of Arkansas, Fayetteville, AR, USA*
KEITH BELK: *Colorado State University, Department of Animal Sciences,, Fort Collins, CO, USA*

The Human and American Gut Microbiome projects have provided critical information about the impact of human gut microflora on health and disease. These projects have provided the framework for studying the influence of microbiomes associated with human and animal foods and their production environments on food safety and quality. Metagenomic sequencing of foods and food environments can provide microbial community profiles and identify genetic attributes such as antimicrobial resistance, pathogen serotypes, and virulence genes. For example, recent studies describing bovine fecal microbiota composition and beef cattle production facilities have revealed potential associations between pathogen transfer and proliferation from farm to animal and throughout the production process. This information could facilitate an understanding of the ecology of microbial communities in each habitat as well as transport of pathogens and antimicrobial resistance genes between foods and food environments. The goal of this symposium is to describe current microbiome research projects focused on pathogen presence and persistence in a variety of farm environments (produce, beef, and poultry) with particular attention to microbiome transfer dynamics in these food processing sectors. We will provide an overview of microbial community profiles in foods and farm environments and describe microbiome shifts induced by preventive controls employed at meat and poultry processing facilities to control foodborne pathogens and spread of antimicrobial resistance genes.

Presenters will discuss the use of microbiome profiling to identify potential indicators of pathogen presence, persistence and transfer on produce farms and across the meat and poultry supply chains. Together, these talks will provide information about the risks associated with the dissemination of antimicrobial resistance and foodborne pathogens imposed by farming and production practices.

S15 Science and Regulatory Guidance Update: Lethality and Stabilization of Meat and Poultry Products

SUSAN HAMMONS: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*
BRADLEY MARKS: *Michigan State University, East Lansing, MI, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*

In 2017, the United State Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) released updated versions of the commonly used lethality and stabilization guidelines and safe harbors known as Appendices A and B. Appendices A and B are used by numerous meat and poultry processors both large and very small. The changes from the previous 1999 versions to the 2017 versions identified opportunities for additional research and increased guidance for the control of *Salmonella*, *C. perfringens*, *C. botulinum* and more. After the release of the 2017 versions of Appendices A and B, both industry and government also identified components that proved to be problematic for the industry to implement and an opportunity for improvement. Shortly after their release both USDA (FSIS and Agricultural Research Service) and industry have commissioned numerous efforts targeting the gaps in the current scientific body of knowledge. The theme of this symposium is for stakeholders in academia, industry and government involved in the research, production and oversight of the production of meat and poultry products that are heat-treated and cooled. The purpose of this symposium is to update the scientific community on the ongoing and soon to be completed research and guidance targeting *Salmonella*, *C. perfringens*, and *C. botulinum* in heat-treated meat and poultry products.

S16 May Contain Allergens – A Risk-based Approach for Determining the Use of Precautionary Allergen Labelling (PAL)

JOSEPH BAUMERT: *University of Nebraska-Lincoln, Lincoln, NE, USA*
BRENT KOBIELUSH: *Cargill, Inc., Wayzata, MN, USA*
DAVID CLIFFORD: *Nestlé USA, Inc., Dublin, OH, USA*

The use of Precautionary Allergen Labeling (PAL) statements such as May Contain, Made on Shared Equipment or Contains Traces on food labels is a source of confusion and frustration for many allergic consumers and the clinicians that treat them. This symposium begins with a discussion on the potential sources of allergen cross-contact in the supply chain (agricultural comingling, distribution, storage) and the potential risk this scenario presents. The second presenter will focus on the cleaning of manufacturing equipment with a specific focus on allergen cleaning validations in wet and dry clean protocols as a means to minimize the food safety risk associated with allergen cross contact. Lastly, a speaker will present on allergen thresholds and their application when using VITAL (Voluntary Incidental Trace Allergen Labeling) to determine if PAL is warranted based on risk.

S17 Managing Large Multidisciplinary/Multi-Institutional Food Safety Projects – Effectively, Impactfully, and with Integrity

DENIS GRAY: *North Carolina State University, Raleigh, NC, USA*
KIMBERLY COOK: *USDA ARS, Beltsville, MD, USA*
LEE-ANN JAYKUS: *Department of Food, Bioprocessing, and Nutritional Sciences, North Carolina State University, Raleigh, NC, USA*
EDITH WILKIN: *Leprino Foods, Denver, CO, USA*
LINDA J. HARRIS: *University of California-Davis, Department of Food Science and Technology, Davis, CA, USA*

Food safety is a complex and multidisciplinary challenge. Therefore, federally funded food safety projects, and even industry-centered projects, increasingly involve large, multidisciplinary/multi-institutional collaborative teams. However, very few individuals thrust into these roles have formal education or training in managing such projects. This symposium brings together a unique and diverse cohort of presenters, ranging from an expert on assessing the effectiveness and impact of research collaborations and centers (with experience on multiple food safety project teams) to experienced managers of such projects (in government, academic, and industry) to a representative of the Scientific Integrity Consortium. The speakers will describe measures for evaluating the effectiveness of such large-scale collaborations, identify common features of successful collaborations, share best practices for forming and managing such teams, and outline essential foundational principles for ensuring the quality and integrity of the resulting research. A panel discussion is included to maximize opportunities for attendee interaction with the multiple perspectives provided by the speakers. After this session, attendees will have a better appreciation of how to play together well in the research sandbox.

S18 Is Cell Cultured Meat *Really* Meat?

PAUL MOZDZIAK: *North Carolina State University, Raleigh, NC, USA*
ROBERTA WAGNER: *U.S. Department of Agriculture – Food Safety and Inspection Service, Washington, DC, USA*
JEREMIAH FASANO: *Food and Drug Administration Center for Food Safety and Applied Nutrition, College Park, MD, USA*

November 16, 2018, a joint statement was issued by FDA Commissioner Gottlieb and USDA Secretary Perdue on the regulation of cell-cultured food products from cell lines of livestock and poultry. In October, the FDA and USDA met regarding the regulations needed to "both foster these innovative food products and maintain the highest standards of public health." The two agencies determined that "the USDA and the FDA should jointly oversee the production of cell-cultured food products derived from livestock and poultry. Drawing on the expertise of both USDA and FDA, the agencies are today announcing agreement on a joint regulatory framework wherein the FDA oversees cell collection, cell banks, and cell growth and differentiation. A transition from FDA to USDA oversight will occur during the cell harvest stage. USDA will then oversee the production and labeling of food products derived from the cells of livestock and poultry…USDA and FDA are confident that this regulatory framework can be successfully implemented and assure the safety of these products."

Biotechnology is making possible meat production through non-traditional means. This symposium was designed to provide baseline knowledge to attendees regarding what cell-cultured meat is, an overview of animal-derived cell culture technology, related food safety concerns particular to these new methods of production, and information regarding regulation and oversight of "clean meat" facilities which produce varieties of beef, chicken, pork, and fish using this new technology. These products present certain regulatory challenges, which will be discussed, as they are not derived from an animal carcass, and will require some adjustments to statutory and regulatory definitions.

What is It? Where is It From?

How are cells harvested from animals and how is meat grown from those cells? A clean meat facility is like a clean, fermentation-based food processing plant and allows meat to be harvested from cells rather than from raising animals to provide animal products to our growing population.

Is there a strategy for regulating these novel food products? What role will USDA-FSIS play?

As a first step toward addressing how Federal regulatory agencies will assure the safety and accurate labeling of human food produced using animal cell culture technology and the inspection of establishments involved in the production of these products, the U.S. Department of Health and Human Services Food and Drug Administration and the U.S. Department of Agriculture Office of Food Safety entered into a formal agreement on March 7, 2019. This formal agreement describes the roles of DHHS-FDA and USDA's Food Safety and Inspection Service (FSIS) with respect to the regulatory oversight of these products. This presentation will include a discussion of the aforementioned formal agreement and next steps including the formation of several cross organizational workgroups. In addition, this presentation will include a brief discussion on USDA-FSIS plans for applying the food safety and labeling provisions of the Federal Meat Inspection Act and the Poultry Products Inspection Act to cell-cultured food products.

Is there a strategy for regulating these novel food products? What role will FDA play?

FDA is responsible for implementing and enforcing the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, and the Fair Packaging and Labeling Act. FSIS is responsible for implementing and enforcing the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act. Each agency has an important role in the oversight of human food derived from cell lines of USDA-amenable species and required to bear a USDA mark of inspection. A previous talk described the joint FDA/USDA agreement outlining the roles and responsibilities for oversight of such food as well as FSIS's plans for application of their statutory authorities under the agreement. This talk will discuss FDA's statutory authorities, prior relevant experience in multiple regulatory domains, and considerations informing the development of the premarket assessment process and postmarket oversight of cell culture described in the joint agreement.

S19 Beyond Slide Decks and Classrooms: Novel Approaches to Food Safety Learning

ANDY YEOMAN: *Focus Games, Glasgow, United Kingdom*
CAROL LEAMAN: *Axonify Inc., Waterloo, ON, Canada*
AUSTIN WELCH: *Sage Media, Thornton, CO, USA*
MEGAN KENJORA: *The Hershey Company, Hershey, PA, USA*

The old-style lecture using slide decks is a boring approach to food safety training. Adding videos, in-class discussions and clicker response systems can reduce monotony and increase learner interest and engagement, yet there's so much more that can improve learning and, ultimately, food safety culture. Novel approaches are being used in the food industry to enhance learning, interest and engagement at different levels. This symposium will raise awareness about three of these approaches.

Board games which have been used for millennia to pass the time have been developed and used to transfer and reinforce food safety messages while encouraging interaction with team members during training sessions and breaks. Companies are also changing the dynamics of learning by including micro-learning approaches, often in combination with internal competition or online games, to improve engagement of associates and positive food safety outcomes. Micro-learning builds on evidence from neuroscience that shows knowledge retention increases when information is presented in short targeted training, online or off-line, which addresses different learning styles. Employee behavior changes when learning is effectively reinforced within the learning platform and on-the-job.

Of course, senior leaders have different mindsets and needs for food safety information so learning approaches must engage their brains in a way that works for them. Furthermore, new senior leaders coming from outside the food industry often have limited understanding of food safety risks. The ground-breaking use of escape rooms, investigative approaches and other experiential learning techniques are building essential knowledge and tapping into critical thinking skills to change behavior, thereby protecting brands and consumers.

Different options are available to the food industry to improve learning outcomes and change behavior to ultimately strengthen food safety culture. Presenters in this symposium will spark ideas about the potential application of novel approaches in workplaces to advance food safety practices and culture.

S20 International Food Defense Preparation for FSMA and Beyond

AMY KIRCHER: *Food Protection and Defense Institute, University of Minnesota, St. Paul, MN, USA*
RYAN NEWKIRK: *U.S. Food and Drug Administration, College Park, MD, USA*
KARLEIGH BACON: *Kraft Heinz Company, Glenview, IL, USA*

The first FSMA Intentional Adulteration Rule compliance date is July 26, 2019. With this deadline fast approaching, the food industry is working to prepare food defense programs that comply with the regulation and that protect the public from the threat of intentional adulteration. The food system is a globally connected supply chain where a disturbance at one point could resonate throughout the world. Therefore, collective global vigilance is necessary to protect the system from intentional adulteration. The objective of this symposium is to share best practices and lessons learned from international food defense outreach and program development initiatives. The intended audience includes food safety, quality, and regulatory personnel in the food industry, government, and academia responsible for contributing to global food defense efforts. The session will start by describing the need and current state of food defense internationally, followed by cases of food defense initiatives performed around the world. The speakers will offer industry, government, and academic perspectives on the successes and challenges of international food defense including best practices and lessons learned regarding regulatory culture, stakeholder buy-in, and sustaining food defense efforts.

S21 Applying Lessons Learned: Keeping STEC Off Our Lettuce

MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
MIA MATTIOLI: *Centers for Disease Control and Prevention, Division of Foodborne, Waterborne and Environmental Diseases, Atlanta, GA, USA*
TERESSA LOPEZ: *Arizona LGMA, Phoenix, AZ, USA*

Leafy greens like other produce are grown in an agricultural environment and can become contaminated with pathogens through a number of routes. In 2018, the CDC, FDA, state, and local public health agencies investigated a multistate outbreak of *E. coli* O157: H7 illnesses linked to romaine lettuce sourced from the Yuma, Arizona growing region. As of June 27, there were 210 people infected from 36 states with 96 hospitalizations and 5 deaths reported. This isn't the first outbreak of *E. coli* O157 linked to lettuce.

The FDA, along with CDC and state partners, conducted an environmental assessment in the Yuma growing region and collected samples of water, soil, and manure. Samples of canal water tested positive for the outbreak strain. The canal is in close proximity to a concentrated animal feeding operations and traceback indicates a clustering of romaine lettuce farms nearby. FDA will be issuing an environmental assessment report with their findings. In response to the outbreak, key industry partners formed a Leafy Greens Task Force designed to assess and address issues associated with recent foodborne illness outbreaks attributed to consumption of leafy greens and to prevent such a tragedy from occurring in the future.

This symposium will provide a brief overview of the 2018 *E. coli* O157 outbreak linked to romaine lettuce grown in the Yuma region and focus more on the environmental assessment findings including water testing, lessons learned, and both federal and industry prevention strategies moving forward.

S22 Breaking the Mold: Using Foods to Protect Against Food Allergy

WESLEY SUBLETT: *University of Louisville School of Medicine, Louisville, KY, USA*
MARTIN CHAPMAN: *Indoor Biotechnologies, Inc., Charlottesville, VA, USA*
SCOTT COMMINS: *University of North Carolina, Chapel Hill, NC, USA*

Recent breakthroughs in food allergy from the Learning Early About Peanut Allergy (LEAP) trial show that introducing food (peanut) into the diet during weaning can prevent peanut allergy. This symposium will describe how these studies have transformed treatment of food allergy. A variety of peanut foods are now being developed for allergy prevention in infancy. The approach is also being applied to other food allergens. The symposium will provide a primer on what makes a food an allergen and discuss the allergenic composition of foods and food 'prevention' products. New technology for quantification of allergens in foods will be presented, including ELISA for specific allergens (e.g., Ara h 2, Ara h 6 etc.) and multiplex arrays that measure multiple food allergens simultaneously. This innovative technology can be used to monitor therapeutic doses of allergens in foods and to improve safety and risk assessment. Finally, the symposium will address clinical and regulatory guidelines and policies that are under consideration for food prevention products and their potential relevance to the food industry.

S23 Microbiological Method Verification in Food Manufacturing: Are Your Methods "Fit Enough" for Purpose?

BRADLEY ZIEBELL: *Conagra Brands, Chicago, IL, USA*
J. DAVID LEGAN: *Eurofins Microbiology Laboratories, Madison, WI, USA*
MORGAN WALLACE: *Rheonix, Ithaca, NY, USA*

The Food Safety Modernization Act (FSMA) requires food manufacturers to verify that their preventive controls are effective. They do this by testing product and environmental samples. FSMA additionally requires that methods be scientifically suitable, or "fit for purpose," and this responsibility also lands on the manufacturer. There is very little guidance available to the manufacturer or the commercial laboratory that defines "fit for purpose" in practical terms. This is different from the situation for regulatory laboratories and diagnostic test kit makers who have detailed validation schemes and guidance documents available. Presenters will review the issues involved with selecting and verifying methods and describe some practical approaches to understanding their "fitness for purpose."

S24 2018 State and Local Foodborne Illness Investigations

LISA HAINSTOCK: *Michigan Department of Agriculture, Lansing, MI, USA*
D.J. IRVING: *Tennessee Department of Health, Nashville, TN, USA*
JAMIE DEMENT: *Florida Department of Health, Tallahassee, FL, 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 environmental 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.

S25 You Cannot Audit Food Safety Culture – Wrong, Here's How!

ANDREW CLARKE: *Loblaw, Brampton, ON, Canada*
HELEN TAYLOR: *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom*
LONE JESPERSEN: *Cultivate Food Safety, Hauterive, Switzerland*

A positive culture of food safety provides the foundation for food safety management systems, without the necessary people engagement, competencies and behaviours of the most detailed and complex systems become fragile, ineffective and hard to manage. The symposium will provide an overview of some benefits of maintaining a positive and effective culture of food safety, some of the challenges faced in both auditing and assessing food safety culture in various food handling and processing environments. You will learn from practitioners on some methods used to assess and audit food safety culture in large and small companies, and the challenges this can cause from both sides of the auditing desk. The session will include specific audit case studies and discuss how to remove the subjectivity from the process and assign accountability beyond that of the technical lead to engage the entire site.

S26 A South/Latin American Perspective on Microbiological Safety and Regulatory Guidelines for Fruit Juices: Issues and Opportunities

JOSHUA GURTLER: *U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA, USA*
FÉLIX RAMOS GUERRERO: *ICCCIA-Ricardo Palma University, Lima, Peru*
MARÍA DÍAZ ZÚÑIGA: *ICCCIA-Ricardo Palma University, Lima, Peru*

Tropical and sub-tropical fruit juices produced in South/Latin American represent one of the main sources of this product category in international trade due to their distinctive color, taste and aroma, in addition to the presence of important bioactive compounds (phytonutrients). Foodborne illness can occur if the interventions employed to inactivate the contaminating pathogens are not adequate or if the storage conditions are favorable for the growth/survival of the bacteria. New products based on tropical and sub-tropical fruits are being frequently commercialized in response to consumer demands. Microbiological issues have triggered the need for specific controls by the industry and by the government to guard against these problems. This symposium will discuss the main problems of safety and quality related to fruit juices, the challenges for processors to comply with the current regulations of South/Latin American countries and legal requirements in the international trade, as well as the opportunities for new treatments to extend fruit juices shelf life.

S27 Utilization of Metagenomics Technologies to Enhance Produce Safety and Quality

MARIA BRANDL: *USDA - FSIS, Albany, CA, USA*
KAREN JARVIS: *U.S. Food and Drug Administration – CFSAN, Laurel, MD, USA*
MIEKE UYTTENDAELE: *Laboratory of Food Microbiology and Food Preservation, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium*

Omics technologies, including metagenomics and transcriptomics, have transformed how researchers are able to study the safety and quality of foods. This symposium will focus on one of the main types of omics technologies – metagenomics – and how the use of this technique can be employed to enhance produce safety. Metagenomics can be utilized to identify a diverse array of microbial species within a particular food sample (microbiome). Since microorganisms can have both beneficial and deleterious effects on food, understanding the types and populations of these organisms can provide significant insights into food safety. Specific examples of how metagenomics technology can be used to produce safety will be presented. First, a study will be presented on the use of metagenomics to identify the lettuce microbiome from the farm through storage. Secondly, the use of this technology to inform public health decisions will be examined. Lastly, the application of metagenomics technologies will be scrutinized for the control of human foodborne pathogens associated with produce. Overall, the goal of this symposium is to demonstrate the applications of metagenomics to produce safety assessment and quality control.

S28 Looking to the Future: What Do Decision Makers Want to Know or Need to Know about Managing Chemical Contaminants in Food?

CONRAD CHOINIERE: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
TUNDE AKINLEYE: *Consumer Reports, Yonkers, NY, USA*
STEVEN HERMANSKY: *Conagra Brands, Omaha, NE, USA*

Among IAFP meeting attendees, there is an increasingly greater interest in the constructive use of knowledge about risks and the outcomes of evidence-based risk assessments to inform decision making. Decision making takes place in not only regulatory and industry contexts, but also in the dietary choices consumers make that may be driven by their perception of risks. What the decision maker wants to know or needs to know may share similarity or differ in emphasis to address challenges associated with managing risks, or perceived risks, from chemical contaminants, such as heavy metals. This proposed symposium will bring together an FDA speaker, an industry speaker and a speaker from a consumer advocacy group to present real-world case studies and experiences on how they have used science and risk assessment to inform the various risk mitigation strategies and recommendations regarding consumption, regulation or industry changes. The proposal aims to start the dialogue on how to help make science useful and accessible so that regulators, industry and consumers make informed decisions, taking into account scientific evidence and risk perception.

S29 Statistical Methods for Microbial Data and Process Validation: The *P*-value is What?

JOHN IHRIE: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
IAN HILDEBRANDT: *Michigan State University, East Lansing, MI, USA*
ANTHONY GUALTIERI: *Kellogg's, Battle Creek, MI, USA*

Many different experimental protocols aim to quantify growth or inactivation of microorganisms. Microbial quantification relies heavily on statistics, yet outcomes are often distorted given the inexact nature of microbial methods used for enumeration. *P*-hacking, an attempt to force statistically significant results, and the misuse of data analysis have become rampant. Further, logistics and economics often limit the number of trials and samples tested in an experiment. In some instances, validation studies are conducted with only one true replicate. Therefore, conclusions are frequently drawn from extremely limited data, and variability is ignored. This symposium will discuss what it takes to produce statistically sound conclusions from microbial data, designing process validation studies based on statistical power and reliability, and practical restraints to validation testing.

S30 The Use of Rapid Microbial Methods by Government Agencies for "Official" Testing

PAUL IN'T VELD: *Netherland Food and Product Safety Authority, Utrecht, Netherlands*
FATIMA FIKREE: *Food Safey Dubai, Dubai, United Arab Emirates*
BOBBY KRISHNA: *Food Safey Dubai, Dubai, United Arab Emirates*
THOMAS HAMMACK: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
MARION CASTLE: *New Zealand Ministry for Primary Industries, Wellington, New Zealand*
CONSTANZA VERGARA ESCOBAR: *Chilean Food Safety and Quality Agency, ACHIPIA, Ministry of Agriculture, Santiago, DF, Chile*
JOSE EMILIO ESTEBAN: *USDA FSIS Office of Public Health, Washington, DC, USA*

Microbiology methods used by government agencies for "Official" testing have traditionally been cultural-based methods – many, which are decades old, time-consuming, and have many procedural steps that can be challenging for members of a laboratory to conduct with consistency.

Over the last 30+ years, method developers have introduced newer, faster methods (technologies) that have improved: time-to-result, method repeatability, sensitivity, etc. In addition, several globally recognized validation/certification bodies have developed and harmonized well-defined schemes that have been used by many method developers to validate these rapid methods and demonstrate performance equivalence to recognized reference (official) methods.

Many foods manufacturers and testing labs around the globe, would like to use these validated/certified rapid methods more routinely, but an important question remains:

Will government agencies accept the use of these methods for "Official" testing?

This IAFP symposium will focus on speakers from government agencies who will be asked to follow a defined presentation format, so country criteria can more easily be compared across each global region being presented. Each speaker will answer the following key questions.

Does their agency:

1. Allow for the use of rapid microbial methods for Official testing?
2. Require these rapid microbial methods to be first validated through recognized global certification bodies such as: AOAC INTERNATIONAL, MicroVal Certification, NF Validation via AFNOR Certification, NordVal Certification?
3. Have additional requirements that these rapid microbial methods must meet?
4. Have any defined restrictions/limitations for the use of these rapid microbial methods?

A final slide summarizing the answers to these 4 questions from all six participating agencies will be projected, with time planned for Q&A.

S31 New Research Findings – Control of *Listeria* in Dairy

KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
DENNIS D'AMICO: *University of Connecticut, Department of Animal Science, Storrs, CT, USA*
KEVIN KEENER: *Iowa State University, Ames, IA, USA*
KENDRA NIGHTINGALE: *Texas Tech University, Lubbock, TX, USA*
STEPHAN RITCHIE: *University of Alabama, Tuscaloosa, AL, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*

The Innovation Center for U.S. Dairy (IC) provides a forum for the dairy industry to work together pre-competitively and is focused on broadening the adoption of current tools and best practices. In 2015 the IC initiated a research consortium to expand dairy's tool kit by leveraging both industry and academic experts to identify gaps and charter precompetitive research. These research efforts are creating new tools and practices to control *Listeria* in the finished product and plant environments with the goal of protecting consumers. This symposium will highlight the research findings of the consortium which range from fundamental knowledge of understanding the cell envelope of *Listeria* to methods to control *Listeria* in high-risk cheeses.

S32 A Precarious Balancing Act: Co-managing Preharvest Environments to Maximize Food Safety, Sustainability, and Economic Viability of Farm Operations

MICHELE JAY-RUSSELL: *Western Center for Food Safety, University of California-Davis, Davis, CA, USA*
PATRICK BAUR: *University of Californa, Berkeley, Berkeley, CA, USA*
MATTHEW JONES: *Washington State University, Pullman, WA, USA*

Recent studies have raised concerns about the impact of food safety measures on ecosystem health in agricultural environments. For example, following the 2006 *E. coli* outbreak in spinach, growers reported increased pressure from buyers, auditors, and other groups to minimize wildlife intrusion into produce fields (e.g., non-crop vegetation removal). However, as several papers published since 2006 have reported, many of these measures can negatively effect on-farm ecosystem health, including pollinator health and soil quality. This may force growers to choose between sustainability and food safety concerns, even though there is limited scientific data that farmers can use to weigh the food safety and sustainability trade-offs associated with specific management decisions. Thus, there is a specific need for research and discussion on the trade-offs between food safety and sustainability in preharvest environments.

Developing tools and approaches for co-managing (i.e., managing a farm for multiple aims, such as food safety and conservation) farms is a complex problem that requires input from all stakeholders, including food safety specialists and extension agents. It is therefore critical that members of the IAFP community who interface with farmers are at the forefront of co-management research. Much of the current research on co-management is currently being pursued by researchers from outside the IAFP community. Therefore, this symposium is a unique opportunity to bridge the gap between the researchers on the forefront of co-management and food safety specialists, as well as introduce a conceptual framework to facilitate the development of science-based co-management strategies. The objectives of this proposal are to (i) provide an overview of the impact of food safety practices on ecosystem health, (ii) present perspective on grower needs and current co-management efforts, (iii) present a case study on co-managing produce farms for water quality and food safety.

S33 Emerging Hazards Associated with Seafood

MELANIE GAY: *ANSES, Boulogne sur Mer, France*
JACQUELINA WOODS: *U.S. Food and Drug Administration, Dauphin Island, AL, USA*
STACEY MCLEROY: *U.S. Food and Drug Administration, College Park, MD, USA*

Seafood is one of the riskiest food products from a consumer health perspective. There are a wide variety of seafood products consumed, including finfish, crustaceans, and molluscan bivalves. Each has food safety hazards associated with them. And, some are known to be riskier than others.

Sometimes that is because of where the seafood is harvested; sometimes because of how it is consumed. While some hazards, such as bacteria, can be reduced by cooking; others such as toxins, are heat resistant. Parasites can be killed by freezing the seafood before reaching the consumer; while the freezing preserves many of the parasites antigenic components any enteric viruses that may be present. So, it is no wonder that seafood is a common route of foodborne illness. Even with all we know, ever-changing consumption patterns, the spread of marine organisms, and diversity of harvest give rise to emerging problems. This includes new geographical sources of seafood, as well as interest in different seafood products, and a switch to more frequent consumption of raw seafood. This session will highlight some examples of recently emerged seafood safety hazards. In addition, the effectiveness of current food safety controls for these emerging hazards and the need for new, or modified, control measures will be discussed.

S34 Fact or Fiction: Combatting Consumer Perceptions of Food Safety Myths with Data

DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
ELIZABETH L. ANDRESS: *University of Georgia, Athens, GA, USA*
AARON LAVALLEE: *USDA Food Safety and Inspection Service, Washington, DC, USA*

The internet is rife with messages around safe food handling, some correct, but many created without scientific data or support to back them. These food safety myths are widely circulated on social media and other internet-based outlets perpetuating misinformation on proper food safety practices and allowing them to persist or even spread among consumers. As the dialogue around food safety evolves to more targeted discussions, food safety professionals need to be generating data and developing sound evidence-based messages to engage with the public on these issues. Changing consumers' minds is difficult, though not impossible, task to undertake and requires strong data on the myth in question along with effective communication strategies and repetition of the message itself. Performing research to generate data regarding the validity of such myths could allow food safety professionals to more effectively engage with consumers that believe or practice behaviors inspired by such narratives. However, there is an abundance of food safety myths and relatively few of them have been tackled and debunked effectively. Those that have are often initially met with resistance by consumers and disbelief in their resistance by food safety scientists. This session aims to explore why these food safety narratives persist and spread, discuss several examples where specific food safety myths were researched, such as the 5-second rule, double dipping, and home food preparation-based myths, followed by how such data can be generated, how messaging is developed from that data, and how those messages are ultimately received. The session will close with a panel discussion on other myths not specifically discussed and what data would need to be generated to address them moving forward.

S35 Future Pains: Assessing the Long-term Consequences of Foodborne Exposure to Microbial and Chemical Hazards

KRISTEN POGREBA-BROWN: *University of Arizona, Tucson, AZ, USA*
BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*
SUZANNE FITZPATRICK: *U.S. Food and Drug Administration, College Park, MD, USA*

Foodborne exposures to microbial and chemical hazards are major causes of morbidity and mortality globally. In the U.S., foodborne pathogens cause an estimated 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually. In addition to these acute illnesses, many foodborne bacteria, viruses, and parasites, as well as chronic exposures to chemical contaminants can result in long-term sequelae of gastrointestinal, immune, nervous, respiratory, cardiovascular, and other organ systems. A few examples of such long-term consequences include chronic kidney disease following hemolytic uremic syndrome caused by Shiga-toxin producing *E. coli*, Guillain Barré syndrome following campylobacteriosis, irritable bowel syndrome, dyspepsia, inflammatory bowel disease, reactive arthritis, and diabetes. Pregnant women infected with *Listeria monocytogenes* or *Toxoplasma gondii* can give birth to babies born with lifelong impacts on cognitive function and other health systems. Chronic aflatoxin exposure can cause liver cancer, while arsenic is associated with multiple types of cancer, as well as developmental, neurological, respiratory, and other effects. Because many of these manifestations may not arise for weeks, months, or even years after infection, they can be difficult to characterize and are too often neglected by clinicians and public health officials.

Long-term health outcomes caused by microbial and chemical hazards cause considerable disease burden and should be considered in food safety prioritization and policy development. To do so, we need sustained research and tools aimed at identifying, characterizing, and quantifying the likelihoods, durations, and severities of long-term health consequences. This symposium will present a number of different approaches to assessing these impacts, including the use of literature review, cohort surveys, analysis of medical claims data, and novel predictive toxicology tools such as organs-on-chips.

S36 Challenges of Sanitation in Dry Processing Environments: What are the Evolving Methods?

LAUREN JACKSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
QUINCY SUEHR: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
ELIZABETH GRASSO-KELLEY: *Illinois Institute of Technology, Bedford Park, IL, USA*
ALEX JOSOWITZ: *Sterilex Corporation, Hunt Valley, MD, USA*
KEVIN LORCHEIM: *ClorDiSys Solutions, Inc, Lebanon, NJ, USA*

Sanitation, as a preventive control or a corrective action, is a significant aspect of food safety. With FDA's Current Good Manufacturing Practice, Hazard Analysis, and Risk-based Preventive Controls for Human Food rule in enforcement phase, and recent outbreaks involving flour and whey powder, sanitation in low-moisture food environments is of great importance. Biofilms and associated microorganisms can survive in dry environments, presenting a further challenge to microbial control in low-moisture processing. Sanitation in dry food processing environments continues to be challenging, both for the removal of chemical contaminants such as food allergens and/or foodborne pathogens. To make it more complicated, each processing environment is different with different specifications. In some cases, wet-cleaning must still be implemented along with dry cleaning/sanitation to fully eliminate chemical or microbial contaminants. Truly dry sanitation methods are available for environmental microbial control on floors and entryways to prevent cross-contamination. Some dry-cleaning protocols may also include purging equipment lines with clean material and using alcohol-based cleaners/sanitizers. These methods have limitations and are not applicable to all systems. In some instances, a complete replacement of equipment with one that has an easily cleanable design may be required. The focus of the symposium will be on some current approaches to sanitation in a dry environment and the challenges associated with it. Methods employed for both allergens and microorganisms will be discussed, along with case studies, practical applications and problems related to implementation.

S37 *Campylobacter*, Health Impact, Performance Objectives and Effectiveness of Sampling Plans

JEFFREY FARBER: *University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada*
LEON GORRIS: *Food Safety Expert, Nijmegen, Netherlands*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*

Campylobacter is the zoonotic bacterium with the largest public health impact in many countries. Since the organism does not grow in the food chain, main points of control are in primary production, during slaughter and in the food preparation area. Absolute control is not possible at these stages, therefore balanced and targeted control measures should be implemented along the chain. The Codex/ICMSF approach of setting a food safety objective and linking it to performance objectives, process criteria, and microbiological criteria along the chain is instrumental in reaching a public health objective in a structured and flexible manner. Until recently, microbial criteria in chicken meat were not used, but since 2018 a process hygiene criterion has become mandatory in the European Union (EU). Notably, this is a dynamic criterion of which the stringency will increase in the next 7 years (m = 1000 CFU/g, n = 50, c = 20 from 1.1.2018 and with c = 15 from 1.1.2020 and c = 10 from 1.1.2025). In this symposium the health impact of *Campylobacter* and its epidemiology will be described, potential control along the food chain by making use of performance criteria will be exemplified and the effectiveness of the current and future sampling plans in the EU will be discussed. The symposium is of relevance for governments, academia as well as for food industry.

S38 When the *Enterobacteriaceae* Hits the Fan: Wind and Particulate-associated Distribution of Foodborne Pathogens

DUMITRU MACARISIN: *U.S. Food and Drug Administration, College Park, MD, USA*
DE ANN DAVIS: *Church Brothers Farms, Salinas, CA, USA*
DAVID INGRAM: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*

Wind, dust and particulate matter suspended in the represent an understudied vehicle for foodborne pathogen dispersal in agriculture/ animal operations and food processing facilities. Dust storms, farm operations, soil erosion and many other factors can result in the redistribution of foodborne pathogens across vast distances. The consequence of these events can amount to outbreaks and recalls, with the vehicle and source of the pathogen remaining ambiguous. Dust dispersal can affect plant physiology, microbial physiology and the plant microbiome. The consequences of wind propagated dispersal of foodborne pathogens can affect a wide variety of produce such as fruits, leafy greens and tree fruit. Understanding the role of dust, wind and climatic conditions that can cause their deposition of foodborne pathogens on the surfaces of produce is of high importance as it can help in better risk evaluation, mitigation and preventative control measures.

The talks presented in this symposium will focus on:

- Role of airborne dispersal of foodborne pathogens in tree fruit production environments.
- Grower's concerns associated with dust storms and dust dispersal.
- Regulatory adaptations that can help mitigate risks associated with dust dispersal.

S39 What Do We Know about Microplastics in Food and Their Impact on Human Health?

GARTH COVERNTON: *University of Victoria, Dept. of Biology, Victoria, BC, Canada*
J. EVAN WARD: *University of Connecticut Dept. of Marine Sciences, Groton, CT, USA*
BART KOELMANS: *Wageningen University and Research, Wageningen, Netherlands*

Plastic waste and pollution are becoming a huge global problem. It is estimated that by 2030, 111 million metric tons of plastic waste will be displaced because of China's January 1, 2018 import ban. (China previously used or resold recycled plastic and was the largest importer of plastic waste.) The food industry uses large amounts of single-use plastic to package food and beverages. Plastic waste slowly degrades into small particles (micro and nanoplastics) and fibers which can find their way into food and consumers. While the risk of microplastics in food is probably highest in seafood because of plastic pollution in oceans and freshwater, microplastics have been found in beer and honey, and pig and poultry feed contains fishmeal.

Are you frightened, curious or skeptical about reports of plastic contaminants in food and beverages? Do you communicate with consumers about food safety issues and need to know how to answer their questions about microplastics? If so, join us as we tackle this topic for the first time at an IAFP Annual Meeting and receive information you need to be a more empowered food safety professional. This symposium will describe: types of microplastics, their origin, and where they are found; quality control issues with analytical methods used to detect and quantify microplastics in foods and discrepancies in how results are reported; foods that contain microplastics; information on human health impacts; as well as knowledge gaps that impede a fuller understanding of this subject. It is fervently hoped that presentations and discussions during this symposium will spark meaningful dialogue on how best to evaluate the threat of microplastics to our food supply and trigger plans and actions to minimize it.

S40 The Mitigation and Regulation of Heat-formed Substances Produced in Foods during Cooking: What are the Unintended Consequences on Microbial Safety and Public Health?

STEVEN HERMANSKY: *Conagra Brands, Chicago, IL, USA*
SCOTT HOOD: *General Mills, St. Paul, MN, USA*
MICHAEL DOURSON: *TERA, Cincinnati, OH, USA*

A growing field in food safety is the focus on the potential risk of heat-formed substances produced during cooking. Compounds that are known as human health hazards are being increasingly identified as heat-formed substances present in food. Two prominent examples of this are acrylamide and furfuryl alcohol, both of which are present in significant amounts in a wide array of foods. This session will help inform how the risk assessment process of heat-formed substances can incorporate the benefits of cooking and cooked food. It will highlight the genetic changes that allowed humans to consume cooked food. The session will then explore the unintended consequences in mitigating heat formed substances, such as introducing microbial hazards. It will address how to assess and communicate these risks to food processors and consumers. The potential impact and implications on the food industry and, ultimately, the end consumer, of using current approaches to assess the potential public health impact of compounds formed during routine cooking of food will be debated.

S41 Strategies to Prevent Pathogen Contamination in Post-lethality Dry and Wet Environments

LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
LILIA SANTIAGO: *Kellogg's, Battle Creek, MI, USA*
JOHN HOLAH: *UK:IE EHEDG & Holchem Laboratories Ltd., Bury, United Kingdom*

The enactment of the Food Safety Modernization Act has paved the way to establish modern food safety standards and prevention practices. Many food safety plans have now incorporated microbial preventive controls where a foreseeable biological hazard is identified. The food industry has also enhanced efforts to ensure that there is scientific justification for the process controls used to mitigate potential biological hazards, including in-plant validation studies using surrogate organisms. However, conducting in-plant validation studies may not be feasible for some pathogen inactivation strategies, such as drum drying, spray drying, extrusion, chemical disinfection, etc. used in industrial operations. The lack of suitable surrogate for certain processes, challenges in designing appropriate inoculation techniques and procedures for recovery of the surrogate, and the financial burden of commissioning a validation study for a number for food matrices can be daunting and may hinder the plant's ability to maintain a robust food safety program. Many supplier verification audits have already incorporated audit criteria to assess the adequacy of preventive controls to deliver the target lethality. Food recalls, however, continue to occur for products with well-recognized thermal or chemical preventive measures. Pathogen recontamination from the plant environment after the kill step is often identified as the root cause for a number of these recalls. What food safety programs and/or preventive controls, beyond a pathogen inactivation step, can be effectively be implemented, monitored and maintained? This symposium will identify opportunities for pathogen recontamination for certain wet and dry processes, understand how design and cleanability of equipment can contribute to the occurrence of potential food safety risks and the utility of establishing baseline data for the appropriate indicator organism.

S42 Challenges in *Campylobacter* Detection and Control

HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands*
BENJAMIN DUQUÉ: *UMR1014 Secalim, INRA, Oniris, Nantes, France*
MICHAEL WILLIAMS: *U.S. Department of Agriculture-FSIS, Washington, DC, USA*

Campylobacteriosis is the most frequently reported zoonosis worldwide. Control of *Campylobacter* along the food chain is troublesome and there is a clear relationship between the prevalence of *Campylobacter* in broiler flocks and public health risk. Chickens serve as reservoirs, and *Campylobacter*'s ability to survive on chicken fillet depends on its ability to survive the scalding (i.e., heating step), chilling and subsequent storage steps. Strain variability and cell history affect the robustness of *Campylobacter* along the chain and quantitative knowledge is needed to refine microbiological exposure assessment models and to evaluate the impact of industrial control interventions. The effectiveness of control measures is verified by microbiological testing. Testing efficacy is however hampered by the fact that *Campylobacter* is often damaged in food and may only represent a small fraction of the total microflora in food. Analytical testing methods, therefore, incorporate an enrichment procedure to recover and selectively amplify *Campylobacter* to higher concentrations allowing subsequent detection. These enrichment-based detection methods are in practice not perfectly selective and sensitive. This symposium focuses on the challenges to control and detect *Campylobacter* along the food chain. It will highlight the advances to predict the robustness of *Campylobacter* to survive along the food chain using molecular markers taking into account cell history and strain variability. It will also discuss the effects of strain variability and competitive flora on the outcome of enrichment-based detection procedures, and it will give an update about the lessons learned and the difference between qualitative and quantitative standards to control *Campylobacter* in products.

S43 Are There Instructions Included? The Role of Regionality and Experimental Choices on the Survival of Foodborne Pathogens in Manure-amended Soils

MANAN SHARMA: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
MICHELE JAY-RUSSELL: *Western Center for Food Safety, University of California-Davis, Davis, CA, USA*
KEITH SCHNEIDER: *University of Florida, Gainesville, FL, USA*

Animal manure is a commonly used organic fertilizer for cultivation of fruits and vegetables. Land application of manure is a useful management technique but also can present the potential to introduce foodborne bacterial pathogens from manure to fruits and vegetables. Many investigators have collected data to examine current and proposed USDA and FDA standards on the interval between manure application and harvest of fruits and vegetables to minimize the risk of foodborne illness. This symposium will address the factors (agricultural, spatiotemporal, weather) which can affect the survival of foodborne pathogens in manure-amended soils. These factors, along with manure types commonly used, application method and rate, vary among geographic region in the U.S, and proper contextualization can provide insight into the survival duration of pathogens in manure-amended soils. Speakers will describe similarities and differences in dynamics and duration of pathogen survival based on agricultural and environmental factors in the Mid-Atlantic, Western, and Southeastern U.S. Data comparing greenhouse and field studies will illustrate advantages and limitations of studying pathogens in these environments, and how these conditions affect results and conclusions made by investigators. Shared methodology, including statistical modeling, will be included so that useful comparisons of data on survival durations and survival dynamics of enteric pathogens in manure-amended soils can be made. This symposium will explore lessons learned in methodology from multi-regional, multi-year research since the landmark *JFP* publication (Harris et al., 2013): A Framework for Developing Research Protocols for Evaluation of Microbial Hazards and Controls during Production That Pertain to the Application of Untreated Soil Amendments of Animal Origin on Land Used to Grow Produce That May be Consumed Raw. It will describe the complexity and commonalities associated with bacterial pathogens in manure-amended soils across different regions. It will benefit researchers, extension professionals, government regulators, and produce growers and handlers.

S44 Updates on the Impact of Sampling Plans on Food Safety

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AIXIA XU: *USDA-ARS-ERRC, Wyndmoor, PA, USA*
URSULA A. GONZALES-BARRON: *School of Agriculture, Polytechnic Institute of Braganza, Braganza, Portugal*

Sampling and subsequent microbiological testing are widely used by government and industry as part of their regulatory programs to ensure safe food products. The major limitation associated with the ling of foods is that the only way to absolutely ensure that no serving of food contained a pathogenic microorganism would be to test every serving. Since the microbiological testing of foods is a destructive process, this would leave no food for consumption. It is, therefore, the common practice to select a fraction of the food product for analysis, assuming the samples collected are representative. Sampling can occur at many stages in the farm-to-fork chain. Microbiological sampling is one of the tools used to verify on an ongoing basis that a food safety system is working as intended. Using a realistic sampling scheme, it is possible to test for absence of a pathogen in a batch of food to a specified level of confidence, but this can lead to large type II errors when not carried out correctly. Considering the large batch sizes, low frequency and the levels of pathogens in the food product, as well as the heterogeneity of pathogen distribution, it is critical to design appropriate sampling plans

to improve the statistical power of the microbiological testing. A variety of sampling schemes can be employed, either individually or in combination. Choice of sampling plans depends on nature and quality of the food batch, likely sources of contamination and cost or operational concerns related to testing. This symposium is to review the performance of the current sampling plans as well as the development of novel sampling schemes to improve the detection rate for ensuring microbiological safety.

S45 Updates to the Conference for Food Protection and the Food Code

DAVID MCSWANE: *Conference for Food Protection, Martinsville, IN, USA*
GIRVIN LIGGANS: *U.S. Food and Drug Administration, College Park, MD, USA*
BRENDA BACON: *Harris Teeter, Matthew, NC, USA*

The Conference for Food Protection (CFP) originated in 1971 to provide members of industry, regulatory, academia, consumer and professional organizations equal input in the development and/or modification of Food Safety Guidance. Such guidance is incorporated into food safety laws and regulations at all levels of government throughout the United States including the FDA Food Code which aims to reduce the risk of foodborne illnesses within food establishments, provide uniform standards for retail food safety that reduce complexity and better ensure compliance, eliminate redundant processes for establishing food safety criteria, and establish a more standardized approach to inspections and audits of food establishments. The symposium will outline important changes to the Food Code over recent years included in the 2017 edition, and what the CFP is planning for its 2020 meeting in Denver.

S46 Is *Bacillus cereus* the Next Big Thing to Worry about in the Food Industry?

FLORENCE POSTOLLEC: *ADRIA - UMT ACTIA19.03 ALTER'iX, Quimper, France*
JASNA KOVAC: *The Pennsylvania State University, University Park, PA, USA*
SANDRA TALLENT: *U.S. Food and Drug Administration, College Park, MD, USA*

Bacillus cereus sensu lato, also known as the *B. cereus* group, includes closely related Gram-positive, spore-forming and aerobic bacilli, widely distributed in the environment and food matrices. Besides characteristic colonies on Mossel agar, these species exhibit highly divergent properties and their distinction remains challenging. Presently their classification relies mainly on distinctive phenotypic traits, such as pathogenic potential to mammals (*B. anthracis, B.cytotoxicus*, emetic/diarrheic strains of *B. cereus*) and insects (*B. thuringiensis*), enzymatic ability causing food spoilage (*B. weihenstephanesis, B. wiedmannii*), thermotypes, as well as colony morphology (*B. (pseudo)mycoides*). Recently, *Bacillus toyonensis*, *Bacillus manliponensis*, *Bacillus gaemokensis* and *Bacillus bingmayongensis* have been recognized as plausible members of this group.

While food and raw material generally show low spore contamination, food poisoning outbreaks are mainly due to improper conditions of use and storage of food after cooking. Careless food handling, especially time and temperature abuse, of products such as cooked rice, sauces, soups, and ready-to-eat products allows production of bacterial toxins associated with *B. cereus* foodborne illnesses.

The issue that continuously exasperates the food industry and food safety officials is how to separate the bacteria that can cause food spoilage from the strains that can cause human illness.

This is a complete nightmare for food business operators and public health agencies. This session will give an update on available knowledge and tools related to *B cereus* issue.

S47 Advancing the Science of Risk-based Criteria for Agricultural Water Quality

EMILY GRIEP: *United Fresh Produce Association, Washington, DC, USA*
DON STOECKEL: *Cornell University, Geneva, NY, USA*
DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
CHARLES GERBA: *University of Arizona, Tucson, AZ, USA*

Through FSMA, Congress directed the FDA to develop a Produce Safety Rule (PSR) including science-based minimum standards for the safe growing, harvesting, packing, and holding of produce. The water quality criteria in the PSR, that originate with USEPA recreational water quality criteria, have been challenged. In response, the FDA is re-evaluating the water quality requirements of the PSR and compliance dates for these regulations have been pushed forward to 2022 or later (proposed). The purpose of this symposium is to discuss a potential path toward a sound scientific basis for criteria governing the quality of water used in produce production that is protective of public health.

Public health outcomes from eating contaminated produce cannot be estimated by direct observation without human subjects. Those experiments are no longer done. More acceptable is the retrospective analysis of outbreaks. Retrospective analysis requires knowledge about the source term, including the pathway, timing and magnitude of the contamination event. The pathway of contamination is often difficult to prove by the time a food vehicle is identified and traceback information is compiled. The timing of the contamination event can only be estimated based on the pattern of illnesses and the flow of product through distribution channels. The magnitude of the event can only be known if contemporaneous samples are available for analysis. For agricultural water used in production, conditions often change over time and samples representative of conditions when the contamination event occurred generally are not available. Retrospective analysis is valuable, but the value is also limited because of these uncertainties.

Scientists and other analysts have been around this wheel before. This session will include lessons learned, industry needs, processes for risk-driven models, and retrospective investigation to validate modeled outcomes. Given a target level of consequences and the inputs that result in lower consequences, science-based minimum standards for water quality are possible.

S48 Determining Preventive Controls for Viruses and Parasites

SOPHIE ZUBER: *Nestlé Research Center, Lausanne, Switzerland*
KALI KNIEL: *University of Delaware, Newark, DE, USA*
TIMOTHY JACKSON: *Driscoll's, Watsonville, CA, USA*

There has been an increasing number of foodborne outbreaks and recalls associated with fresh produce contaminated with parasites and viruses both in the United States and worldwide. When pathogens are recognized as contaminants of a particular type of fruit or and vegetable they need to be identified in the hazard analysis in a HACCP plan and be addressed accordingly with preventive controls. This symposium will highlight some of the possible preventive controls that could be developed for the most epidemiologically important foodborne viruses and parasites related to the fruits and vegetable sector. It will also address advances and challenges in control methods currently in use for safe produce as they relate to viruses/parasites. Experts from the fields of food parasitology and virology will present in detail possible preventive controls for such agents and clarify any misconceptions. In addition, speakers from industry will discuss potential or already in place systems to control such emerging pathogens

S49 Communicating Benefit/Risk Analysis of Food Processing

CHRISTINE CRINCOLI: *Cargill, Inc., Minneapolis, MN, USA*
CANDACE DOEPKER: *ToxStrategies, Newport, KY, USA*
ANTHONY FLOOD: *IFIC, Washington, DC, USA*

The public's concern about chemicals in food is on the rise. Whether consumers are seeing media coverage about acrylamide in coffee or hearing about arsenic in rice from their neighbors, this concern is exacerbated by the multitude of warning labels that often appear on their favorite foods and beverages. Warning statements, required by regulatory actions, can appear on foods that contain unavoidable and often harmless levels of contaminants. Many of which are naturally occurring or are formed during processing. These concerns ultimately influence consumer behavior to seek out and consume minimally processed products or foods labeled as preservative-free – products that run counter to best practices of food safety. This session will provide 1) information on biological and emerging chemical risks and contaminants formed during processing; 2) overview of BRAFO – a risk assessment framework designed exclusively for Risk Managers; and 3) best practices for communicating food safety in the context of food production and chemical risks.

S50 *Clostridium difficile*: A Food Safety Risk?

KEITH WARRINER: *University of Guelph, Guelph, ON, Canada*
J. ANTONIO TORRES: *Tecnologico de Monterrey, Monterrey, NL, Mexico*
GENEVIEVE FLOCK: *U.S. Army Combat Capabilities Development Command Soldier Center, Natick, MA, USA*

*Clostridium difficile i*s a gastrointestinal pathogen of significant concern in hospital settings. In addition, the continued increase in outbreaks outside the hospital environment has prompted investigations into food as a potential source of the pathogen. Given that *C. difficile* is inherently present in the environment, there could be a potential for it to enter the food supply such as other environmentally ubiquitous foodborne pathogens (e.g., *Listeria monocytogenes*). *C. difficile* rates of isolation in foods and the environment have varied widely from as low as 0% up to 50% in meats in the United States. This symposium will discuss recent development and control strategies to guard against the hazards associated with *Clostridium difficile* in foods.

S51 Challenges and Promises of Using Quantitative Data for Controlling *Salmonella* in Poultry

STEVEN RICKE: *University of Arkansas, Fayetteville, AR, USA*
ERIC EBEL: *U.S. Department of Agriculture-FSIS-OPHS, Fort Collins, CO, USA*
ANGELA SIEMENS: *Cargill Meat Solutions, Wichita, KS, USA*

Despite the best efforts of food safety professionals, non-typhoidal salmonellosis continues to be a major cause of gastrointestinal illnesses and an enormous economic burden in the U.S. and worldwide. These trends are compelling regulatory agencies and industry to consider alternative approaches for controlling *Salmonella*, especially in raw poultry. Quantitative microbial risk assessment (QMRA) has been proposed as a viable tool with the potential to bridge the gap in our understanding of factors for controlling *Salmonella* in raw poultry. An effective QMRA could identify critical control points along the food production chain which could be used to verify process control and mitigate the public health risk. The myriad of factors that can influence *Salmonella* prevalence across different food industries, including meat and poultry, warrants the use of precise data that can make QMRA more effective. Methods for *Salmonella* enumeration have been available for some time now, and new technologies provide opportunities for rapid results and reduced cost. However, major questions remain about the use of these methods, including quality/reproducibility, sampling, modeling and statistical considerations. The availability of a rapid, reliable and cost-effective *Salmonella* enumeration method could not only mean better control within the processing facilities, but it could also provide an ability to monitor the *Salmonella* prevalence upstream at farms. *Salmonella* enumeration for upstream monitoring has been tried by various poultry companies, but it remains an experimental approach. The proposed symposium will discuss current enumeration methods, comparisons with newer enumeration methods for use in QMRAs.

S52 Foodborne Disease Outbreak Update

BROOKE WHITNEY: *U.S. Food and Drug Administration - Coordinated Outbreak Response and Evaluation Network, College Park, MD, USA*
PONGPAN LAKSANALAMAI: *Maryland Department of Health & Mental Hygiene, Baltimore, MD, USA*
JESSICA JONES: *U.S. Food and Drug Administration, Gulf Coast Seafood Laboratory, Dauphin Island, AL, USA*
ZSUZSANNA SRÉTERNÉ LANCZ: *Food Microbiological National Reference Laboratory, Budapest, Hungary*
LAURA GIERALTOWSKI: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
EMILY HARVEY: *Massachusetts Department of Public Health, Jamaica Plain, MA, USA*
DOUG NOVEROSKE: *U.S. Department of Agriculture-FSIS, Washington, DC, USA*
LAURA GIERALTOWSKI: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

This symposium covers five different kinds of products involved with outbreaks in 2018 or late 2017: cut melons, corn, crabs, coconut and ground beef, plus one outbreak scenario that is late breaking. CDC, public health and regulatory officials in several states, and the U.S. Food and Drug Administration investigated a multistate outbreak of *Salmonella* Adelaide infections with 77 people infected from nine states. The source of the outbreak (one supplier) will be discussed along with the impact on many major retailers. Epidemiologic, laboratory, and traceback evidence indicated that crab meat labeled as fresh or precooked and imported from Venezuela infected at least 26 people with *Vibrio parahaemolyticus* from seven states and D.C. over 4 months; because of the outbreak investigation, FDA increased testing of fresh crab meat from Venezuela. An extensive outbreak of invasive *Listeria monocytogenes* infections confirmed by whole-genome sequencing and linked to frozen corn and possibly to other frozen vegetables originating from Hungary occurred in five EU Member States since 2015. By June 2018, 47 cases were reported with a case fatality rate of 19%. The strain persisted in the environment of the processing plant even after standard cleaning and disinfection. Frozen shredded coconut imported from Viet Nam, contaminated with many different *Salmonella* serovars, sickened 27 persons in 9 states; most had drunk an Asian-style dessert drink containing the coconut. Consumption of undercooked beef was found to be significantly associated with *E. coli* illness in >200 Marine recruits in San Diego. Poor hygiene practices among recruits and inconsistent cooking temperatures within dining facilities were revealed and lawsuits were brought against the contract supplier. The many recommendations will be discussed.

Symposium

S53 The Impact of Packaging Materials on Food Safety: Testing, Modeling and Regulation

LUKE ACKERMAN: *U.S. Food and Drug Administration, College Park, MD, USA*
MELVIN PASCALL: *The Ohio State University, Columbus, OH, USA*
TBD TBD: *TBD, TBD, AL, USA*
NAEEM MADY: *Intertek, Boca Raton, FL, USA*
CHARLES NESLUND: *Eurofins, Lancaster, PA, USA*
MAEVE CUSHEN: *CremeGlobal, Dublin, Ireland*

Packaging materials continue to play a critical role in maintaining the quality and ensuring the safety of food products. New research and applications have led to the development of packaging that offers increased protection from oxygen, light, heat, and microbial growth. For example, the use of nanomaterials and antimicrobial coatings are gaining wider use and acceptance because of the benefits offered.

With the advancement of knowledge in toxicology and carcinogenicity and the use of new chemical formulations in packaging comes a greater concern about the unintended consequences of chemicals migrating from packaging to food. This has resulted in increased regulation and testing of existing and new packaging materials.

This symposium will focus on the following topics:

- Assessment of chemical contaminants in food packages, current regulations and hot-topic compounds like BPA and PFAS
- Risk assessment for new and old food packaging materials, documenting chemical safety, chemical exposure assessment, risk communication
- Testing materials in contact with food and the effectiveness of test methods for new materials
- Chemical migration modeling as guidance or an alternative to testing
- Regulatory aspects of food packaging materials and safety

Attendees will learn, from a diverse collection of experts in this field, how advances are being used to build a better understanding of the impact packaging materials have on food safety.

S54 Agricultural Water and Emerging Pathogens in the Age of FSMA: Do We Need to Worry?

KALI KNIEL: *University of Delaware, Newark, DE, USA*
SOCRATES TRUJILLO: *U.S. Food and Drug Administration, College Park, MD, USA*
JIM BRENNAN: *SmartWash Solutions, LLC, Salinas, CA, USA*

The contamination of agricultural water with foodborne pathogens represents a major concern in produce safety. Several disease outbreaks have been reported worldwide which could have been linked to contaminated agricultural water used in upstream or downstream farm operations. This symposium will provide updated information about the status of agricultural water regarding the prevalence of viruses and parasites. In addition, we will discuss how effective the current safety standards are in assuring that the agricultural water is free of such pathogens. Speakers from Federal U.S. agencies, academia and industry will participate in this symposium.

S55 Environmental Monitoring – A Cost-effective Tool or Expensive Waste of Resource?

ANETT WINKLER: *Cargill, Inc., Munich, Germany*
ROY BETTS: *Campden BRI, Chipping Campden, United Kingdom*
RICHARD BROUILLETTE: *Commercial Food Sanitation, South Burlington, VT, USA*

It is clear that Environmental Monitoring (EM) costs the industry much time and money and is widely practiced. However whilst well thought out and valid EM systems are of great value and can be a great friend to us, poorly designed systems where little thought is given to design, handling results and corrective actions, are of little value and are our foe.

Over the last year's environmental monitoring (EM) gained a lot of attention from both the industry and regulators. Many documents (e.g., published by Codex Alimentarius, FDA, GMA) underline its importance, provide general guidance and even require EM as part of Food Safety management. However, EM will only form an integral part of food safety systems when it is set up adequately – assuring that the environment won`t contribute to contamination of products. This symposium will focus on the practical aspects of EM to ask what we can learn from the past – incidents with both good and bad examples of EM practice will be considered.

It will also pose questions such as: How do we get the most information out of the time and money we invest in that activity? How to set meaningful sampling strategies and criteria? How useful are the indicators? How to interpret the results? Are there adequate corrective actions defined? How can these be verified?

S56 Poultry Vaccines: What is Working, What are the Gaps, and What is on the Horizon?

CONNIE SCHMELIK-SANDAGE: *USDA APHIS, Ames, IA, USA*
STEVIE HRETZ: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*
CHRISTINE SZYMANSKI: *University of Georgia, Athens, GA, USA*

Control of biological hazards begins long before a bird is presented for harvest; the success of interventions used inside an establishment is directly impacted by what comes in the door. This symposium will explore the successes, unintended consequences, and upcoming goals for this key pre-harvest intervention. With a somewhat chronological approach, we will explore the history and current status of live vaccines, the vulnerability of attenuated vaccines with regard to regulatory testing, and the future of vaccines to improve the range of protection and strategies to better control perpetuation of vaccine strains in birds headed for harvest.

S57 Biofilm Removal as a Critical Part of Spoilage and Pathogen Contamination Prevention

ABIGAIL SNYDER: *The Ohio State University, Columbus, OH, USA*
LAURENT DEHALLE: *REALCO, Ottignies-Louvain-la-Neuve, Belgium*
CHRISTOPHER MCNAMARA: *Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA, USA*

Biofilms are heterogenous microbial communities adhered to surfaces in a porous extracellular polymeric matrix. Each biofilm represents an ecosystem were pathogenic and spoilage bacteria, yeasts and molds coexist and interact, increasing their resistance to sanitizers and cleaning operations. Biofilm contamination of foods and beverages can lead to spoilage and represents a food safety hazard. There is a lack of targeted strategies for

efficient removal of biofilms in industrial plants. Biofilm formation in crevices, corners, dead zones, valves or areas where the mixing rate is low is almost inevitable. In industrial settings, surface disinfection is usually focused on the use of biocides, aiming to inactivate the microorganisms. Since biofilms are complex biological structures adhered to a surface, these strategies often fail, as the removal of the base layer is neglected. Eliminating and preventing formation of the base layer is fundamental for controlling biofilm development. Currently, strong oxidizing agents such as chlorine, chlorine dioxide and bromide or organic biocides such as isothiazolones are used, which generate high levels of hazardous by-products. Another strategy for biofilm detachment is the use of various physical treatments such as ultrasound, thermal shock, or mechanical treatments using pigs or shear stress induced by the fluid hydrodynamics. Enzymatic treatment is an emerging intervention that functions by decreasing the biofilm cohesion and destroying the physical integrity of the matrix while having no identified negative impact on the environment. If biofilm removal is not complete, rapid re-growth can occur. It is necessary to find solutions that promote significant detachment including the basal layer. The combination of two or more of these treatments may be necessary for the complete removal of biofilm.

In this symposium, different novel techniques for biofilm removal in the food and beverage industries will be discussed. The symposium will start with an introduction on the role of biofilm on spoilage and pathogen contamination of foods and beverages that will cover an overview of the critical factors for biofilm formation, removal, and verification. Then, enzymatic of biofilm removal and prevention as well as natural compounds for the control of biofilms on food contact surfaces will be discussed in depth.

S58 Protecting Probiotics: Detecting Hazards and Confirming Formulation Accuracy

JOSEPHINE D. GREVE-PETERSON: *Eurofins Food Integrity & Innovation, Madison, WI, USA*
SOMSUVRA GHATAK: *U.S. Pharma Lab Inc, North Brunswick, NJ, USA*
CARMEN TARTERA: *U.S. Food and Drug Administration – CFSAN, Laurel, MD, USA*

The earliest probiotics were fermented dairy products such as yogurt and kefir. Elie Metchnikoff published observations on the longevity of Bulgarian peasants who regularly consumed these foods as long ago as 1908. Today, cultured dairy products remain a significant portion of the probiotics market, but other forms including dietary supplements and foods with added probiotic cultures have become increasingly popular: so much so that the global probiotics market in all forms is estimated at $41 bn (USD) in 2017. The market is growing rapidly with a compound annual growth rate (CAGR) estimated at over 7%. As consumption of probiotics grows, the need to maintain their excellent safety record becomes increasingly important. However, they pose unique challenges for safety and quality managers because detecting pathogens against the background probiotic population is difficult, and culture components may contain allergens, particularly milk or soy proteins. Moreover, there is an economic component to consumer protection because probiotic benefits are linked to a narrow range of identified strains with individually high value. Accidental or deliberate strain substitution may damage probiotic brand value and harm consumers economically. This symposium will explore some of the issues and opportunities involved in protecting probiotic consumers and brands.

S59 Extraintestinal Pathogenic *Escherichia coli* (ExPEC): Urinary Tract Infections, Sepsis, and Avian Colibacillosis

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MELHA MELLATA: *Iowa State University, Ames, IA, USA*

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are responsible for various diseases in humans and animals. The proposed subpathotypes of ExPEC include avian pathogenic *E. coli* (APEC), uropathogenic *E. coli* (UPEC), sepsis-associated *E. coli* (SEPEC), and neonatal meningitis causing *E. coli* (NMEC). ExPEC-associated diseases in humans include urinary tract infection (UTI), inflammatory bowel disease, sepsis, and neonatal meningitis. APEC is responsible for avian colibacillosis, a significant disease in poultry flocks. Community-acquired urinary tract infection caused by UPEC is one of the most common infectious diseases in the United States (U.S.), affecting approximately seven million women and costing approximately 11.6 billion dollars annually. Complications from UTI result in ca. 23,000 deaths annually in the U.S., in contrast, to ca. 35 deaths associated with Shiga toxin-producing *E. coli*. NMEC is one of the leading causes of neonatal bacterial meningitis. Sepsis is the 6th leading cause of death in the U.S. The ExPEC are often resistant to multiple antibiotics, including fluoroquinolones and extended *beta*-lactamases. They are present in many foods, such as poultry meat, red meat, and fresh produce. Research has connected poultry and poultry meat to disease (e.g., UTI) in humans. The objective of this symposium is to review the evidence connecting food to human disease, describe the genetic characteristics of ExPEC, discuss the latest research on vaccine development, and the methods for post-harvest control of ExPEC in food and water. This symposium will be of benefit to poultry breeders, meat and poultry processors, as well as the public health community and consumers.

S60 A New Paradigm: Cutting Pathogens Off at the Pass by Understanding Their Evolution Dynamics

MANAN SHARMA: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
TERESA BERGHOLZ: *North Dakota State University, Fargo, ND, USA*
FRANCISCO GARCÉS-VEGA: *(Independent Consultant), Cali, Columbia*

Understanding the evolution and dissemination dynamics of foodborne pathogens in food environment is critical to gain insights into the likeness of the emergence of new pathogens in these scenarios and preventing such events from happening. Coupled use of molecular tools alongside bacterial culture is critical to a complete assessment of these issues. Mathematical models improve our understanding of pathogen dynamics by providing a theoretical framework in which factors affecting prevalence, transmission, and control of the pathogens can be explicitly considered. In the past mathematical modeling has provided more profound knowledge of microbial data. Whole genome sequencing (WGS) generates complex data set, making functional analysis difficult. Effective description and analysis of such data set are of considerable importance in the post-genomic era.

However, little is discussed about the role of WGS data on predictive or mathematical modeling and risk assessment. Combining information could provide a different perspective to genomic/WGS data alone and may answer critical data gaps. The goal of this symposium is to provide a framework of mathematical modeling in predicting foodborne pathogen dynamics in the environment as well as at the genetic level.

Presenters will discuss the past, present, and future of mathematical modeling and WGS. The symposium begins with the application of mathematical modeling using pathogen persistence and prevalence data in the food production environment, specifically to identify spatiotemporal, agricultural, and weather effects which warrant further investigation to determine survival durations of pathogens in pre-harvest environments. It continues with an analysis of pathogen evolution and virulence gene transmission and finishes with insights on using complex WGS data to generate risk-assessment profiles.

S61 Resurgence of Less Recognized and Presumptive Pathogens: Food Safety Implications

ALVIN LEE: *Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA*
PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
ROY BETTS: *Campden BRI, Chipping Campden, United Kingdom*
KEITH LAMPEL: *U.S. Food and Drug Administration (retired), Laurel, MD, USA*

Reported incidence of emerging, less recognized and opportunistic pathogens linked to outbreaks of food and waterborne illness in Europe and the U.S. have been increasing. It is increasingly recognized that many otherwise commensal organisms can become pathogens under right conditions, in a right host and, if consumed in sufficient quantities. Recent outbreaks linked to *Cyclospora cayetanensis, Toxoplama gondii,* Hepatitis E, and other less recognized/presumptive foodborne pathogens and their food safety implications. The global sourcing of food and ingredients, especially, fresh produce, fruits and vegetables as well as RTE, minimally processed foods and supply chain issues have allowed the emergence of some of these pathogens with opportunistic tendency to come to the forefront of food safety. More troubling is the incidence of these pathogens in novel food sources. Speakers from industry, academia and regulatory agencies will review less recognized emerging pathogens and discuss their food safety implications.

S62 Novel and Emerging Technologies for Improving Sanitation

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DALE GRINSTEAD: *Diversey, Racine, WI, USA*
SIMA HUSSEIN: *Ecolab Inc., Greensboro, NC, USA*

Novel and Emerging Technologies for Improving Sanitation: The way that cleaning and sanitation (C&S) is conducted has not changed extensively over the past 50 years. Commonly used technology, products, and practices that are in use today would be recognizable by someone who was conducting C&S programs in the 1960s or 1970s. Most cleaning is conducted with traditional liquid surfactant cleaners. While there are some differences in the specific chemistry of those products, those differences are largely variations on a theme. Similarly, the suite of antimicrobial agents used has not changed extensively for many decades. Quaternary ammonium chloride, peroxyacetic acid, chlorine bleach, and a few others are still the "go to" agents used to control microorganisms. Although the common practice has largely stood still for more than 2 generations, science has not. There have been many technological improvements that can improve sanitation results. This session will focus on several of those improvements and how they may be able to be used to improve sanitation practices and food safety outcomes. Technologies that will be discussed include improved cleaning and microbial control using surface modification to change the nature of surfaces in food environments to make them easier to clean and sanitize and antimicrobial surfaces that can actively prevent the attachment or growth of microorganisms on those surfaces between cleaning cycles. We will discuss new technology to control biofilms including use of beneficial or harmless bacteria to out-compete harmful bacteria and synergists that can make biocides more effective against biofilms.

Sanitation is about more than just chemistry. The most effective and innovative chemicals will not work if they are not used or not used properly. Sanitation failures are often human failures; they are failures to do the right thing. Therefore, this session will also address new ways to drive and verify compliance of cleaning and sanitation processes. Process improvement technologies discussed will include new training procedures such as augmented reality and process verification tools such as digital chemical use monitoring.

S63 Application of Principles of Failure Mode Effects Analysis (FMEA) for Effective Verification and Implementation of Food Safety Plans

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LILIA SANTIAGO: *Kellogg's, Battle Creek, MI, USA*
AARON UESUGI: *Kraft Heinz Company, Glenview, IL, USA*

The Food Safety Modernization Act (FSMA) requires hazard analysis, a key component that manufacturing facilities need to consider for effective implementation of risk-based preventive controls. Hazard analysis consists of hazard identification and hazard evaluation, both of which are essential to determine if an identified reasonable foreseeable hazard requires a preventive control. Current HACCP based systems often rely on qualitative approaches to understand if food safety risks are controlled across the supply chain and hence there is a need for a powerful systematic preventive method for risk management to verify if the preventive controls are significantly minimizing or preventing food safety hazards. Failure Modes and, Effects and Analysis (FMEA) is a semi-quantitative risk assessment methodology designed to identify and address all potential failure modes failures during manufacturing of a process or product. A FMEA risk assessment tool can be used to verify the effectiveness of the implementation of preventive controls in a manufacturing facility's food safety plan. In the FMEA methodology, potential risks of the processes are detected and assessed in every step based on likelihood of occurrence of the hazard (O), severity of the hazard (S) and possibility to detect the failure (D) before affecting the customer or consumer, ideally before consumption. A Risk Priority Number (RPN = O X S X D) is calculated and corrective actions are suggested for potential failures that have an RPN value larger than the selected threshold value to reduce and eliminate the potential failures from the system. Thus, incorporation of FMEA analysis within the verification procedure of HACCP based system may be a convenient tool for better food safety assurance. The symposium will present an overview of FMEA process and practical examples demonstrating the application of FMEA principles to control biological, chemical and physical hazards. We anticipate that the examples shared will spur discussions with attendees to collectively benchmark current practices among participants.

S64 Attributing Illnesses to Food Sources in the Face of Uncertainty

MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*
MICHAEL BATZ: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
CARY CHEN PARKER: *U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA*
BEAU BRUCE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ARIE HAVELAAR: *University of Florida, Gainesville, FL, USA*

A major challenge for a risk-based food safety system is attributing and quantifying the amount of illness caused by pathogens in specific foods, i.e., foodborne illness source attribution. Foodborne illness source attribution estimates are necessary to understand the burden of foodborne disease and provide critical information for risk-informed priority setting and policy evaluation. However, attribution studies often use data with considerable underlying variability and various systematic biases. In addition, there are uncertainties with different modeling methods that must be considered when conducting attribution studies or interpreting their results. Characterizing the uncertainty around attribution estimates is important, although often difficult because uncertainty affects how attribution estimates are interpreted and used in food safety decision making.

This symposium will include a diverse and international set of speakers from academia, public health agencies, and regulatory authorities. It will include four presentations on new and evolving methods of source attribution and the challenges and implications of uncertainty in the underlying data and attribution methods. The presentation topics will include a scoping review of the literature, structured expert elicitation, and various analyses of outbreak data. This session is being organized by the Interagency Food Safety Analytics Collaboration (IFSAC), a partnership between the U.S. Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and USDA Food Safety and Inspection Service (FSIS).

S65 Safety of Animal Source Foods in Low and Middle Income Countries

ARIE HAVELAAR: *University of Florida, Gainesville, FL, USA*
HUNG NGUYEN VIET: *ILRI, Hanoi, Viet Nam*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia*
BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*

Globally, 35% of the burden of foodborne disease is associated with animal source foods (ASF), but this varies widely between different countries and regions. The burden of ASF is highest in low-and middle-income countries (LMIC), specifically in Africa and South East Asia. Yet, in these regions, promoting the consumption of ASF is considered one of the best approaches to reduce stunting, which still affects 155 million children worldwide. The full health benefits of ASF consumption can only be achieved with a concomitant improvement of their safety.

This symposium will presents results and new initiatives to promote the safety of ASF by international organizations (WHO, FAO), USAID funded Feed the Future Innovation Labs and a program jointly sponsored by the Bill and Melinda Gates Foundation and the UK Department for International Development. It will also offer new opportunities for researchers from LMIC to present their results and expand their network at the major global food safety event, and will strengthen the global scope of IAFP.

S66 Let's Hear from Next Generation Food Safety Scientists on Pathogen Behavior in Ready-to-Eat Foods

NEDA NASHERI: *Health Canada, Ottawa, ON, Canada*
VIVIAN LY: *University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada*
KAYLA MURRAY: *University of Guelph, Guelph, ON, Canada*
VICTOR JAYEOLA: *North Carolina State University, Raleigh, NC, USA*

A current research collaboration between Health Canada, the University of Guelph and the University of North Carolina are investigating the survival and inactivation of *Listeria monocytogenes, Salmonella,* and foodborne viruses during the storage of low- moisture foods. This is a wide-ranging research consortium funded by the ILSI North America Food Microbiology Committee and includes a number of developing research scientists who will also present their findings. The ILSI North America Food Microbiology Committee is committed to proactively improving the understanding and control of microbial food safety hazards to enable scientifically informed decision making. The Committee achieves its mission by funding research that is conducted at institutions who also train the next generation of food safety scientists.

Ready to Eat low-moisture products such as nuts, dried fruits, cereal products, and chocolate are often ingredients used in the manufacturing of many food products. They carry significant potential for the amplification of outbreaks and recalls over a wide variety of products. The research consortium represented by this next generation of food safety experts is studying several aspects of pathogen behavior in low-moisture ready-to-eat foods and goes beyond traditional thermal mitigation strategies.

Objectives include:

(1) survival and potential changes in virulence and the microbiome of the food with time and temperature fluctuations during storage.

(2) effectiveness of nontraditional mitigation processes in killing cells of bacterial pathogens or viruses

(3) genetic alterations in genomes during long term storage

S67 Antibiotic Reduction, Alternatives, and the Relationship to Food Pathogen Outbreaks

WILLIAM CHANEY: *Diamond V, Cedar Rapids, IA, USA*
SCOTT GUSTIN: *Tyson Foods, Springdale, AR, USA*
LINNEA NEWMAN: *Merck Animal Health, Madison, NJ, USA*
RICHARD GRIFFITHS: *UK Poultry Association, London, United Kingdom*

Current and new marketplace food trends and consumer demands related to antibiotic-free and antibiotic reduction are changing the way animals are raised for food production. Food industry stakeholders (producers, retailers, food service, etc.) are taking steps to address antimicrobial stewardship and identify alternatives to antibiotics. The sudden reduction and/or elimination of antibiotics, without considering alternatives, can be reflected in increased animal disease that can be translated in more potential of a food pathogen outbreak. Some companies have taken steps and learned valuable lessons in antibiotic reduction that are worth sharing with the large audience at IAFP. As a continuation to last year's Pre-harvest roundtable "Responsible Use of Antibiotics. Are We Making Progress?", we will invite experts to this symposium to share and discuss 1) Areas of concern when reducing and/or eliminating the use of antibiotics; 2) Best alternatives to mitigate issues that antibiotic reduction could heighten (global perspective); 3) Food safety concerns due to antibiotic reduction—How have countries that have pioneered in this area such as Denmark address these issues?; 4) How antibiotic alternatives could address food safety concerns in pre-harvest stages.

S68 Using Food Microbiomes

NUR HASAN: *CosmosID, Rockville, MD, USA*
MENU LEDDY: *Orange County Water Board, Orange City, CA, USA*
LAWRENCE GOODRIDGE: *University of Guelph, Guelph, ON, Canada*
GREGORY SIRAGUSA: *Eurofins Microbiology, New Berlin, WI, USA*

Are you still wondering about how and why food microbiomes should be in your food microbiologist's tool bag? Food Microbiologists rely on the standard aerobic plate count and other indicators to assess product quality, spoilage/pathogen potential and hygiene. Many consider culture to be the 'King of Methods.' We are comfortable with our abilities to know when to suggest plating methods be used, to understand the basic process for sample handling, and in interpreting the results. However, we know a culture is biased and captures only a small fraction of viable bacteria, yeasts and molds in samples. This can lead to ambiguous data and faulty conclusions.

Microbiomes are now a mainstay across almost all fields of microbiology and becoming more so in food microbiology. From a single assay, a microbiome tells us identity and proportions of microorganisms and can identify specific genes in samples.

This symposium will present microbiome case examples and uses from the perspectives of microbiome users. It is our goal is that attendees will begin to feel comfortable adapting this cutting edge technology to answer contemporary issues in their respective industries.

S69 Biofilm and Low-water Activity Foods

DIANE WALKER: *MSU Center for Biofilm Engineering, Bozeman, MT, USA*
KATARZYNA LEDWOCH: *Cardiff University, Cardiff, United Kingdom*
KARL THORSON: *General Mills, Minneapolis, MN, USA*
ALIYAR FOULADKHAH: *Public Health Microbiology Laboratory, Tennessee State University, Nashville, TN, USA*

Dry-surface biofilms are an increasing concern in the food industry especially related to low-moisture foods. Although biofilms have traditionally been associated with wet surfaces, research has shown that microorganisms can survive for extended periods in a desiccated state on dry surfaces. The health-care industry has also been tackling this issue on medical equipment and environmental surfaces, indicating that bacteria can survive and then be transferred from dry surface biofilms to the hands of healthcare workers and patients as an important role in transmission. In the food industry with the Current Good Manufacturing Practice, Hazard Analysis, and Risk-based Preventive Control for Human Food the focus on sanitation as a preventive control is an increasing concern. This symposium will focus on the characteristics and approaches to control of dry-surface biofilms on surfaces processing low-water activity.

S70 Polypropylene Permaculture? Microplastics in Terrestrial Agricultural Systems

ESPERANZA HUERTA LWANGA: *El Colegio de la Frontera Sur/Wageningen University and Research, Campeche, Mexico*
SHANNON BARTELT-HUNT: *University of Nebraska-Lincoln, Omaha, NE, USA*
MARION BRODHAGEN: *Western Washington University, Bellingham, WA, USA*

Microplastics are a well-documented source of contamination in surface water and seafood, but little research has investigated the potential of microplastics to influence terrestrial food systems. The hydrophobic nature of these compounds in the environment can cause them to absorb toxic pollutants such as polybrominated diphenyl ethers, endocrine-disruptors, and pharmaceuticals and personal care products. Furthermore, biomagnification of these contaminants through terrestrial food chains may exacerbate the exposure potential to humans. However, research is only beginning to explore the consequences of their deposition in agricultural systems.

Concern about environmental impacts of traditional nutrient and pest management approaches, as well as reduced availability of traditional irrigation water sources, has led to increased interest in alternative agricultural inputs. As a result, adoption of reclaimed wastewater for irrigation, biosolids and composts for nutrient management, and biodegradable plastic row cover for weed suppression has increased. However, these inputs are potential reservoirs for microplastics. Additionally, the risk that microplastics and their associated chemical contaminants pose to consumers if present in agricultural systems is not well understood.

This symposium will bring together cutting-edge research working to address the potential of microplastics occurring in terrestrial agricultural systems and the long-term consequences of their deposition including relevance to human health. By doing so, we hope to create a platform to address and refine the questions concerning mitigation of chemical contaminant risk in the agricultural environment.

The specific objectives of this symposium are to 1) present an overview of sources of microplastics, transmission routes to terrestrial systems, and human health risks associated with exposure to this xenobiotic 2) evaluate current research on microplastic transfer to agricultural systems via alternative inputs such as biosolids and biodegradable plastic row cover 3) explore current efforts to mitigate terrestrial food production systems exposure to microplastics.

S71 Revolutionary Diagnostic Changes are Shifting the Epidemiological Landscape and Posing Challenges for Outbreak Identification

MELISSA MILLER: *University of North Carolina, Chapel Hill, NC, USA*
HEATHER CARLETON: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ROBYN ATKINSON-DUNN: *State Laboratory, Salt Lake City, UT, USA*
MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*

Foodborne disease outbreak surveillance plays a pivotal role in our nation's food safety system. It has primary responsibility for identifying problems in the food supply chain that would not otherwise be recognized, limiting the extent and impact of outbreaks, and providing key information to the food industry for risk management. The primary source of data for surveillance comes from clinical diagnostic laboratories. The detection methods for foodborne pathogens in stool samples have been evolving from traditional microbiological cultivation to novel culture-independent diagnostic tests

(CIDT). This trend accelerated recently with the introduction of CIDT syndromic panels that test for multiple pathogens. The shift has been driven by the demand for faster protocols that can provide physicians information to treat patients. The adoption of CIDT by clinical laboratories has increased dramatically and it is now estimated that more than 20% of all bacterial infections are tested using only a CIDT. This major technological move has multiple epidemiological implications. Without isolates, the detection of clusters and outbreaks as well as the identification of resistance and virulence trends will be notably diminished. The third important consequence of increased CIDT adoption is the difficulty of measuring disease incidence rates due to different performance compared to culture methods. The proposed symposium is intended to: 1) provide an overview of the commercially available CIDT instruments and kits, 2) describe benefits and extent of CIDT-adoption by the diagnostic and public health sector, 3) report on the already observed epidemiological trends resulting from CIDT, and 4) provide a selection of possible solutions that the CDC, other public sector agencies and industry are pursuing to minimize their impact on outbreak detection and etiological agent characterization. The topic of CIDT has been largely missing from previous IAFP meetings and because of its importance, it is critical to include in 2019.

S72 Distribution of Foodborne Pathogens – Geographical Insight from the Use of WGS

MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
HEATHER CARLETON: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
MARC ALLARD: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*

The use of Whole Genome Sequencing (WGS) techniques for food safety is increasing around the world and a greater number of institutions are generating and sharing sequences of the most common foodborne pathogens. For some of these pathogens, there is now a sufficient number of publicly-shared sequences to allow for studies on the geographic distribution of certain genetic strains. With the globalization of the food supply and international travel becoming commonplace, there is the potential for distinct geographical signals to become blurred as foodborne pathogens colonize new environments. Much like the application of WGS for uncovering human ancestry, the combination of sequence data and other relevant information – or metadata – can help to uncover the distribution, movement, and spread of pathogens in our food supply around the globe.

This roundtable will bring together experts in this field to 1) give an update of how widely used WGS is around the world and how much of what is being sequenced is presently shared publicly; 2) How much do we know about the geographic distribution of certain strains; 3) How far can we go into the analysis of pathogen movements around the globe; and 4) Present work on the WGS analysis of recent international outbreaks and what we can learn from it.

SF1 Predictive Microbiology and Risk Assessment Software Fair: Spotlight on Case Studies

YUHUAN CHEN: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
MARK TAMPLIN: *Centre for Food Safety & Innovation, Tasmanian Institute of Agriculture, University of Tasmania, Hobart, TAS, Australia*
FERNANDO PEREZ-RODRIGUEZ: *University of Cordoba, Cordoba, Spain*
PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece*
MARK TAMPLIN: *Centre for Food Safety & Innovation, Tasmanian Institute of Agriculture, University of Tasmania, Hobart, TAS, Australia*
FLORENCE POSTOLLEC: *ADRIA - UMT ACTIA19.03 ALTER'iX, Quimper, France*

Predictive microbiology and risk assessment tools are useful to accelerate and enhance microbial food safety assessments. The application of predictive models have been scientifically validated as rapid response tools and the approach is recognized by the Codex alimentarius, national and international regulations to assess the exposure to microbiological contaminants and support management decisions.

The International Committee on Predictive Modelling in Foods (ICPMF) with the support of the MMRA PDG is delighted to organize a Software Fair at the IAFP Annual Meeting in 2019 to ensure the dissemination and the use of predictive microbiology and risk assessment tools by the food industry.

The top six software currently available to perform these assessments will be presented at the symposium. These are ComBase, CB Premium, FDA-iRisk, MicroHibro, GroPin and Sym'Previus.

The symposium will be divided into two parts. In the first part (1.5h), the six software will be briefly presented focusing each on a specific example based on real industrial case studies including shelf-life determination, thermal processing, HACCP and formulation, sampling and full exposure or risk assessments. Then, in the second part of the symposium (1.5h), the participants will get the chance to practice using the software of their choice and their preferred case study. This live demo will ideally take place in a dedicated and equipped space with 6 different areas: one for each software (tables for small groups and screens). Each developer will have a small group and will perform the demo on the previously presented software tool and interested participants could also try on their own laptops.

Attendance audience: Food Laboratories, food consultants, food safety authorities, researchers with interest in microbial food quality and safety, food quality and safety departments from food companies.

Roundtable Abstracts

RT1 Is It Time for Food Safety Performance Standards Since Zero Risk is Not an Option?

CANDACE DOEPKER: *ToxStrategies, Newport, KY, USA*
DONNA GARREN: *American Frozen Food Institute, McLean, VA, USA*
SCOTT HOOD: *General Mills, Golden Valley, MN, USA*
ANGELA SIEMENS: *Cargill, Inc., Towanda, KS, USA*
CRAIG HEDBERG: *University of Minnesota, School of Public Health, Minneapolis, MN, USA*

Food safety systems rely on verification activities to determine if the system is working as designed and validated. Microbiological performance standards can be used to verify if a processing system is adequately controlling a specific hazard. Performance standards should be set to protect public health. Sampling protocols and microbiological testing methods must be appropriate for the food being tested. In the U.S. poultry industry, performance standards have been in place to measure the prevalence of *Salmonella*. Over time, the performance standards have changed to reflect the improved conditions in the industry. Prevalence based performance standards may work for other product categories, especially in dry products of raw agricultural products such as wheat flours and the produce area, especially for frozen fruits and vegetables. This roundtable discussion will explore the current and potential future uses of performance standards in foods where it is not reasonable to expect zero presence of pathogens.

RT2 Today's RTE Redefined – Managing Environmental Controls and the Risk of the "Reasonably Foreseeable"

SEAN LEIGHTON: *Cargill, Inc., Wayzata, MN, USA*
PEYMAN FATEMI: *The Acheson Group, Pleasanton, CA, USA*
SCOTT HOOD: *General Mills, St. Paul, MN, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
ERIC BROWN: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*
DAVID ACHESON: *The Acheson Group, Big Fork, MT, USA*

No-bake, flour-based cookies. Raw potato salad. Asparagus smoothie. Your food may not be specifically listed as ready to eat (RTE) by FDA, but is it "reasonably foreseeable" that a consumer would eat it without further processing? What is RTE? What is required of these foods? And where and how should you establish an environmental monitoring and control program to manage that risk?

Today's food manufacturers are being held to ever-tightening regulatory and customer controls while, at the same time, consumer uses of foods continually expand beyond standard definitions. Even while the food industry is seeing emerging risks in pathogenic contamination, the trending consumer belief toward less processed-more healthy is taking a vast array of raw and minimally processed foods closer and closer to RTE. This ambiguity and ever-emerging food trends put the food industry at increasing risk which many don't realize or know how to control.

Bringing in industry-leading regulatory, manufacturing, legal, and consultation experts, this roundtable will focus on the challenges of defining RTE and non-RTE; the regulatory expectations; how to manage an FDA "swab-a-thon" and how to conduct your own; use of whole genome sequencing (WGS); and design of a good environmental monitoring and control program, including equipment and plant design, to reduce risk.

RT3 Emerging Foods: Seaweed; Superfood, Health & Safety, Challenges & Opportunities

PATRICIA BIANCHI: *Aquaculture Stewardship Council, Utrecht, Netherlands*
BALUNKESWAR (BALU) NAYAK: *School of Food & Agriculture, University of Maine, Orono, ME, USA*
WILLIAM BURKHARDT: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, Mobile, AL, USA*
ANOUSHKA CONCEPCION: *Connecticut Sea Grant and Department of Extension, University of Connecticut, Groton, CT, USA*
ANDREA (TREY) ANGERA: *President, Springtide Seaweed, LLC, Gouldsboro, ME, USA*

Seaweed aquaculture makes up a significant portion of organisms cultured worldwide, ~28 million metric tons, with a value ~US $10 billion. In addition to other uses (i.e., hydrocolloids and feed) for cultivated seaweed, *Undaria, Porphyra* and *Laminaria* are extensively used as food for direct human consumption. Seaweed foods capitalize on the trend for natural and organic products, and are a growing trend in the U.S. and internationally.

China is the world's largest producer of edible seaweed; additional production comes from Korea, Indonesia, Japan and the Philippines. There are growing businesses in more than 50 countries (FAO State of the World's Fisheries and Aquaculture). In the U.S., collaboration within Connecticut and Maine industry and institutions are at the forefront for the development of the seaweed aquaculture industry. Maine leads with its cold, clean waters, and coastal infrastructure with many established companies that sell processed or fresh, wild-harvested or farmed, local, sea vegetables through retail and direct sales.

The potential of seaweeds as sources of natural and healthful food is widely recognized and known as "superfood" with its nutrient content and free from pesticides. In comparison with land vegetables, seaweeds are potentially rich sources of polysaccharides, minerals, and certain vitamins. However, there are regulatory gaps affecting safety concerns, risk assessments and guidelines.

This roundtable will cover the key insights gained through research, production, and compliance for the development and safety of seaweed as an emerging superfood. Our panelists shall discuss the regulatory and supply chain challenges. They will reveal the opportunities for industry, and government to further advancement in sustainability, farming and processing best practices including HACCP and other food safety protocols, the role of water quality (pre-and post-harvest), product testing for contaminants, nutrition, Best Management Practices (BMPs) and third party certifications for market access and community development.

RT4 Cyclospora: It's Not Just an Imports' Issue

TRISHA ROBINSON: *Minnesota Department of Health, Minneapolis, MN, USA*
MICHAEL OSTERHOLM: *University of Minnesota, Minneapolis, MN, USA*
WALTER RAM: *Giumarra Companies, Tuscon, AZ, USA*
JENNIFER MCENTIRE: *United Fresh, Washington, DC, USA*
SAMIR ASSAR: *U.S. Food and Drug Administration, College Park, MD, USA*

Recurring outbreaks of *Cyclospora caytanensis* have historically traced back to fresh produce items imported into the United States. In 2018, illnesses totaling over 2,000 resulted from consumption of produce items that were both internationally and domestically supplied. In this session, we'll discuss the impact improved diagnostic testing has on identifying illnesses, generate hypotheses on how domestic vs. imported foods become contaminated, and discuss potential hazard controls to prevent contamination of produce items.

RT5 #FoodSafety: Practical Advice for Digital Communication and Science Storytelling

AUBREY PARIS: *Institute on Science for Global Policy, Princeton, NJ, USA*
ADAM YEE: *My Food Job Rocks, Sacramento, CA, USA*
TRACIE SEWARD: *Association of Schools and Programs of Public Health, Washington, DC, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
MICHAEL BATZ: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

The past decade has seen an explosion of revolutionary online technologies, leading to increased interest and necessity in leveraging digital tools for proactive and transparent science communication. Digital platforms (i.e., social media, blogs, and podcasts) represent exciting new mediums for global engagement about food safety topics by allowing food safety professionals to interact with the public while engaging in real-time dialogue. Despite the hype digital communications have received, concrete discussions outlining effective utilization of these tools remain absent, often lacking depth, explicit instructions, and concrete case studies. Additionally, as food safety professionals branch out to newer communication avenues, training in how to best frame messages and establish trust is needed. Proactive trust building and science storytelling are critical skills for today's food safety professionals if they wish to engage the public and other scientists.

This roundtable will: (a) provide a practical toolkit for scientists to utilize digital tools in building trust and developing their own digital communication, (b) highlight and integrate the basics of science storytelling with food safety communication, and (c) give participants hands-on experience crafting food safety messages. The roundtable will conclude with a short activity where participants will develop food safety messages aimed at various audiences. Participants and panelists will discuss the presented messages and brainstorm dissemination methods across various digital mediums. At the conclusion of the session, participants will be provided a panelist-reviewed list of "best practices" to jumpstart future endeavours in digital engagement. The goal of this roundtable is to move beyond a surface-level discussion of digital communication tools by empowering participants with the necessary information to engage in various mediums to promote an inclusive food safety discussion.

RT6 Supply Chain Verification of a Sanitation Program

NADIA NARINE: *Lumar Food Safety Ltd., Richmond Hill, ON, Canada*
JESSICA JONES: *Chick-fil-A, Inc., Atlanta, GA, USA*
GORDON HAYBURN: *Trophy Foods Inc., Mississauga, ON, Canada*
RICK STOKES: *Ecolab Inc., Eagan, MN, USA*
RICHARD BROUILLETTE: *Commercial Food Sanitation, South Burlington, VT, USA*
EVAN ROSEN: *Tate & Lyle, Hoffman Estates, IL, USA*

Recent outbreaks associated with restaurant operations and fresh produce have also uncovered the need for greater awareness of supplier activities, tracking and systems, agriculture-oriented hygiene, and monitoring activities. These range from the quality of water and equipment and hygienic handling in transit and more. The Preventive Controls Rules and the Foreign Supplier Verification Program requirements under FSMA provide an opportunity for processors to use third party audits and documentation reviews as a-means of verifying supply chain food safety. These practices are relied on heavily in the industry by many companies. Therefore, a discussion as to the best practices surrounding verification activities is warranted.

Even with all the efforts of food processors, pathogens remain one of the main reasons for recalls and, therefore, this should be a critical part of any supplier verification or supply chain program review. However, there is not much current discussion as to the best practices related to sanitation verification. A review of a sanitation program can be monitoring the effectiveness of sanitation completion, cleaning after maintenance repairs, trending of data for behavioral changes in the sanitation program and using data for continual improvement of the sanitation program. However, this can be a challenge to meet when accepting documentation from suppliers, both domestic and foreign suppliers. The variability in GFSI and other second or third party schemes, combined with a wide variation in processing operations, may pose challenges in executing a supplier verification program surrounding sanitation operations.

This roundtable will bring a range of experts to share challenges, systems issues, as well as best practices and insight on how to best manage the reviewing of a sanitation program on site and through a documentation review exercise. This facilitated panel discussion will provide an opportunity to discuss key indicators to watch for, and those that can help prevent future issues and outbreaks.

RT7 Home Food Delivery: The Last Mile is Not What It Used to be

DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
HOWARD POPOOLA: *The Kroger Company, Cincinatti, OH, USA*
JOSEPH NAVIN: *Uber, San Francisco, CA, USA*
ALLISON JENNINGS: *Amazon, Seattle, WA, USA*
MELANIE ABLEY: *U.S. Department of Agriculture–FSIS, Springfield, VA, USA*

The last mile is not what it used to be. Driving to the grocery store, buying your food, and then taking it home to prepare is not your only option. Today your groceries can be delivered directly to your door, you can have a robot meet you with your groceries, you can have food delivered to another location for pick up, you can get meal kits or specialized components delivered. And thanks to third-party delivery services like you can get ready to eat meals delivered from almost any restaurant. All of these different ways for consumers to get food to their homes and onto their plates raises a number of food safety questions. This roundtable will address these and other questions. Questions include: Is there adequate temperature control of food during the delivery process? Who is responsible for ensuring food safety and proper temperature control? Does home delivery impact the shelf life of the food? Attendees will leave with a better knowledge of the risks associated with the varied forms of home food delivery and gain understanding of how these risks can be mitigated. Panelists will include experts past and present members of Conference for Food Protection committees charged to examine this issue, federal government representatives, as well as experts from industries including retail, e-commerce, and home food delivery.

RT8 Cultural Influences on Food Safety Research and Education Programs in a Global Society

ROSE OMARI: *Science and Technology Policy Research Institute Council for Scientific and Industrial Research and EATSAFE Ghana, Accra, Ghana*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
BOBBY KRISHNA: *Dubai Municipality, Dubai, United Arab Emirates*
MARIA TERESA DESTRO: *bioMérieux Inc., São Paulo, Brazil*
YOHAN YOON: *Sookmyung Women's University, Seoul, South Korea*

An expanding, globalized food system requires food safety conversations that consider cultural differences. Conversing with researchers and experts who interact with diverse communities heightens understanding about food handling behaviors and safety priorities across cultures. This roundtable will engage panelists who provide food safety information to international communities, resonating with individuals who wish to develop or improve culturally aware food safety programs and research in an age where translating and enforcing effective food safety programs is increasingly difficult with open markets and trade.

Dietary preferences, water quality, and technological advances are just some influencers of food safety priorities that conflict with food sustainability, begging questions about balancing food safety and security priorities. "When in doubt, throw it out" is commonly used in the United States to warn against expired food, but discarding foods due to doubt is not sustainable for some communities. Countries with disparities between affluent and marginalized groups that lack basic sanitation struggle to balance food safety and security. Additionally, some countries that rely on food safety methods and technologies developed elsewhere struggle to apply and adapt them to the needs of their community.

Media outlets and food safety programs disseminate food-handling recommendations, but persistence of traditional or trendy methods of evaluating safety and quality can overrun best practices. Open-air markets and street food vendors in certain areas challenge the efficacies of food safety communication and training. The panel will provide a discussion on developing and communicating relevant science-based food safety behaviors with cultural awareness.

As foods and consumers cross borders, so do the ideas of food safety and security that are intricately intertwined with cultural differences. Expert panelists will discuss their experiences working with consumers, food businesses, and other researchers across the globe, shedding light on how to best research and effectively communicate food safety and security issues.

RT9 Challenges in Low-moisture Food Plant Sanitation – A Dairy Perspective

NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
STEPHEN WALKER: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
RICHARD BROUILLETTE: *Commercial Food Sanitation, South Burlington, VT, USA*
RHONDA FRASER: *Fonterra, Palmerston North, New Zealand*
SHANTANU AGARWAL: *MarsWrigley, Chicago, IL, USA*
DEAN TJORNEHOJ: *CDI, Visalia, CA, USA*

There have been recent U.S. recalls and international illness outbreaks attributed to powders including in January 2017 when 41 infants were hospitalized in France/Spain for *Salmonella* from non-fat dry milk. This incident has significantly raised awareness of processors, large buyers, and with regulators. Traditional water-based cleaning/sanitation is not desirable in dry manufacturing facilities so there is not a single set of universally accepted 'answers' for pathogen controls. Practices and standards are also rapidly shifting and driven by FSMA requirements for preventive controls/supplier verification, new whole-genome sequencing tools, metagenomic mapping, export requirements, and increased regulatory activity. This roundtable is intended to hear from dairy industry experts and academics on best practices and share on topics including sanitation, design, environmental monitoring, product testing, how to respond to a presumptive positive swab, and other related topics.

RT10 Food Workers – Technology Approaches and Other Interventions for Keeping Sick People Out of the Operation

ARON HALL: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
VERONICA BRYANT: *NC Department of Health & Human Services, Raleigh, NC, USA*
JASON HORN: *In-N-Out Burger, Baldwin Park, CA, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*

*"A good line cook never shows up late, **never calls in sick**, and works through pain and injury."* - Anthony Bourdain, *Kitchen Confidential*, Page 64

"You want loyalty from your line cooks. Somebody who wakes up with a scratchy throat and slight fever and thinks it's okay to call in sick is not what I'm looking for" - Anthony Bourdain, *Kitchen Confidential*, Page 66

Anthony Bourdain might not have been a researcher, but he gave some insight into the inner workings of the kitchen. We have all met the person that brags about their perfect attendance as a sign of commitment to the company's success.

We all agree that ill food workers need to stay at home while sick. That is easier said than done. What incentives can you put in place to make sure the workers stay at home? What are the barriers that prevent the worker to call out/in sick? Is it cost prohibitive to pay employees sick pay? How do cultural norms affect behavior? How do we leverage technology to make it better for all parties involved?

The FDA requires employers to restrict employees for 48 hours after being asymptomatic for Norovirus. Current mathematical models suggest that 6.7 million illnesses could be avoided annually if both employees and employers would be 100% compliant with current regulation regarding exclusions and restrictions of ill food workers. This round table will answer the central question: How do we do better job of keeping ill employees out of the operations? What does that look like in practice?

RT11 Revitalizing the Future of Food Safety Extension

CATHERINE CUTTER: *Penn State University, University Park, PA, USA*
MELISSA CHASE: *Virginia Tech/Virginia Cooperative Extension, Blacksburg, VA, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
COURTNEY CRIST: *Mississippi State University, Starkville, MS, USA*
CONNIE FISK: *Produce Safety Alliance, Plattsmouth, NE, USA*

Since its 1914 inception to address rural agricultural issues through applicable research and education in agriculture, the U.S. Cooperative Extension Service (CES) has promoted food safety initiatives like consumer education (e.g., home food preservation, safe food-handling practices), training and assistance for the food industry and government (e.g., FSMA training, HACCP certifications, food entrepreneur initiatives), targeted research and dissemination of technologies, and 4-H youth programs. While the original mission of science-based information dissemination to the public is still relevant, the food safety aspects of CES must continue to evolve and adapt in the face of multiple challenges threatening its efficacy, reach, and future.

In response to funding shifts, some states have moved towards a model supporting specialized area agents in order to remain impactful, while other states' agents oversee various programming across a large geographic span. To increase stakeholders, CES collaboration with industry groups and state departments of agriculture has demonstrated efficacy; however, this collaboration differs across states. To improve messaging, CES has re-engineered food safety material development through movements like the eXtension national website and new research-based training strategies like the SafePlates Food Protection Manager Certification. In response to urbanization, other groups are developing alternative communication and information delivery models (e.g., blogs, newsletters, social media accounts) to support a wider interest in food safety. Furthermore, while students in Food Science disciplines are often engaged in facets of research and teaching, they may have little exposure to Extension associated opportunities.

This symposium will focus on collaborations across academia, government agencies, and industry towards Extension by: (a) reviewing its infrastructure to address needs, shifting priorities of staff, and funding; (b) exploring case-studies in which efforts have been reinvented to best serve the population; (c) discussing the fast-paced growth, incorporation, and influence of technology; and (d) recruiting the next generation of professionals.

RT12 Examining the Mutual Benefits of a Defined Supplier Monitoring Program: What is the Value of a Supplier Monitoring Program to the Retailer or Food Service Company and Suppliers?

WHITNEY LANGSDON: *Wendy's, Columbus, OH, USA*
AUSTIN BERNARD: *Chick-fil-A, Inc., Atlanta, GA, USA*
PAM MEIJER: *Mérieux NutriSciences, Chicago, IL, USA*
SHARON BEALS: *CTI Foods, Fort Worth, TX, USA*
PAUL HALL: *Flying Food Group, Lakeland, FL, USA*

This roundtable will address the value and mutual benefits of Supplier Monitoring Program from the perspective the retailer/food service organization, the supplier and the consumer. The focus will be on program design and parameters (specifications) to consider measuring and monitoring as part of a robust program which can result in defined quality and financial benefits for the manufacturer and retail/food service organization, as well as higher consistent quality for the consumer, as measured by an unbiased third party.

Our discussion will cover how effective and robust programs address food quality and safety at multiple levels in the product life cycle, including product development, product safety & quality and supplier quality while covering a variety of food matrices. Incorporation of these multiple matrices and stages will be covered as well as a discussion of what the results of the programs mean to all participating parties.

Key discussion points and benefits addressed will include:

- Verification of continued compliance to product specifications
- Early alerts on changes in product quality, ultimately preventing consumer complaints
- Help assure product purchased is meeting specification
- Create accountability for production of product
- Development strategic relationships with suppliers
- Assist in the development of supplier capability and knowledge
- Assist in the development of supply chain management strategies
- To promote continual improvement

RT13 Scientific Modernization of Meat Inspection – The International High Speed Train – Catch It or Get De-Railed

CARMEN ROTTENBERG: *U.S. Department of Agriculture, Washington, DC, USA*
ROGER COOK: *New Zealand Ministry for Primary Industries, Wellington, New Zealand*
MARTIN APPELT: *Canadian Food Inspection Agency, Ottawa, ON, Canada*

In recent years, countries across the globe have made progress towards the modernization of meat inspection and putting practices into place to address the microbiological hazards that pose the greatest risks to public health. These modernization efforts are grounded in scientific assessments and include an increased collection and analysis of data to support decision making. The United States has finalized regulations to modernize poultry inspection and has proposed a rule to modernize pork inspection.

The U.S. is not alone in taking these steps towards inspection modernization. Many countries are recognizing that long-standing organoleptic inspection systems cannot detect microbiological risks and that new approaches are necessary for successful protection of public health.

Roundtable

This roundtable will explore different approaches from around the world to modernize meat inspection systems, with a focus addressing microbiological risks as part of the inspection process. The panelists will also discuss any potential inspection modernization impacts or considerations as it pertains to international trade and equivalency determinations.

RT14 The Use of Chemicals in Food Hygiene and Linkage to Microbial Resistance

JONATHAN FRYE: *U.S. Department of Agriculture-ARS-USNPRC, Athens, GA, USA*
MARIA HOFFMANN: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
JOHN DONAGHY: *Nestec Ltd., Vevey, Switzerland*
DONNA GARREN: *American Frozen Food Institute, McLean, VA, USA*
LARRY KOHL: *Retail Business Services LLC, an Ahold Delhaize USA Company, Salisbury, NC, USA*

The presence of biocidal agents in the food chain has brought increasing concerns about the development of antimicrobial resistance and its impact on public health. Yet those agents are used for very good reasons, to protect animal health, support good food hygiene practice, and ultimately protect food safety. It can be difficult to make sense of a complex landscape where risks are real and imminent, yet data are sometimes missing to support science-based decisions. In this panel you will hear about the use of biocidal chemicals to protect public health, from regulators on the difficulty of their role as policy makers; representatives from the food industry will share their experience about how the wrong decision in this critical topic can have serious consequences. Members of the recently run GFSI Technical Working Group on Chemicals in Food Hygiene will also share the outcome of their 18-month long work program on how to ensure consumer protection through the appropriate application of sanitizers, disinfectants and cleaning agents from farm to fork, balancing risks and benefits of their use while facilitating global trade of food.

RT15 Cottage Foods: Harmonizing Food Safety Practices for a Growing Entrepreneurial Industry

SERENA GIOVINAZZI: *Florida Department of Agriculture and Consumer Services, Tallahassee, FL, USA*
ERIC EDMUNDS: *The Acheson Group, Boise, ID, USA*
ABIGAIL SNYDER: *The Ohio State University, Columbus, OH, USA*
ELIZABETH L. ANDRESS: *University of Georgia, Athens, GA, USA*
JOELL EIFERT: *Virginia Tech, Blacksburg, VA, USA*
ERDOGAN CEYLAN: *Mérieux NutriSciences, Crete, IL, USA*

USDA's Economic Research Service reported a 180% increase in U.S. farmers' markets between 2006 – 2014. As state and local legislation allowances of entrepreneurs have percolated into the community to satisfy this newfound demand, there has been a rise in community marketplaces in which consumers have a forum to demand more locally produced products. Cottage food laws, existing in 49 states, allow individuals to produce and sell "low-risk" foods made in their home kitchens. Striking differences in the approved low-risk food lists between states adds confusion to food safety guidelines. Differences in sale limits, license/permit requirements, training requirements, and permitted sale locations further compound the uncertainties faced by entrepreneurs, extension agents, and food safety professionals. These variations act as barriers to academic, extension, and regulatory guidance and outreach for operators and consumers, compromising food safety.

While the cottage food industry has been heralded as mutually beneficial for entrepreneurs and consumers desiring locally made products, there is a need to provide adequate food safety training to stakeholders to reduce the risk of unsafe foods entering commerce. Outreach is needed to support FSMA-exempt groups including cottage food operations, food entrepreneurs, and home food preservers. There is also a need for basic research and validation of food processing and preservation techniques with new processes and equipment.

This roundtable seeks to identify, address, and discuss safety concerns pertaining to the cottage food industry by: (a) reviewing current cottage laws and state-to-state inconsistencies; (b) discussing current best practices and successes for food safety training; (c) addressing challenges with emerging technology, food trends, family recipes, publications, and web-based resources available to consumers; (d) identifying the roles, challenges, and knowledge gaps related to cottage law, food business incubators, commissary kitchens for stakeholders; (e) exploring consistent messaging and training opportunities.

RT16 Has the Time Come for Complete Adoption of the Food Code?

VERONICA BRYANT: *NC Department of Health & Human Services, Raleigh, NC, USA*
JASON HORN: *In-N-Out Burger, Baldwin Park, CA, USA*
DARIN DETWILER: *Northeastern University, Boston, MA, USA*
GLENDA LEWIS: *U.S. Food and Drug Administration, Washington, DC, USA*
ANN MARIE MCNAMARA: *Hazel Analytics, Seattle , WA, USA*

The FDA Food Code is a set of guidelines of the latest scientific thought on the prevention of foodborne illness in retail and foodservice settings. Since it is guidance material and not law, states and local health departments can choose to follow these guidance materials, or not. Some states only partially adopt these materials, some states write their own rules.

Currently, there are 8 different versions of the food code being used by states that chose to do so. This makes it difficult for restaurants and grocery retailers operating stores in multiple locations to train and execute against different expectations, some of which are behind the times in the accepted science of food protection.

This roundtable will discuss the pros and cons of complete ("Universal") adoption of the food code, the need for consistency across various states and jurisdictions, and the need for state-of-the-art requirements for protecting public health in retail and foodservice establishments. Discussion shall also focus on whether the food code should be codified into law.

RT17 Finding the Needle in the Cheese Block: How Do We Create Robust Sampling Plans for Dairy Products?

MARION CASTLE: *New Zealand Ministry of Primary Industries, Wellington, New Zealand*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*
MELINDA HAYMAN: *U.S. Food and Drug Association, Washington, DC, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
TIMOTHY FREIER: *Mérieux NutriSciences, Crete, IL, USA*

Have you ever been asked to test your product for Listeria in a 375g sample? Are you struggling to determine what your indicator results mean? This roundtable session will discuss global perspectives from government, academia, and industry on microbiological sampling for detection of pathogens and indicators as a verification for preventive controls. The focus will be on dairy ingredients and finished products, but have applicability to other foods. The panel will discuss:

- Criteria to consider risk-based sampling
- Target microbe(s)
- When to test, testing frequency
- Appropriate sample sizes, including compositing and pooling. What do the sample sizes mean? Are more samples better?
- Statistical considerations: process control and lot acceptance
- Methods and when validation is needed
- Novel rapid methods

RT18 Building a National Integrated Food Safety System (IFSS)

STEVE MORIS: *Kansas Department of Agriculture, Manhattan, KS, USA*
BARBARA CASSENS: *U.S. Food and Drug Administration, Alameda, CA, USA*
BOB EHART: *National Association of State Departments of Agriculture, Arlington, VA, USA*
JERRY WOJTALA: *International Food Protection Training Institute, Battle Creek, MI, USA*
ERNEST JULIAN: *Rhode Island Department of Health, Providence, RI, USA*
JOSEPH CORBY: *Association of Food and Drug Officials, New York, NY, USA*

FDA manages programs and initiatives that build the infrastructure and capacity of the state, local, territorial, and tribal regulatory agencies and promote a national Integrated Food Safety System (IFSS). Building an IFSS is mandated by the Food Safety Modernization Act (FSMA) signed into law January 4, 2012, by President Barack Obama.

The IFSS represents a seamless partnership among federal, state, local, territorial, and tribal agencies (strategic partners) to achieve the public health goal of a safer food supply. An IFSS also actively solicits input and support from stakeholders.

The seamless operation of IFSS strategic partners will:

- Plan and prioritize work to coordinate resources
- Use foodborne illness outbreak data to inform the development of evidence-based food safety policies and programs, and criteria to evaluate their effectiveness
- Implement efficient, prevention-focused, risk-based inspections and sample collections
- Share data among strategic partners
- Promote use of compliance and enforcement tools for achieving compliance with food safety laws and regulations

An IFSS strives to leverage the participation, coordination, resources, and authorities of all regulatory partners to protect the food supply.

This panel will discuss the advancements made in developing an IFSS, successful case studies of integrated efforts, and the benefits created for stakeholders.

RT19 Improving Post-mortem Inspection of Beef for Human Health Protection

BETH RIESS: *The Pew Charitable Trusts, Washington, DC, USA*
MARK RASMUSSEN: *Iowa State Univ, Ames, IA, USA*
MELANIE ABLEY: *U.S. Department of Agriculture–FSIS, Springfield, VA, USA*
ANDREW POINTON: *APFoodIntegrity Pty Ltd, Grange, Australia*

Post-mortem inspection of every single beef carcass is standard practice around the world. This inspection is for human health protection and animal health surveillance. There have been few changes in inspection practices in the last century, though changes for pig (swine) and sheep inspection have been made more readily. Inspectors still examine organs and viscera visually and manually for signs of infectious disease, parasitic infestations and conditions that would render parts of the animal unsuitable for human consumption. Many of the diseases and conditions being inspected are not zoonotic, and inspection may cause microbial contamination to be spread from one carcass to another. This roundtable will address how the functions of post mortem inspection can be improved to detect conditions important to public health (including implementing newer technologies), and animal disease control (using examples from swine, sheep and beef post mortem inspection), without negatively impacting the safety of meat. Understanding and communicating the risks to all stakeholders is an essential component of the change process.

Roundtable

RT20 Application of High-throughput Sequencing by Industry: Potential, Barriers and Opportunities

FABIEN ROBERT: *Nestlé, Dublin, OH, USA*
ROBERT BAKER: *Mars Global Food Safety Center, Beijing, China*
SANJAY GUMMALLA: *American Frozen Food Institute, McLean, VA, USA*
EMILY GRIEP: *United Fresh Produce Association, Washington, DC, USA*
BEHZAD IMANIAN: *Illinois Institute of Technology, Institute for Food Safety and Health, Bedford Park, IL, USA*
ERIC BROWN: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*

A quick look at the past 15 years is enough to arrest us in astonishment at the developmental pace and transformative power of whole genome sequencing (WGS) technology. In the U.S., public health authorities and food safety regulators have adopted WGS (one of the many applications of High-Throughput or Next Generation Sequencing HTS/NGS) as a superior tool in illness outbreak investigation, well aware of its promising prospects as a phenomenal surveillance tool. Impressive and admirable efforts have also been made to harmonize/standardize/validate the interagencies laboratory and analytical methods, with many other governments following suit, laying the foundations for a truly global and all-inclusive system of surveillance and regulations to improve public health on this planet. The earlier detection of outbreaks, prevention of countless illness and more efficient implementation of food safety measures are but a few benefits of the WGS application that we have enjoyed to date with many more, unexplored and undiscovered yet.

While the private sector generally leads and/or adopts innovations associated with our food systems, their response to the HTS/NGS storm has been slow and muted at best. This roundtable will explore the major obstacles that the industry is facing to implement HTS/NGS technology to its fullest potential. In this roundtable, representatives from industry who are intimately familiar with the subject matter, will discuss their experiences in the context of the challenges and opportunities for utilizing HTS/NGS technology to its full potential. An IFSH representative and one of the top U.S. regulators who have played a key role in the implementation of the new technology in FDA, CDC and USDA FSIS will also join the industry panelists to discuss and identify industry's challenges and barriers and to explore potential solutions via immediate and long term collaborations between the industry and the government partners.

RT21 Food Safety and Trade: Colleagues or Competitors

DONALD PRATER: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*
MATT MCKNIGHT: *U.S. Dairy Export Council, Arlington, VA, USA*
ROGER COOK: *New Zealand Ministry for Primary Industries, Wellington, New Zealand*

A panel of governments, industry and other stakeholders will look at food safety and trade. Governments today use a number of tools to support these joint objectives, from Codex standards to export certificates. While the food industry can benefit from expanding markets, they also seek to protect existing markets. This panel will address questions like: What are government's legal obligations when it comes to trade? Is food safety a trade barrier? How do governments ensure food safety and still maintain open markets? Is food safety enhanced by trade, or does it create downward pressure (e.g., the lowest common denominator on food safety standards)? How does trade help food security?

RT22 Fresh-cut Processing and FSMA

JIM BRENNAN: *SmartWash Solutions, LLC, Salinas, CA, USA*
DREW MCDONALD: *Church Brothers Produce, Salinas, CA, USA*
JOHN GURRISI: *Fresh Express, Inc., Orlando, FL, USA*
SAMIR ASSAR: *U.S. Food and Drug Administration, College Park, MD, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*
JENNIFER MCENTIRE: *United Fresh, Washington, DC, USA*

Fresh-cut processing and food safety preventive control is a priority in the industry. The Preventive Controls Rule and the Draft Fresh-cut Guidance provides an opportunity for processors to utilize a systems thinking approach for managing food safety in manufacturing fresh cut. This roundtable will bring a range of experts to share challenges, systems implementation, as well as best practices and insight on how to best manage challenges associated with fresh-cut processing. This facilitated panel discussion will provide an opportunity to discuss key components including supply chain, wash water process controls, sanitation controls, and utilizing a hazard analysis and risk-based preventive control approach to managing fresh-cut processing.

Technical Abstracts

T1-01 Lactic Acid Culture to Suppress *Listeria* Growth and the Decay of Minimally Processed Vegetables

Besnik Hidri[1], Michael Sciberras[2], Gustavo Ramirez[3] and **Veronique Zuliani**[4]

[1]Chr. Hansen, Milwaukee, WI, [2]Chr. Hansen, Victoria, Australia, [3]Chr. Hansen, Mexico, Mexico, [4]Chr. Hansen, Arpajon, France

Introduction: The fresh-cut greens/vegetables' sector is answering today's consumer demands for healthy and convenient food and less waste. For these products, shelf life is mainly defined by appearance. Adding one or more additional day(s) of shelf life provides a significant added value regarding logistics, and answers consumers' demands for longer shelf life.

Purpose: The purpose was to evaluate if the addition of a GRAS approved lactic acid culture with anti-*Listeria* activity may also contribute to extending the shelf life of minimally processed vegetables

Methods: The percentage of decay on spring mix (iceberg/romaine/radicchio/carrots), tender leaf blend, and spinach were evaluated at the end of shelf life on a control batch versus a batch sprayed with a *Lactobacillus curvatus* culture just before packaging. Decay was evaluated based on browning and texture (water loss/loss of crispness). Storage between 2.0 and 5.6°C was applied and atmosphere packaging mimicked suppliers' practices (air or environment enriched with N_2).

Results: For all three tested products the percentage of decay significantly decreased when the culture was applied. For spring mix kept at 4°C, the iceberg was the most sensitive. After 15 days of aerobic storage, 47% and 6% of the total leaves were damaged in the control versus the treated batch, respectively. For spinach kept at 5.6°C under air for 17 days, the decay dropped from 6% to 2% when culture was applied. For MAP packed tender leaf kept 16 days at 5.6°C, the decay dropped from 6% to 4%.

Significance: This study has demonstrated that in addition to *Listeria* control, GRAS approved *Lactobacillus curvatus* can improve the shelf life of minimally processed vegetables by delaying browning and texture change.

T1-02 Prevalence of *Salmonella enterica* and *Listeria monocytogenes* in Irrigation Waters as determined by Culture-based and Rapid Molecular Methods

Eric Handy[1], Cheryl East[1], Rhodel Bradshaw[1], Mary Theresa Callahan[2], Sarah Allard[3], Shirley A. Micallef[2], Shani Craighead[4], Brienna Anderson-Coughlin[4], Samantha Gartley[4], Kali Kniel[4], Joseph Haymaker[5], Chanelle White[5], Fawzy Hashem[5], Salina Parveen[5], Eric May[5], Hillary Craddock[3], Rianna Murray[3], Amy Sapkota[3] and Manan Sharma[1]

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Introduction: Detection of bacterial foodborne pathogens in waters used to irrigate fruits and vegetables can inform growers to quickly mitigate potential contamination of irrigation sources.

Purpose: To determine if rapid molecular (RM) methods are as accurate as culture-based (CB) methods in the detection of *Salmonella enterica* and Listeria *monocytogenes* in irrigation water and if testing larger volumes recovers *S. enterica* and *L. monocytogenes* more frequently.

Methods: Water from six surface or reclaimed sites in the Mid-Atlantic United States were surveyed by filtering three separate volumes (10 L, 1 L, and 0.1 L) through modified Moore swabs (MMS) at each of 107 sampling events from September 2016 to July 2018. For CB methods, MMS was incubated in universal pre-enrichment broth (UPB), followed by enrichment in selective broths and on selective agar for *S. enterica* and *L. monocytogenes* detection. For RM methods, DNA extracted from UPB enrichments was amplified using real-time PCR (RT-PCR) specific for *S. enterica* and *L. monocytogenes* to determine their presence/absence. For CB methods, presumptive isolates were confirmed by RT-PCR.

Results: CB detected *S. enterica* and *L. monocytogenes* in 49.5% and 29% of sampling events (*n*=107), respectively; RM detected *S. enterica* and *L. monocytogenes* in 72.9% and 29.9%, respectively. In 46.7% and 27.1% of sampling events, the 10 L samples contained *S. enterica* and *L. monocytogenes,* respectively, when assayed by CB; when tested by RM, 59.8% and 19.6% of 10 L samples contained *S. enterica* and *L. monocytogenes,* respectively. In 13.1% and 14.0% of sampling events analyzed by CB, all three test volumes (10 L, 1 L, and 0.1 L) contained *S. enterica* and *L. monocytogenes,* respectively. False positive rates for *S. enterica* and *L. monocytogenes* by RM were determined to be 14.3% and 3.4%, respectively.

Significance: *S. enterica* was detected more frequently than *L. monocytogenes* in the Mid-Atlantic United States, and recovery rates were dependent on the pathogen, sampling volume, and method. High false positive rates may limit adoption of the currently evaluated rapid method without modification.

T1-03 Ballpark Figures: Use of a Mathematical Model to Estimate Relative Risk from Agricultural Water to Produce in Pursuit of "Same Level of Public Health Protection" Evaluations

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Introduction: The Food Safety Modernization Act Produce Safety Rule (PSR) established agricultural water criteria that mirror the 2012 United States Environmental Protection Agency (USEPA) Revised Recreational Water Quality Criteria. In 2017 the Food and Drug Administration reacted to stakeholder comment with a policy of enforcement discretion during a re-evaluation period. During this reevaluation, the FDA is considering how to "further reduce the regulatory burden or increase flexibility while continuing to achieve our regulatory objectives."

Purpose: Describe and demonstrate a relative-risk approach that enhances PSR flexibility and addresses regulatory needs for agricultural water quality.

Methods: A process-based mathematical model was created to estimate the risk of illness from consuming produce, assuming irrigation with water at the risk threshold of the USEPA criteria. The associated risk from ingestion of produce was calculated using representative food-borne pathogens (bacterial, viral, protozoan) as the source term and commodity-specific scenarios, including removal or reproduction during the passage of produce from irrigation to consumption. Resulting risk of illness was used to bin scenarios into a high, medium, and low relative risk categories. Variability was accommodated with draws in each model iteration using Monte Carlo simulation.

Results: The modeled analysis allowed evaluation of risk relative to an index scenario, such as *E. coli* O157: H7 on leafy greens. Comparison scenarios addressed different commodities and different practices. Results of this analysis supported the idea that growing conditions and handling practices for some commodities, such as apples and dry bulb onions, are associated with substantially lower risk from irrigation water related to the index scenario.

Significance: This relative risk approach could be valuable when evaluating the "same level of public health protection" to support alternatives and variances within the PSR. Effective use of alternatives and variances leverages flexibility in the PSR, resulting in defensible commodity- or practice-specific equivalent requirements that are less burdensome and do not negatively impact consumer safety.

T1-04 Evaluation of Zero Valent Iron Filtration to Reduce *Escherichia coli* in Agricultural Irrigation Water in Laboratory and Field Trials

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Introduction: Surface water is a critical source of irrigation water which may contain bacterial foodborne pathogens that can contaminate produce intended for human consumption. Zero-valent iron (ZVI) filtration may provide a cost-effective strategy to reduce pathogens in surface water.

Purpose: To evaluate the effectiveness and longevity of ZVI and sand in a unique filter designed to reduce *E. coli* in irrigation water in laboratory and field trials.

Methods: ZVI (50% ZVI and 50% sand) and sand (100% sand) were constructed with Schedule 40 PVC pipes (two-inch diameter). Autoclaved pond water (eight liters) was inoculated with *E. coli* TVS 353 (10^4 CFU/mL) and introduced to ZVI and sand filters, followed by 16 L of uninoculated pond water. The effluent was collected to determine *E. coli* populations. Six laboratory filtration events and two field irrigation trials on spinach plants with ZVI- or sand-filtered water, along with control (unfiltered) water, were conducted.

Results: ZVI filtration resulted in significantly (P<0.001) lower populations in water of *E. coli* than sand filtration. *E. coli* populations were reduced by 70% by ZVI filtration but were not reduced by sand filtration (-10%) over six laboratory trials. ZVI filtration removed *E. coli* populations in early trials one through three (mean reduction: 96%) more effectively than in trials four through six (44%). For field irrigation trials, soil samples irrigated with ZVI-filtered water had *E. coli* populations below the detection limit (less than two log CFU/g) and were significantly lower than *E. coli* in soils irrigated with sand filtration (2.11 log CFU/g) or control water (1.75 log CFU/g). Prevalence of *E. coli* was lower on spinach plants irrigated with ZVI-filtered compared to sand or unfiltered irrigation water.

Significance: ZVI filtration reduced *E. coli* populations in surface water and on irrigated spinach plants, and may aid produce growers in meeting irrigation water standards of the Food Safety Modernization Act.

T1-05 Environmental Inactivation and Irrigation-mediated Regrowth of *Escherichia coli* O157:H7 on Romaine Lettuce When Inoculated in a Fecal Slurry Matrix

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❖ ***Developing Scientist Entrant***

Introduction: Irrigation mediated transfer of fecal material has been identified as a mode of contamination for minimally processed produce. The survival kinetics of pathogenic bacteria under field conditions is not well studied, particularly if these transferred pathogens survive to the point of harvest and ultimately manifest as a food safety hazard.

Purpose: To estimate the *Escherichia coli* O157: H7 inactivation rate on romaine lettuce leaves as a function of time, fecal matrix, irrigation, and environmental factors when applied in a fecal slurry.

Methods: Two ten-day field trials occurred in Salinas, CA during July and October 2012. Lettuce heads, grown under commercial conditions, were inoculated with one of three fecal slurries: rabbit, chicken, or pig, containing an average of 4.1×10^7 CFU *E. coli* O157: H7 (C_0). Lettuce samples (n=576) were collected daily and processed within 4.5 hours. The bacterial concentrations were enumerated using the most probable number technique (C_t). A negative binomial regression model was developed to estimate the survival rate of *E. coli* O157: H7.

Results: Romaine lettuce had quantifiable concentrations of *E. coli* O157: H7 on 559 (98%) of 572 inoculated heads over the two ten-day trials. Compared to initial concentrations (C_0) we observed a mean 2.6- and 3.2-log reduction during the July and October trials, respectively. However, given the regrowth of *E. coli* O157: H7 following foliar irrigation, the mean log reduction nearly doubled to 5.1 to 6.7 in July and October, respectively. Increases in leaf wetness were significantly associated with increased *E. coli* O157: H7 concentration (P=0.0309).

Significance: Substantial regrowth of *E. coli* O157: H7 on romaine lettuce leaves following foliar irrigation was observed. Our findings suggest delaying harvest (>24 hours) following irrigation may reduce the potential increase of *E. coli* O157: H7 if present on romaine lettuce.

T1-06 Pathogen Persistence and Transfer Dynamics as Influenced by Biological Soil Amendments in a Pre-harvest Environment

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Introduction: Biological soil amendments of animal origin (BSAAO) add nutrients to soil but may harbor pathogens. BSAAO type may influence pathogen survival in soils and potential transfer to fruits and vegetables.

Purpose: Investigate the population dynamics of *Escherichia coli* in soils amended with BSAAO and their transfer to cucumbers.

Methods: Plots (three m^2) were amended with raw poultry litter (PL), heat-treated poultry litter pellets (HTPP), composted PL (CPL), or left unamended with inorganic fertilizer (UN). One set (n=10) of plots was covered with plastic mulch and another left uncovered. Each plot was spray-inoculated with one liter of non-pathogenic *E. coli* TVS 355 (six log CFU/ml). Cucumber plants (Supremo) were planted five days post-inoculation (dpi). Composite soil samples (n=234) were collected every 10 days up to 120 dpi, along with cucumbers upon maturation (n=160), to determine *E. coli* populations and presence/absence of *Salmonella* spp., following a modified FDA-BAM protocol. Data were analyzed using one-way ANOVA (P<0.05).

Results: A significant (P<0.05) decrease in *E. coli* populations was observed in all plots compared to initial populations at 120 dpi, at which time all *E. coli* populations were <0.4 log MPN/g. At 60 dpi, *E. coli* populations in soil were <0.4, <0.4, 2.55, and 4.29 log MPN/g in CPL, UN, HTPP, and PL plots, respectively. Although UN plots had lower bacterial populations than PL and HTPP, they supported significantly (P<0.05) greater *E. coli* transfer to cucumbers (1.7 log MPN/g) at 60 dpi (harvest time). *E. coli* was detected on all cucumbers (n=120) from PL, CPL, and HTPP plots at 0.7-1.0 log MPN. *Salmonella* spp. was detected and confirmed in HTPP-treated plots.

Significance: BSAAO type affected *E. coli* survival durations in amended soils, but longer survival durations did not correlate to greater transfer to cucumbers.

T1-07 Establishment of Vegetation Buffer Zone Areas to Reduce Transfer of Enteric Pathogens from Animal Operations to Fresh Produce

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Introduction: Transfer of enteric pathogens from confined animal feedlot operations (CAFOs) to adjacent produce fields continues to be a significant risk in many commercially important horticulture operations.

Purpose: To develop a fast growing and cost-effective vegetation buffer zone (VBZ) area that could prevent the transfer of enteric pathogens from CAFOs to adjacent produce fields.

Methods: A fast-growing and cost-effective VBZ area (15 by 30 m) was planted in between produce fields and dairy or poultry operations. Manure, soil, plant material within the VBZ, air and produce (tomatoes and romaine lettuce) were sampled for 10 months at three-week intervals. The presence of Shiga toxin-producing *E. coli* (STEC) and fecal indicators was determined at each sampling event. Transfer of enteric pathogens from CAFOs to fresh produce was determined by REP-PCR. Soil and produce samples were collected at 10, 61 and 122 m from each CAFO, with four replicates per distance. Air and vegetative strip samples were sampled at 15-day intervals.

Results: In produce fields adjacent to dairy operations, the occurrence in the air of presumptive STEC was higher at 10 m (0.67±0.15 log CFU/15 days) than at 61 and 122 m away (1.57±0.16 log CFU/15 days) from the VBZ (P<0.05). The opposite effect was observed at the poultry operation (P<0.05). Generic *E. coli* was on average 1.2 log CFU/100 g higher in the dairy than in the poultry VBZ (P<0.05). At both locations, there was a positive correlation (P<0.05) between warmer weather and the recovery of STEC from soil (0.5 log CFU/150 g cold weather vs 3.3 log CFU/150 g warm weather). No significant differences in the population of STEC and fecal indicators were observed between locations in produce.

Significance: The VBZ was able to provide significant coverage of produce fields from enteric pathogen contamination, however, effectiveness varied with CAFO operation. Previous soil contamination with manure particles coming from CAFOs increased produce contamination.

T1-08 The Use of Riparian Buffer Zones to Reduce the Risk of *Salmonella* Transmission from Animal Operations to Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Due to the recent outbreaks of *Salmonella* spp. 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 riparian buffer zone (RBZ) by AFOs could prevent the transfer of *Salmonella* spp. to nearby fresh produce fields in sustainable farming systems.

Methods: A five-layer RBZ (15 by 30 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 houses. Samples were collected from manure, air, soil, and fresh produce (romaine lettuce and tomato) sources for a period of 10 months. Four replicates of soil and fresh produce samples were taken from plots located 32 ft., 200 ft., and 400 ft. away from cattle/poultry AFOs. Air and vegetative strip samples were sampled at 15-day intervals.

Results: A total of 37 presumptive *Salmonella* spp. isolates were retrieved from the soil, produce, air, and manure samples from both dairy and poultry farms. Interestingly, 59.5% of presumptive *Salmonella* isolates were from soil samples (n=22). The bulk of isolates were collected during the spring/winter months (n=25). Only three isolates were recovered from lettuce samples in the month of November. *Salmonella* isolates from air samples (n=6) were only recovered from the dairy farm at both the 200 ft. and 400 ft. plots during the summer months. Surprisingly, *Salmonella* isolates (n=6) were only found in manure samples from dairy cattle

Significance: The effectiveness of the RBZ cannot yet be determined. However, it seems that distance and air flow does have an effect on the transmission of *Salmonella*.

T1-09 Investigating the Influence of Streptomycin Sprays on Bacterial Populations in the Apple Carposphere and Orchard Soil

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Introduction: The use of streptomycin in pome fruit production to control fire blight (*Erwinia amylovora*), a devastating disease in humid environments, may select for antimicrobial resistant (AMR) bacteria.

Purpose: Investigate the effect of streptomycin use on the occurrence of AMR bacteria and populations of enterococci and *Staphylococcus aureus* on fruit and in the soil.

Methods: Apple (n=40) and soil (n=14) samples were collected from an orchard at the Western Maryland Research and Education Center, Keedysville, MD. Trees were sprayed with streptomycin sulfate four times during the flowering season, in April. Streptomycin-sprayed 'Fuji' (n=10) and 'Cripps Pink' (n=10) apples, and unsprayed breeder apples of near same lineage (n=10 per cultivar) were collected over two dates in October 2018. Composite soil samples (n=4 per sprayed cultivar and n=6 unsprayed) were also analyzed. All samples were diluted in buffered peptone water and hand massaged for two minutes. *Enterococcus* spp. and *S. aureus* was enumerated by plating on Enterococcosel and Baird Parker agar, respectively. After three hours of incubation at 37°C, extended spectrum β-lactamase (ESBL)- and carbapenemase (CP)-producing bacteria were enumerated on ChromAgar ESBL and Chromagar mSuperCarba agars, respectively.

Results: ESBL-producing bacteria (2.1 to 6.5 log CFU/g) were recovered from 53 (98%) of 54 samples. CP-producing bacteria (1.5 to 6.2 log CFU/g) were recovered from 50 (93%) of 54 samples. Approximately 90% of isolates were PCR-identified as pseudomonads. *Enterococcus* spp. (0.1 to 3.6 log CFU/g) were recovered from 30 (56%) of 54 samples, of which 14 (19%) of 73 (19%) and 3 (4%) of 73 were PCR-identified as *Enterococcus faecalis* and *Enterococcus faecium*, respectively. *S. aureus* (0.1 to 3.7 log CFU/g) was recovered from 34 (63%) of 54 samples. There was no significant difference in counts of any bacterial group between sprayed and unsprayed apples of either variety (P>0.01), but counts were significantly lower (P<0.01) on apples compared to soil.

Significance: Our data suggest that counts of AMR bacteria, enterococci and *S. aureus* in apple orchards are unaffected by streptomycin sprays.

T1-10 *Salmonella* and Indicator Bacteria Profiles of Produce and Meat Products Sold in Northern California Farmers' Markets: Implications for Microbial Food Safety

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Introduction: Small-scale farms and direct marketing of agricultural products are increasing in popularity. The potential microbial food safety risks of these directly marketed products are not well known due to a wide range of on-farm practices, distribution, and farmers market requirements (e.g., hygiene and sanitation training).

Purpose: The purpose of this study was to evaluate *Salmonella* prevalence and microbiological profiles of produce and meat products sold at farmers markets in Northern California.

Methods: Produce (leafy greens, root vegetables, and fruits) and meat (chicken, pork, beef) were sampled at 44 certified farmers markets across nine counties in northern California from February to July of 2018. Produce and meat products were tested qualitatively for *Salmonella* and quantitatively for generic *E. coli* by Most Probable Number (MPN). Recovery of isolates was achieved by selective and differential culturing methods; presumptive isolates were confirmed by PCR.

Results: Overall, six (2.8%) of 211 meat samples were positive for *Salmonella*, with three positive isolates from beef 3 (2.9%) of 105, two (2.6%) of 78 from pork, and one (3.6%) of 28 from chicken. All produce samples (*n* = 127) were negative for *Salmonella*. Forty (31.5%) of 127 of produce samples were positive for generic *E. coli* including two of two tomato samples, 26 (34.2%) of 76 leafy greens, and 14 (28.6%) of 49 root vegetables. The average concentration of generic *E. coli* from produce samples was 14.0 MPN/g (range 0 to 920 MPN/g).

Significance: As the number of farmers markets and direct-to-consumer marketing channels continues to increase, it is crucial to evaluate the risks associated with animal products and fresh produce sold directly from the farmer or vendor. Identification of mitigation strategies will enhance both the economic interests of the producers, as well as food safety.

T1-11 The Whole is Greater Than the Sum of Its Parts: Building Cooperative Monitoring Programs among Farms

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Introduction: Under the Food Safety Modernization Act, the United States Food and Drug Administration established a data sharing provision allowing farms to share water sampling data under certain circumstances; i) growers must be on a shared source, ii) sampling sites must be representative of the source, and iii) there must be no "reasonably identifiable source of likely microbiological contamination" between sampling sites. However, the provision excluded specific guidance on the scale or spatial extent that would determine a farms ability to use this provision.

Purpose: We sought to explore the environmental conditions and levels of microbial variability experienced within surface water systems of the western United States over several irrigation seasons. Investigating the scales at which adjacent sites share similar water quality will enable us to aid farmers interested to making use of this provision while providing feedback and data to the FDA as they continue to develop guidance materials.

Methods: Indicator bacteria (*E. coli* and enterococci*)* were enumerated in water samples collected from 169 sites in central Washington state and northern California during the 2016 to 18 irrigation seasons (*n*=1558). Cooperative clusters (groups of adjacent sampling sites) (*n*=23), were created based on proximity and lack of a likely source of microbial contamination between sites. Data were analyzed within and between cluster variability. Statistical models were used to explore conditions associated with elevated microbial risks to irrigation water.

Results: Twenty-one (95%) of 23 cooperative clusters are statistically similar regardless of proximity, source type or adjacent land uses. Sampling sites for all years were below the statistical threshold value (STV) for *E. coli* for FSMA agricultural water requirements; the average STV was 2.19.

Significance: Based on our extensive microbial water quality survey farms can form cooperative monitoring programs which drastically reduce the regulatory burden of the FSMA agricultural water requirements.

T1-12 Development of the On-Farm Readiness Review to Prepare Farms for Produce Safety Rule Implementation

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Introduction: The National Association of State Departments of Agriculture, in partnership with Food and Drug Administration, state departments of agriculture, and extension developed an On-Farm Readiness Review (OFRR) tool and assessor training to provide outreach to farms with questions about meeting Produce Safety Rule PSR) requirements.

Purpose: The purpose of this study was to evaluate the impact of the OFRR assessor training and obstacles faced by producers to come into compliance with the PSR.

Methods: OFRR assessor training began in January 2018 to train staff from states, cooperative extension, and FDA to conduct OFRRs. Fourteen OFRR assessors training were conducted nationwide in 2018. Participants were trained to utilize curriculum that covered: assessor expectations, on-farm etiquette, and egregious conditions. Participants observed a demonstration OFRR on a farm and then conducted an OFRR on another working farm. Post OFRR training surveys were conducted for each training. Trainees began conducting OFRRs in April 2018.

Results: Over 300 trainees completed the OFRR training in 2018 and are qualified to conduct OFRRs; 96 completed an assessment of the training. As of December 1, 2018, 394 OFRR post farm visit survey responses representing 32 states and one United States territory was collected. National OFRR post farm visit survey results will be utilized to guide the educational priorities of produce safety educators.

Significance: Seventy-eight percent of training participants indicated that the training had a positive impact on their ability to inspect for the PSR and 87% indicated that they would use the OFRR resource tool. Of the OFRR post farm visit survey responses, 33% meet the FSMA PSR requirements while 54% needed minor improvements. OFRR assessors indicated that 31% of farms need more time, 26% need technical assistance, 15% need facility upgrades and 4% need significant monetary investments to meet minimum PSR requirements.

T2-01 Phage-like Plasmids Transfer Antibiotic and Heavy Metal Resistance Genes by Transduction, Transformation and Conjugation

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Introduction: Bacteriophage-like plasmids (PLPs) are an emerging class of mobile genetic element that contain elements of both phages and plasmids. PLPs have been shown to carry antibiotic and heavy metal resistance genes, raising questions regarding the horizontal gene transfer (HGT) mechanisms by which PLPs disseminate resistance determinants.

Purpose: PLPs AnCo1 (extended spectrum β-lactamases), SJ1 (mercury), and MA725 (tellurite) carrying antibiotic and heavy metal resistance genes were examined for their ability to transfer resistance determinants to recipient cells by transformation, transduction and conjugation.

Methods: For transformation experiments, PLPs, isolated from bacterial cells using a plasmid isolation kit, were transformed to competent *E. coli* DH10B by electroporation. For transduction experiments, PLPs were induced from their bacterial hosts with mitomycin C, followed by incubation with recipient bacteria, to allow infection. For conjugation studies, bacteria containing PLPs and a helper plasmid (donor bacteria) were incubated with recipient bacteria. Recipient bacteria containing resistance determinants were isolated by plating the bacteria on media supplemented with appropriate antibiotics and heavy metals.

Results: All PLPs were transferred to recipient bacteria by transformation and transduction. Two PLPs (AnCo1 and SJ1) were transferred to recipient cells by conjugation, but PLP MA725 could not be transferred. All PLPs conferred either antibiotic resistance (AnCo1) or heavy metal resistance (SJ1, MA725) to recipient cells. The MIC of AnCo1 was 3 mg/ml of cefotaxime, the MIC of PLP SJ1 was 50 µg/ml of mercury chloride, and the MIC of MA725 was 40 µg/ml of potassium tellurite. The level of resistance of the individual PLPs to cefotaxime, mercury chloride and potassium tellurite remained the same regardless of the HGT mechanism.

Significance: These results indicate that PLPs can be transferred to bacteria by all three horizontal gene transfer mechanisms, and may play a major role in dissemination of antibiotic and heavy metal resistance genes.

T2-02 Bio-based Sanitizer Delivery Systems for Improved Sanitation of Bacterial and Fungal Biofilms

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Introduction: Biofilms can persist in food processing environments due to their relatively higher tolerance and resistance to antimicrobials including sanitizers.

Purpose: In this study, a novel bio-based sanitizer composition was developed to effectively target biofilms and deliver chlorine-based sanitizers to inactivate bacterial and fungal biofilms.

Methods: The bio-based composition was developed by encapsulating chlorine-binding polymer in a bio-based yeast cell wall particle (YCWP) microcarrier. The effect of this sanitizer composition on the inactivation of bacterial and fungal cells in simulated wash water and inhibition of biofilm formation was evaluated in this study.

Results: This study demonstrates the high affinity of bio-based compositions to bind target bacterial and fungal cells and inactivate five log of model pathogenic bacteria and fungi in wash water without and with high organic load (COD=2000 mgl) in 30 s and five min, respectively. For the sanitation of biofilms, this bio-based sanitizer can inactivate seven log of pathogenic bacteria and three log of fungi after one hour of treatment, while the one-hour treatment using conventional chlorine-based sanitizer can only achieve two to three log reduction for bacterial biofilms and one to two log reduction for fungal biofilms, respectively. The enhanced antimicrobial activity can be attributed to three factors: i) localized high concentration of chlorine bound on the YCWPs; ii) high affinity of YCWPs to bind diverse microbes, and iii) improved stability in an organic-rich aqueous environment.

Significance: In summary, these unique attributes of bio-based carriers will significantly enhance the sanitation efficacy for biofilms, reduce persistence and transmission of antimicrobial resistance microbes, limit the use of antimicrobial chemicals, and improve the cost-effectiveness of sanitizers.

T2-03 A Novel Antimicrobial Film for Preventing Cross-Contamination of Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Cross-contamination of fresh produce can lead to nationwide foodborne outbreaks. Several studies have shown that produce handling bins are contaminated with bacteria despite current sanitation practices. Thus, there is an unmet need to prevent cross-contamination of fresh produce from bins during postharvest handling.

Purpose: The purpose of this study was to develop a chlorine-rechargeable film and evaluate its efficacy in preventing cross-contamination of fresh produce.

Methods: Poly(vinyl alcohol-co-ethylene) (PVA-co-PE) halamine films were prepared by a combination of PVA-co-PE powders, diallylmelamine, and dicumyl peroxide. This was extruded to form pellets and they were hot pressed to form thin films, followed by immersion in bleach for chlorination. Cross-contamination was simulated by using spinach with five log CFU/cm^2 *Listeria innocua* to contact the uncharged/charged films, which then contacted another fresh leaf at different contact times (five to 1200 s) and applied forces (one or 9.8 N). Bacteria on surfaces were quantified by plate counting, and color, texture and total phenolic content of leaves were measured for quality tests. Experiments were done in triplicate, and the significant differences (P<0.05) were determined using ANOVA.

Results: The active chlorine content in the film reached 614.4±9.9 ppm within one hour chlorination and was maintained at the same concentration for at least three recharging cycles. The charged film was self-cleaning (greater than five-log reduction of inoculated bacteria) after a 20 min contact, and reduced approximately two log of inoculated *L. innocua* on leaves. The antimicrobial film was also effective for reducing the cross-contamination of leaves as it prevented the bacterial transfer from contaminated leaves to non-contaminated leaves. There were no significant differences in the color, texture, and total phenolics between fresh and treated leaf samples.

Significance: PVA-co-PE halamine films can effectively prevent leaf-to-leaf cross-contamination when used as liner materials. In summary, this material provides a cost-effective solution to reduce cross-contamination of fresh produce.

T2-04 Developing High Performance, Low cost and Rechargeable Antimicrobial Coatings for Food Safety Applications

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Introduction: Each year in the United States, more than 90% of foodborne illnesses are due to microbiological contamination. Environmental surfaces, including equipment and facilities, are important avenues for microbial habitation and transmission in food processing establishments. Making environmental surfaces with antimicrobial functions is of great importance for food safety and prevention and control during food processing.

Purpose: We recently developed an *N*-halamine polymer-based antimicrobial coating material which can be easily scaled up to coat on various substrate surfaces (e.g., metals, plastics, glasses, paints), regardless of chemical composition, size, and shape.

Methods: A new antimicrobial polymer was synthesized and coated on various substrate materials including metals, plastics and glasses. The stability and rechargeability of coating on surfaces were tested by a chemical titration method. Moreover, a series of microbiological tests (sandwich, live/dead staining, inoculation/swabbing) were designed to prove the efficacy and rechargeability of the developed coating on a stainless steel substrate.

Results: The *N*-halamine functional group on the coating surface could be easily activated through treating the surface with chlorine bleach. These *N*-halamine moieties could provide a potent and rechargeable antimicrobial function for the coating. For instance, the coated stainless steel surface completely inactivated 6 log CFU of both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli* O157:H7) bacteria within 10 min of contact and more than 70% of original chlorine on the surface could be recovered even after 10 equivalent recharge-discharge cycles. In addition, we successfully scaled up the process to coat various equipment components (metal pipes and plastic belts) that were commonly used in the food industry.

Significance: Our new *N*-halamine polymer-based antimicrobial coating material has great potential to produce a high-performance, low-cost, and easy-to-apply coating on food-associated environmental surfaces for food safety, prevention, and control applications.

T2-05 Antioxidant-Antibacterial Properties and Nutrition Value of Some Varieties of Libyan Date Palm Fruits (*Phoenix dactylifera*)

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Introduction: The date palm belongs to the family *Palmae* (Arecaceae). It has important social, environmental, and economic value for many people in countries where it grows.

Purpose: In this work, we evaluated separately the antibacterial activity, antioxidant properties, and nutritional value of the cortex, pulp, and the seed of Libyan "Bekrari" and "Bronci" date varieties.

Methods: The DDPH assay method was used to evaluate antioxidant activity, and the agar well diffusion method was used to evaluate the antibacterial activity of extracts. The nutritional values of cortex, pulp, and seed powder of both date varieties were evaluated using standard analytical methods.

Results: The antioxidant activity assay proved that the methanolic extracts of seeds of both varieties possessed strong antioxidant activity compared with the pulp and cortex extracts. The IC_{50} for Bekrari and Bronci seed extracts ranged from 0.092 to 0.047 mg/ml, respectively. Additionally, the IC_{50} values of Bekrari and Bronci pulp extracts were 8.81 to 4.98 mg/ml respectively. The methanolic extracts of Bekrari seeds at 100, 50, 25, and 12.5% concentration showed antibacterial activity against the gram-positive bacteria *Staphylococcus aureus* with inhibition zones of 15.6, 13.3, 13, and 12 mm, respectively, and methicillin-resistant *S. aureus* (MRSA) with zones of 18.3, 16, 14, and 11.5 mm, respectively. Furthermore, the extract of Bronci seed inhibited the growth of *S. aureus* and MRSA. Zones of inhibition were 16, 14.5, 12.5, and 10 mm for *S. aureus* and 16.5, 14.2, 12.5, and 11 mm for MRSA. Nutritional value evaluation revealed that Bronci and Bekrari seeds contained 6.59±0.02 and 6.02 ±0.01% respectively of crude protein. The percentages of fat, moisture content, total ash, and total fibers in Bronci seeds were 5.48, 8.36, 1.12, and 38.45% and 6.60, 8.25, 1.16, and 39.60% in Bekrari seeds, respectively. The percentages of crude protein, fat, moisture content, total ash and total fibers in the Bronci pulp were 3.47, 0.05, 7.67, 1.72, and 2.26% respectively. Whereas, in Bekrari pulp they were 2.25, 0.04, 7.84, 1.85, 2.74%. The percentages of crude protein, fat, moisture content, total ash and total fibers in Bronci cortex were 4.87, 0.65, 7.46, 2.74, and 17.55%. In Bekrari cortex they were 3.82, 0.29, 4.66, 1.59, and 9.79% respectively.

Significance: Date seed and cortex are considered waste. This work provides evidence that they are rich in nutritive compounds and minerals. In addition, the seed has potent antioxidant activity. Therefore, we recommend to incorporate it into food and nutraceutical formulations.

T2-06 Activity of Lavender (*Lavandula officinalis*) Essential Oil Against *Listeria monocytogenes* and Sensory Acceptance of the Effective Concentrations in Fresh-cut Mango

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Introduction: Outbreaks involving fresh-cut fruits contaminated with *Listeria monocytogenes* has been reported worldwide. Mango is largely retailed after minimal processing, which must include a sanitization step to ensure safety. *Lavandula officinalis* essential oil (LOEO) is a food flavoring agent with antimicrobial activity. The efficacy of LOEO against *L. monocytogenes* and the sensory acceptance of mango sanitized with this substance remain unknown.

Purpose: This study determines the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LOEO against *Listeria monocytogenes* C1-387 and the sensory acceptance of mango sanitized with effective concentrations of LOEO.

Methods: MIC of LOEO was determined by microdilution broth method (Brain Heart Infusion) using a range of concentrations (32 to 0.5 µl/ml) and interpreted according to Clinical Laboratory Standards Institute procedures. MBC was determined by plating MIC assay dilutions on *Listeria* agar (48 h; 37°C) and defined as the lowest concentration that reduced ≥99.9% the initial inoculum. Disinfected mangoes were peeled, cut in pieces (three cm^3) and sanitized by immersion (one minute) in MIC or MBC of LOEO. The sensory evaluation was performed after Ethics Committee approval with fifty untrained panelists. A nine-point structured hedonic scale was used for evaluation of appearance, odor, consistency, taste, aftertaste, and overall acceptance. The purchase intent was assessed using a five-point structured hedonic.

Results: MIC and MBC values of LOEO were four and eight µ/m, respectively. Mango sanitized with the MIC of LOEO received the score "like moderately" or "like very much", while that sanitized with MBC of LOEO received the score "dislike slightly" or "neither like, nor dislike" for flavor, taste, texture and aftertaste attributes. Responses for purchase intent were "probably buy" and "might or might not buy" for mango sanitized with MIC and MBC of LOEO, respectively.

Significance: LOEO can be used as an anti-*Listeria* agent for sanitization of fresh-cut mango, however, the sensory impacts should be cautiously evaluated.

T2-07 Application of Bacteriophages on Beef and Leafy Greens as a Natural Intervention against *E. coli* O157

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Introduction: *Escherichia coli* O157 remains a great concern for the beef and leafy greens industry. Bacteriophages have the potential to be an additional safe and effective intervention against *E. coli* O157.

Purpose: The objective was to determine the efficacy of a commercially available bacteriophage cocktail (PhageGuard E) as an intervention against *E. coli* O157 on refrigerated beef and vegetables.

Methods: Lysis activity of natural phage isolates was assessed by spotting serial dilutions on 88 *E. coli* O157 strains. For beef, two cold (4°C) beef cuts of nine cm^2 were contaminated with 1×10^5 CFU/cm^2 *E. coli* O157, while for romaine lettuce, zucchini and spinach 18 cm^2 areas were contaminated with 1×10^6 CFU/cm^2 *E. coli* O157. Subsequently, samples were treated with 3×10^7 or 3×10^8 PFU/cm^2. Controls were treated with tap water. Samples were then incubated at 4°C, after which bacteria were retrieved at two, six, 24, 30, and 54 hours post phage treatment. Bacterial reductions on phage-treated samples were calculated relative to controls at the corresponding time point. Reductions in three independent experiments were used for statistical analysis (Unpaired *t*-test).

Results: A cocktail of two selected phages, lysing 90% of all *E. coli* O157 strains tested, showed bacterial reductions from 1.5 to 1.9 log ($P<0.05$) on three different strains when cold beef was treated with 3×10^8 PFU/cm^2, while 0.8 to 1.5 $\log_{10}$ ($P<0.05$) reductions were observed with 3×10^7 PFU/cm^2 at 24 hours post phage treatment. Similarly, reductions between 1.45 to 2.97 log ($P<0.05$) and 2.33 to 3.86 log ($P<0.05$) were observed after 24 hours on contaminated vegetables treated with 3×10^7 or 3×10^8 PFU/cm^2, respectively. In all experiments, the maximum reduction was already achieved six hours post phage application.

Significance: The phage cocktail described above can be used by the industry as a natural, safe, and effective intervention to fight *E. coli* O157.

T2-08 Nutrient Stress as a Means to Enhance Robustness in *Lactobacillus plantarum* B21 for Improved Food Protection

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❖ ***Developing Scientist Entrant***

Introduction: *Lactobacillus plantarum* B21 is a bacteriocin-producing strain of lactic acid bacteria. Bacteriocin is a proven natural food preservative and antimicrobial peptide. Incorporating B21 into food products has great potential benefit for long-term storage of food. Producing stable cultures of this microbe that can survive industrial processing is therefore of great interest.

Purpose: In this study, the effect of nutrient stress, in particular, omission of a carbohydrate source from the growth medium of *L. plantarum* B21, was tested as a means to increase long-term stability.

Methods: *L. plantarum* B21 was grown in the absence of glucose and/or Tween 80. These treated cells were spray dried with 10% (w/w) whey protein isolate and the microencapsulated bacteria were stored at 4, 22 and 30°C for eight weeks. The viability of the cells (CFU/g) were assessed as % survivability; morphological changes were observed with electron microscopy. Inhibitory activity using well diffusion assays and electron microscopy was observed. Experiments were performed in triplicate and results assessed via two-way ANOVA. A *P*-value of <0.05 was considered statistically significant.

Results: Carbohydrate stress was found to significantly improve B21 cell stability. During an eight-week storage period, 10 g of powdered cells showed a survival rate of 19.05% (±3.23%) compared to unstressed cells (1.14±0.74%) and Tween 80-stressed cells (0.80±0.27%) at 22°C. Carbohydrate-stressed cells also retained functional bacteriocin activity. The omission of Tween 80 in the growth medium resulted in filamentous rod-shaped cells, with significantly lower survival rates of 13.83% (±2.50%), compared to unstressed (84.13±5.70%) and glucose-stressed (81.90±13.6%) cells after spray-drying.

Significance: The results provide detailed insight into the ideal growth conditions needed to produce robust and stable B21 microcultures for use in food protection and industrial purposes.

T2-09 Impact of Static and Turned Pile Composting of Dairy Manure on the Persistence of Pathogenic *E. coli* and Transfer to Spinach Leaves

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Introduction: The Produce Safety Rule has two methods for composting biological soil amendments of animal origin. Knowledge gaps exist regarding the effectiveness of these systems in eliminating antimicrobial resistant (AMR) microorganisms.

Purpose: To evaluate the efficacy of aerobic composting of dairy manure in removing AMR pathogenic *E. coli* and preventing the transfer of these microorganisms to organically grown spinach.

Methods: Organic dairy manure coming from three steers was collected over a period of six months prior to composting. Feed (no antibiotics) and manure accumulation were controlled for the duration of the experiment. Composting followed produce safety rule standards. Compost, aged manure, sterile soil and a mixture of these treatments were used to grow spinach for up to 42 days in mesocosms. Spinach seed, sterile soil, aged manure, compost and spinach plants were screened for the presence of 3rd-generation-cephalosporin-resistant (CR) (0, 4, 8 and 16 ug/ml) *Enterobacteriaceae*, *E. coli*, coliforms and Shiga toxin-producing *E. coli* (STEC) at seven-day intervals. All samples were screened for multidrug resistance to 15 different antimicrobials.

Results: Minimum times and temperature rule requirements for composting were met. Populations of cephalosporin-resistant *Enterobacteriaceae* were identical between concentrations ($P>0.05$). Spinach plants grown with aged manure presented the highest populations (4.99 log CFU/150 g) of cephalosporin-resistant *Enterobacteriaceae* when compared to those grown with compost (3.75 log CFU/150 g) ($P<0.05$). Populations of cephalosporin-resistant coliforms were higher (one log CFU/150 g) than non-resistant strains ($P<0.05$). Cephalosporin-resistant coliforms were recovered at higher populations at D28 (4.23 log CFU/150 g) when compared to D3 (2.94 log CFU/150 g) ($P<0.05$). Populations of cephalosporin-resistant STEC were recovered from aged manure and compost. Spinach plants grown with aged manure presented higher cephalosporin-resistant STEC populations (1.8 log CFU/150 g) than plants grown with compost (0.5 log CFU/150 g) ($P<0.05$).

Significance: AMR *Enterobacteriaceae*, coliforms and STEC were recovered from both compost treatments and spinach. Current composting methods may not reduce the transfer of these pathogens to produce.

T2-10 Effects of *Origanun vulgare* on Physiological Functions of *Salmonella* Enteritidis Sessile Cells in Mature Biofilms

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Introduction: The survival of *Salmonella* Enteritidis in the food chain is related to its ability to form biofilms on surfaces. *Origanum vulgare* L essential oil (OVEO) is suggested as a sustainable disinfectant to replace chloride-derived agents because of its strong antimicrobial activity. However, little is known about the action mechanism of OVEO on *Salmonella* Enteritidis sessile cells in mature biofilms.

Purpose: This study assesses the effects of OVEO on physiological functions and counts of *Salmonella* Enteritidis sessile cells of mature biofilms formed on food-contact surfaces.

Methods: Food-contact stainless steel surfaces (2 by 2 by 0.2 cm) were immersed in brain heart infusion broth (BHI) inoculated with *Salmonella* Enteritidis (final count six log CFU/mL) and incubated at 37°C. After 72 h, surfaces were washed twice with distilled water and exposed to OVEO (2.4 µl/ml), NaCLO (250 mg/ml) or distilled water for 15 min. Afterward, coupons were again washed and submitted to ultrasound (40 kHz, five min). Cells were resuspended in PBS and labeled with the fluorochromes: propidium iodide for membrane integrity; bis-(1,3-dibutyl barbituric acid) trimethine oxonol for membrane potential and ethidium bromide for efflux activity, all measured by flow cytometry (FC). Resuspended cells were also serially diluted and enumerated on BHI agar.

Results: No viable sessile cells were recovered from surfaces exposed to OVEO or NaClO (less than one log CFU/cm^2). Among the non-cultivable sessile cells exposed to OVEO or NaClO, ~40% and 80% showed compromised membranes and efflux activity, respectively. Around 20% of the non-cultivable sessile cells exposed to OVEO showed depolarized membrane, while 51.6% of those exposed to NaClO were depolarized. No physiological damages related to membrane or efflux activity was observed in >90% of sessile cells exposed to distilled water.

Significance: OVEO compromised the viability of *Salmonella* Enteritidis sessile cells in mature biofilms formed on stainless steel surfaces by affecting the membrane function and the efflux activity.

T2-11 Disrupting Irreversible Bacterial Adhesion and Biofilm Formation with an Engineered Enzyme

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❖ ***Developing Scientist Entrant***

Introduction: Biofilm formation is a major cause of post-harvest microbial persistence on fresh produce and processing surfaces, as the biofilm matrix has been shown to provide pathogens with protection from common disinfection approaches.

Purpose: The potential of an enzyme-based biocide was investigated as a supplement to common disinfection practices for preventing bacterial adhesion and removing mature biofilms.

Methods: *E. coli* O157: H7, *E. coli* 25922, and *Salmonella Typhimurium* biofilms were grown in minimal media in 24-well polystyrene plates at 32°C for 48 hours and treated with 0.1 mg/ml purified enzyme during or after growth. Biofilm formation was quantified by using a standard crystal violet staining assay. A microfluidic flow cell was also used to directly observe and quantify the impact of enzyme rinses on *E. coli* O157: H7 cells adhered to spinach leaf surfaces. Mechanisms of the enzyme actions were characterized by transmission electron microscopy (TEM) and cell surface hydrophobicity using the microbial adhesion to hydrocarbons (MATH) test. All experiments were performed at least in triplicate.

Results: The enzyme significantly inhibited biofilm growth (P<0.05) of *E. coli* O157: H7 (-41±7%) and 25922 (-39±6%). The enzyme was also effective at removing mature *Salmonella* biofilms (P<0.05); providing a 90% reduction versus rinsing with water. In the flow cell, enzyme rinses resulted in significantly greater cell removal than water (P<0.05), representing a reversal of initial phases of biofilm formation. After the enzyme treatment, the relative hydrophobicity of the cell surface was significantly reduced (P<0.01) for all three strains. *Salmonella* showed the largest decrease (43.4±14.2% to 6.7±4.4%). TEM images further reveal major modification to surface polysaccharide structures.

Significance: These results present a strong case for further development and optimization of enzyme activity to be applied as a novel supplement to disinfection protocols to minimize food safety risks associated with bacterial pathogens.

T2-12 Surfactant Type Plays an Important Role in Antimicrobial Efficiency

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Introduction: Plant fatty acids, such as pelargonic acid, are potent and safe antimicrobials; however, their use by the food industry is limited because of inherently low miscibility in water.

Purpose: Antimicrobial activity of water suspensions of pelargonic acid, prepared with various surfactants (Tween 80, Triton X-100, sodium dodecyl sulfate (SDS) and quillaja saponin), were comparatively assessed against *Salmonella.*

Methods: Surfactants were used at a concentration of 0.1% and 1% (w/v) along with dilution of pelargonic acid down to ≥2 mM. An emulsion was produced by mixing surfactant with pelargonic acid on a vortexer for one min. The minimum inhibitory concentration (MIC) of the emulsion was tested against *Salmonella* serotypes Newport, Oranienberg and Typhimurium using a modified plate dilution method with resazurin as an indicator of bacterial growth.

Results: Significant (P<0.05) differences between MICs of emulsions were observed based on the surfactant that was used as well as the concentration it was used. SDS based emulsions (0.1% SDS) demonstrated the strongest inhibitory activity with a MIC of 5.8±0 mM against *Salmonella* Newport and 10.9±0 against *Salmonella* Oranienberg and Typhimurium. Emulsions generated using 1% Triton had the lowest inhibitory concentration against *Salmonella* with a MIC of 62.5±0 mM, 72.91±47 mM and 83.3±36 mM against *Salmonella* Newport, Oranienberg and Typhimurium, respectively. Quillaja saponin (0.1%) based emulsions had a MIC of 20±9 against *Salmonella* Newport and 31.25±0 mM against both Oranienberg and Typhimurium. Emulsions made using 0.1% Tween had a MIC of 26.04±9 mM against all three serotypes tested.

Significance: Selection of surfactant and target serotype are significant (P<0.05) determinants of antimicrobial efficacy for pelargonic acid emulsions.

T3-01 Prevalence and Fitness of Produce-Outbreak Associated *Salmonella enterica* in Tomato Plants

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Introduction: Numerous outbreaks of *Salmonella* have been linked to the consumption of raw tomatoes.

Purpose: To investigate the efficiency of colonization and internalization of *Salmonella* into tomato plants by routes of contamination consistent with the pre-harvest environment.

Methods: Red round market tomato (*Solanum lycopersicum*) varieties 'Florida 47R' and 'Mountain Glory' were grown from commercial seed and maintained in the greenhouse. *Salmonella* contamination (single or a cocktail of serovars *Salmonella* Javiana, Montevideo, Newport, Saintpaul, and Typhimurium) was introduced via blossoms (3.9 log CFU/blossom) or soil (8.4 log CFU/root zone). Tomatoes were analyzed for *Salmonella* by enrichment in accordance with modified FDA-BAM methods. Five randomly chosen colonies from each *Salmonella*-positive sample were serotyped using the Agilent 2100 bioanalyzer following multiplex PCR. Data were analyzed for the prevalence of contamination and serovar predominance in stems and fruit.

Results: Soil inoculation resulted in 19 (19%) of 100 of plants demonstrating translocation of *Salmonella* to the lower stem after seven days post-inoculation (dpi). *Salmonella* Javiana, Montevideo, Newport and Saintpaul translocated the farthest up the stem (ca. 5.0 to 6.5 cm) while *Salmonella* Typhimurium translocated the least distance up the stem (ca. 1.5 to 2.0 cm). Of the total tomato fruit harvested from *Salmonella*-inoculated (cocktail) blossoms (n=200), 28 (14%) of 200 were contaminated and five (2.5%) of 200 had *Salmonella* internalized into the fruit; of the presumptive-*Salmonella* positive colonies screened, 70 (50%) of 140 and 38 (27%) of 140 were identified as *Salmonella* Montevideo and *Salmonella* Javiana on the surface, respectively and 13 (52%) of 25 were identified as *Salmonella* Montevideo inside. When single serovars were used to inoculate blossoms, *Salmonella* Montevideo had the highest prevalence at five (17.2%) of 29 on the surface of tomato fruit while *Salmonella* Newport (three of 49) and *Salmonella* Saintpaul (one of 59) were the only serovars found to internalize into the fruit.

Significance: Lower levels of inoculum on blossoms resulted in reduced colonization and internalization of *Salmonella* on tomato; however, a risk to public health remains.

T3-02 Logistical Challenges and Lessons Learned in an International Supply Chain Study to Evaluate the Influence of Packaging Type on Broccoli Quality and Food Safety Attributes

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Introduction: Due to concerns about food safety, packing broccoli in ice is no longer an acceptable practice. Packing broccoli without ice may change the cooling rate, thus influencing the final quality and food safety of the produce

Purpose: The objective of this study was to monitor the temperature and relative humidity of dry-packed broccoli in various packing types over the supply chain, from the field, packing in Guadalajara, Mexico to delivery at a produce distributor in St. Paul, MN.

Methods: Loggers were calibrated and tested to assure robustness before shipping to Guadalajara, Mexico. Broccoli was dry packed into cases made up of a variety of packaging materials: corrugated waxed cardboard (CWC), reusable plastic bins (RPB), and Eco Pack System, with and without lids (EPL and EP, respectively) containing loggers to measure temperature and relative humidity. All cases were packed into a single pallet for hydro-cooling and refrigerated shipment to the United States. Packaging surfaces and broccoli were sampled in the field and at the final produce distributor to establish microbial levels (SPC and coliforms).

Results: The most challenging portion of the study was shipping loggers with lithium-ion batteries into Mexico and then assuring reentry to the United States. Packaging type influenced the amount of field heat dissipated during the hydro-cooling step. The amount of time taken to reach 5°C or below varied by packaging type; RPC took 5.8±0.25 h, EP took 6.8±3.8 h, EPL took 13±7.0 h, and CWC took 57±7.5 h. Higher coliform levels (P<0.05) were found after transport in broccoli packaged in EPL and CWC.

Significance: Cooling after produce harvest is essential for food safety, and product quality. This study has shown that packing materials can influence cooling during the dissipation of field heat and transport.

T3-03 Detection and Prevalence of *Listeria* in Produce Packing and Fresh-cut Operations.

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* contamination of produce can often be traced back to environmental sources in packing and fresh-cut operations. Environmental monitoring programs (EMP) are of increasing importance, as they are a key strategy for identifying environmental *L. monocytogenes* sources that could lead to finished product contamination.

Purpose: The goal of this project is to work with production operations to develop *Listeria* EMPs in order to gather baseline data on *Listeria* prevalence and distribution over one year.

Methods: We developed and implemented sampling plans in eight produce operations and collected samples (n=2000) over one year, a minimum of six times per facility. Each operation was visited at the end of year one for a validation sampling by an outside expert that had never seen the operation previously. Samples were tested using the FDA BAM method. SigB sequencing was used as a preliminary typing method.

Results: Among the sponge samples collected, there was a statistically significant (P<0.05) difference between positives observed during routine (43 of 2000) versus validation (26 of 392) samplings. Additionally, 24 of 85 samples with more than one *Listeria* isolate sequenced had more than one allelic type present. We also found that 35 of 114 samples were found only to be positive at one enrichment time (24 versus 48 h). *L. monocytogenes* prevalence overall varied, from zero (<0.40%) of 249 to 11 (6.9%) of 159.

Significance: Our data indicate that the classical culture-based methods that plate after two different enrichment times provide enhanced sensitivity, and that collection of multiple isolates is necessary to capture *Listeria* diversity present. The results also show the value of validation sampling by an outside party, which can both increase confidence in results or identify potentially problematic sampling schemes. While our data cannot be accurately extrapolated to other operations, they indicate overall a need for robust sampling techniques and testing procedures and individualized sampling plans.

T3-04 Prevalence and Antimicrobial Resistance of *Listeria* spp. from Pacific Northwest Produce Processing and Handling Environments

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (*Lm*) can survive and establish itself in diverse food processing and handling environments. Identifying niche locations and strain characteristics through routine and investigative environmental programs is of utmost importance to producers of ready-to-eat foods and raw agricultural commodities.

Purpose: Determine the prevalence and characteristics of *Listeria* spp. in Pacific Northwest (PNW) produce processing and handling (PPH) facilities.

Methods: Surfaces (e.g., drains, equipment, forklifts) near high-traffic entry areas (n=50/facility) were swabbed using ready-to-use sponges in six PPH facilities, followed by a "swab-a-thon" (n=100) in one PPH. *Listeria* spp. were isolated using a modified ISO 11290-1 method, speciated (Microgen® *Listeria*-ID), and antibiogram-typed (CLSI) by disc diffusion assay using 17 antimicrobials.

Results: *Listeria* spp. were recovered from 15 swabs (n=400; 3.8%). Isolates (up to 3) from each positive sample were speciated as *Lm* (n=29) and *L. innocua* (*Li*; n=13). *Lm* was frequently (7/11 swabs) seen in drains and forklift traffic areas. All isolates showed sensitivity to amikacin, ampicillin, erythromycin, gentamicin, imipenem, kanamycin, rifampin, co-trimoxazole and tetracycline. Resistance to cefoxitin (100%), chloramphenicol (CHL; 10% *Lm*), clindamycin (CLI; 100% *Li*; 72% *Lm*), nalidixic acid (100%), novobiocin (NOV; 15% *Li*; 14% *Lm*), and penicillin (PEN; 8% *Li*; 14% *Lm*) was observed, as was reduced susceptibility to CHL (15% *Li*; 7% *Lm*), CLI (8% *Li*; 28% *Lm*), NOV (38% *Li*; 72% *Lm*), PEN (92% *Li*; 86% *Lm*), streptomycin (62% *Li*; 48% *Lm*) and vancomycin (46% *Li*; 76% *Lm*).

Significance: Our data show that areas with heavy traffic and close to outside environments are conducive to *Listeria* spp. hotspots. Preventive controls efforts should be focused on these areas. A proportion of isolates possessed reduced susceptibility or resistance to clinically-relevant antibiotics, though none were resistant to antibiotics used in listeriosis treatment. Considering the ubiquity of *L. monocytogenes* and potential severity of listeriosis, antimicrobial resistance trends should be continuously monitored in food-chain isolates.

T3-05 Impact of Various Post-harvest Wash Water Conditions on the Performance of Peracetic Acid over Time

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Introduction: Peracetic acid (PAA) is a known antimicrobial solution that reduces the pathogenic risk of postharvest wash water, but many products in the market vary the chemical ratio of PAA and hydrogen peroxide (H_2O_2) which makes sourcing challenging for processors. There is a need to evaluate the accuracy and limitations of different compositions of PAA under traditional postharvest wash water processing conditions.

Purpose: This study investigated PAA's stability and efficacy against *Escherichia coli* O157:H7 upon reinoculation and their dependence on H_2O_2:PAA ratio (0:100, 25:75, 50:50, 75:25, 100:0), concentration (0, 30, 60, 80 ppm), organic load (0, 100, 750 COD), temperature (4, 20, 37°C), and pH (5.0, 6.5, 9.0).

Methods: Postharvest wash water samples were inoculated with nalidixic acid-resistant *E. coli* O157: H7 (ATCC 43894) to compare the stability and efficacy of different H_2O_2/PAA treatments. *E.coli* O157: H7 were enumerated by plating an aliquot of the postharvest wash water on nalidixic acid-treated tryptic soy agar. Sanitizer stability was measured using a two-part iodometric titration performed using a Hanna Instrument titrator HC901C. Experiments were conducted in triplicate and statistical analysis of variance (ANOVA) was performed.

Results: Sanitizers with PAA as the dominant ingredient were more efficient at reducing *E. coli* O157: H7 levels over time when compared to those with H_2O_2 as the dominant ingredient. The 50:50 H_2O_2: PAA at 80 ppm wash water was the most stable over time and had the highest efficacy against *E. coli* O157: H7 with organic load capacity below 750 COD. Organic loads at 750 COD interfered with *E. coli* O157: H7 inactivation after 3.5 hrs, compared to all parameters tested.

Significance: Sanitizer composition, concentration, organic load, temperature and pH can significantly decrease the efficacy and stability of PAA systems over time, indicating that these parameters should be monitored in production to ensure that water quality and safety are maintained.

T3-06 Evaluation of Abiotic Bacterial Surrogates for Validation and Verification of One-pass Produce Wash Systems

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Introduction: The routine measurement of the lethality of a produce wash process is difficult to impossible given the normally low pathogen load and the variability of other microbial flora on the incoming product. In response, finished product for testing for pathogens is often used as a final check for safety. However, this testing does not validate the wash process itself. It is slow and can sometimes lead to costly test and release programs. Additionally, pathogen testing has low sensitivity for detecting contaminants due to sample sizes and non-uniform distribution of pathogens.

Purpose: Demonstrate the measurement of lethality with abiotic bacterial surrogates (SafeTraces AquaTags)

Methods: In one-pass produce wash system at 15 ppm chlorine, the recovery of abiotic *E. coli* surrogates (AquaTags) was compared to the recovery of *E. coli*. Iceberg lettuce was inoculated with both *E. coli* and the surrogates. Samples were tested before and after wash for each run for the presence of both *E. coli* and the surrogates.

Results: The abiotic surrogates had similar log reduction to *E. coli* in the one-pass wash system. Over four runs with 10 samples of 25 g each, mean log reductions of 1.45±0.28 and 1.41±0.15 were observed for the abiotic surrogate and for *E. coli,* respectively. *E. coli* was measured using 3M Petrifilms. The surrogates were measured using quantitative PCR. These results reinforce previous correlations in flume wash systems.

Significance: The log reduction of the abiotic surrogates and *E. coli* strongly correlate, suggesting that the lethality of wash systems can be measured with the abiotic surrogate. These surrogates can enable validation and routine verification of wash process lethality and reduce reliance on finished product testing. The PCR assay for the test of the surrogates takes approximately 15 min and a validation study can be completed in less than one hour.

T3-07 Disinfection of Minimally Processed Pineapple Using *Enterococcus faecium* as a Surrogate for *Salmonella enterica*

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Introduction: Pineapple is a widely consumed fruit globally; however, there is little information regarding the effectiveness of decontamination treatments. *Enterococcus faecium* is a bacterium that has been used as a surrogate for *Salmonella* to evaluate heat treatments; however, it has not been used for disinfection treatments.

Purpose: The study was conducted to evaluate the efficacy of chemical disinfectants treatments to control *Salmonella enterica* using *E. faecium* as a surrogate in minimally processed pineapple.

Methods: Growth curves were made for *S. enterica* and *E. faecium* (resistant to rifampicin) in trypticase soy broth (TSB) at different pH (3.5, 4.0 and 4.5) at 20 and 35°C, as well as in pineapple at 35°C. Portions of pineapple (10 g) were inoculated with six log CFU of *E. faecium* and *S. enterica* (different portions for each microorganism). The inoculated pineapple was disinfected with sodium hypochlorite (50 and 200 ppm), electrolyzed water (25 ppm), peracetic acid (40 and 80 ppm), and a disinfectant based on citrus seed extract (0.1 and 0.2%). The surviving cell count was performed on trypticase soy agar supplemented with rifampicin (200 ppm). The comparison of disinfectants was carried out by ANOVA test followed by a Tukey comparison ($P \leq 0.05$).

Results: No significant differences were found between the growth of *S. enterica* and *E. faecium* in TSB and pineapple ($P \geq 0.05$). Regardless of the disinfectant and the concentration, no significant differences were found ($P \geq 0.05$) and neither regarding the use of *S. enterica* and the surrogate. The population of *E. faecium* decreased 2.23, 1.52, 1.82 and 1.71 log CFU/g after the application of chlorine, peracetic acid, citrus seed extract, and electrolyzed water, respectively.

Significance: The results show the viability of using *E. faecium* as a surrogate of *S. enterica* in disinfection treatments. However, the decrease in the bacteria population did not reach the five log CFU recommended reduction. Therefore, it is necessary to design effective alternatives for decontamination of minimally processed pineapple.

T3-08 Application of Ultraviolet Light in Combination of Peracetic Acid Washing to Inactivate *Salmonella* on Shredded Iceberg Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce has been implicated in many *Salmonella* outbreaks. Washing with chlorinated water is commonly used to decontaminate fresh produce and prevent cross-contamination via wash water. In this study, we explored the potential of using ultraviolet light (UV) with peracetic acid as an alternative to chlorine washing for fresh produce.

Purpose: The purpose was to determine the effect of UV with/without peracetic acid (PAA) washing on inactivation of *Salmonella* on lettuce and in the wash water.

Methods: Shredded iceberg lettuce was dip-inoculated with a four-strain *Salmonella* cocktail to final levels of six to 7.5 log CFU/g and air-dried. They were then washed in tap water containing six percent lettuce extract (turbidity >60 NTU; COD >2000 ppm) while being treated by 20 ppm free chlorine, PAA (40 and 80 ppm), UV (10, 20 and 30 mW/cm^2), and a combination of UV and PAA for one, two, and five min. The *Salmonella* counts of the lettuce and water samples were determined.

Results: Among all the single treatments, the 30 mW/cm^2 UV treatment achieved the highest *Salmonella* reduction (two log) on lettuce. However, the single UV treatment was not able to completely eliminate *Salmonella* in the wash water. The combined treatment, 30 mW/cm^2 UV and 80 ppm PAA, resulted in the highest *Salmonella* reduction (2.52 log±0.38) on lettuce and completely eliminated *Salmonella* in the wash water. Extending the washing time from one to five min for the combined treatment increased the *Salmonella* reduction on lettuce from 2.48 log±0.52 to 3.24 log±0.24. The sample size of lettuce between 200 g, 500 g, and 1,000 g did not significantly impair the inactivation of *Salmonella* on lettuce for the combined treatment.

Significance: The combined UV and PAA treatment could be used as an alternative to chlorine washing for fresh produce decontamination.

T3-09 A Mathematical Model for Chlorine Kinetics and Pathogen Cross-Contamination in Fresh Produce Wash Processes

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Introduction: Produce wash could induce pathogen cross-contamination due to lowered efficacy of chlorine-based sanitizers at higher organic matter content (COD) in water. Free chlorine (F_r) will bind to COD released by fresh-cut produce instead of binding to bacteria. Even at variable COD levels, F_c levels must be continuously regulated at desired levels to allow better sanitization.

Purpose: To develop a mathematical model which accurately predicts cross-contamination of pathogen levels and chlorine kinetics during produce wash cycles.

Methods: Chopped iceberg lettuce was washed (five kg/run; three runs) in a tap water bath (pH ~6.5) with initial F_c ~10 ppm. Simultaneously, red leaf lettuce (two wt%; dwell time ~ 30 s) inoculated with *E. coli* O157: H7 (five log MPN/g) was introduced to the tank. Turbidity, COD, pH, total and free chlorine, bacteria levels in red leaf and iceberg lettuce and wash water were measured every two min (three independent repeats). A mathematical model to simulate chlorine kinetics and cross-contamination in the washing process of fresh produce was developed.

Results: At high lettuce amounts, F_c levels decreased rapidly while COD and turbidity increased linearly; pH slightly decreased due to F_c depletion. Chlorine consumption rate increased in second and third runs. A strong relation between F_c and COD was noted, and our model predicted F_c levels with > 90% accuracy. At F_c of two ppm, bacteria persisted in the *ic*eberg lettuce. The overall reduction of bacteria in red leaf lettuce was one log and F_c had no effect on bacteria reduction in red leaf lettuce. Our mathematical model accurately predicted bacteria levels in iceberg lettuce and wash water.

Significance: Our mathematical model could accurately predict bacteria cross-contamination and chlorine reaction kinetics during produce wash cycles.

T3-10 Fate of Injured *Salmonella* and *Escherichia coli* O157:H7 on Granny Smith Apples after Cold Plasma and Organic Acid Treatment

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Introduction: Inability of chlorine-based antimicrobial treatments to kill all bacterial populations on fruit surfaces designated for fresh-cut preparation is a food safety problem. There is a need for an alternative sanitizer treatment that can achieve a better inactivation without damaging the surface characteristics and the atheistic appeal of the treated fruits.

Purpose: To estimate the percent of injured bacterial pathogens after cold plasma treatments and how these injured populations will react upon further treatments with short-chain organic acids.

Methods: Granny Smith apples were inoculated with 10^7 CFU/ml *Salmonella* and *Escherichia* coli O157: H7. All inoculated apples were exposed to cold plasma treatment for 40 s, followed immediately by dipping in a solution of organic acids for five min. Bacterial populations on apple surfaces before and after treatments were enumerated using selective and non-selective agar media. Injured populations by cold plasma treatments were assayed before and after organic acid exposure.

Results: After inoculation of the apples, bacterial pathogens recovered on the apples averaged 4.6±0.18 log CFU/g and 4.8±0.14 log CFU/g for *Salmonella* and *Escherichia* coli O157: H7, respectively. Cold plasma treatments for 40 s led to average 0.5-log inactivation for the pathogens and the percent injured bacteria among the survivors averaged 18 and 20% for *Salmonella* and *Escherichia* coli O157: H7, respectively. Cold plasma treatments followed immediately by dipping apples in an organic acids solution for five min led to 3.8-log inactivation, and the injured populations were less than one percent.

Significance: These results suggest that the injured pathogens on apple surfaces treated with cold plasma can be totally inactivated by immediate treatment with an organic acid solution, suggesting that this treatments procedure will enhance the microbial safety of treated apples designated for fresh-cut preparation.

T3-11 Influence of Bacteriophage in the Control of Stress-adapted *Listeria monocytogenes* Inoculated on Fresh-cut Produce

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Introduction: *Listeria monocytogenes* develops different mechanisms to survive in fresh-cut produce under different preharvest and postharvest conditions

Purpose: To investigate the influence of bacteriophage control on stress-adapted *L. monocytogenes* inoculated on fresh-cut tomato and carrot

Methods: Fresh-cut carrot and tomato inoculated with 10^8 CFU/ml of *L. monocytogenes* (acid-adapted-AA, chlorine-adapted-CA, heat-adapted-HA) in comparison with the non-adapted strain (NA) were treated with 10^8 PFU/ml of Listex P100 bacteriophage and stored at four, ten, and 25°C for 10 days. Effect of treatment on quality attributes (pH, total soluble solids, titratable acidity and color), and morphology was also investigated.

Results: All stress-adapted and non-adapted *L. monocytogenes* were ($P \leq 0.05$) susceptible to bacteriophage control. With phage treatment, the population of *L. monocytogenes* on fresh-cut tomato reduced by less than one-fold log cycle, while greater log reductions (twofold) were observed for a fresh-cut carrot. Phage treatment reduced NA, AA, CA and HA *L. monocytogenes* populations by 0.57, 0.81, 0.86 and 0.95 log CFU/ml on fresh-cut tomato and by 2.26, 2.41, 2.49 and 2.54 log CFU/ml on fresh-cut carrot respectively. Quality attributes of both types of fresh-cut produce were not significantly affected by the phage treatment.

Significance: Bacteriophage biocontrol can offer eco-friendly means of addressing possible *L. monocytogenes* contamination under different preharvest and postharvest conditions in fresh produce

T3-12 Some Steps Toward Validating a Fresh-cut Process to Meet the Food Safety Modernization Act Requirements

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Introduction: To be Food Safety Modernization Act compliant, processors need to know their process, know why it is their process and know that they achieved their process. Compliance requires both process metrics and process control parameters. Cross-contamination control is a process metric. When this control is inadequate, a contamination event can be amplified.

Purpose: Demonstrate a cross-contamination metric and a performance-based approach to determining the appropriate duration of a validation trial where this procedure would be applied.

Methods: Cross-contamination was examined indirectly by APC capture sterile sheets of nonwoven fabric (MicroTally swabs) suspended in the product stream. Archival process data is used to establish an appropriate length for a validation study based on variance.

Results: In pilot plant experiments at zero, three, seven, and 15 ppm free effective chlorine, the metric for cross-contamination showed a 2.5-log reduction in APC uptake at three ppm relative to six-log uptake with no chlorine. At higher chlorine levels uptake approached the sensitivity of the assay (about 3.5-log reduction). APC levels determined by grab sampling were highly variable before and after processing. The log of the geometric mean of the post process APC at the highest chlorine level with a silver ion pretreatment (SmartWash Boost) was 5.2±1.3. This variability is characteristic of APC data on fresh-cut produce and limits the utility of APC for directly measuring lethality. The running standard deviation the free effective chlorine level stabilizes at about ±0.6 ppm with an interval of four to five hours indicating that normal variation has been included.

Significance: A practical noninvasive procedure for assessing cross-contamination control would allow fresh-cut processors to assess the performance of their processes during commercial operations. This approach needs further validation, but it is the basis against which other approaches can be evaluated.

T4-01 Antimicrobial Resistance in Retail Ground Beef with and without a "Raised without Antibiotics" Claim

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Introduction: Raised without antibiotics (RWA) ground beef is perceived to harbor lower antimicrobial resistance (AMR) levels than conventional products.

Purpose: Determine AMR levels in retail ground beef with and without an RWA claim.

Methods: Retail ground beef samples were obtained from six United States cities, and 299 samples had an RWA claim. Samples lacking an RWA claim (n=300) were assigned to the conventional production system (CONV). Each sample was cultured for the detection of five antimicrobial resistant bacteria (ARB). Metagenomic DNA was isolated from each sample and qPCR was used to determine the abundance of ten antimicrobial resistance genes (ARGs). The impacts of the production system and city on ARB detection were assessed by the Likelihood-ratio chi-squared test. The impact of production system on ARG abundance was assessed by two-way ANOVA.

Results: Tetracycline-resistant *E. coli* (CONV=46.3%; RWA=34.4%) and erythromycin-resistant *Enterococcus* (CONV=48.0%; RWA=37.5%) were more frequently (P<0.01) detected in CONV. Detection of 3rd generation cephalosporin-resistant *E. coli* (CONV=5.7%; RWA=1.0%), vancomycin-resistant *Enterococcus* (CONV=0.0%; RWA=0.0%) and methicillin-resistant *Staphylococcus aureus* (CONV=1.3%; RWA=0.7%) did not differ (P=1.00). The bla_{CTX-M} ARG was more abundant in CONV (2.4 vs. 2.1 log copies/gram, P<0.01) but the *tet*(A) (2.4 vs. 2.5 log copies/gram, P=0.02) and *tet*(M) (3.6 vs. 3.9 log copies/gram, P<0.01) ARGs were more abundant in RWA. *aadA1*, bla_{CMY-2}, *mecA*, *erm*(B), and *tet*(B) abundances did not differ significantly (P>0.05).

Significance: United States retail CONV and RWA ground beef harbor generally similar levels of AMR since only five of 15 AMR measurements were statistically different between production systems. Three AMR measurements were higher in CONV, while two AMR measurements were higher in RWA.

T4-02 Antimicrobial Effect of Major Components of Berry Phenolic Extract against *Campylobacter*

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Introduction: Bioactive extracts of berry fruits or byproducts (pomace) were found to be a potential alternative to synthetic antimicrobials in reducing growth and colonization of *Campylobacter jejuni* in the poultry gut and improving weight gain. As berry pomace comprises numerous bioactive compounds including phenolic acids like gallic acid (GA), vanillic acid (VA), and protocatechuic acid (PCA), it is important to know which one has the potential effect in reducing growth and altering different pathogenic traits of *C. jejuni*.

Purpose: The purpose of this study was to evaluate the effectiveness of individual phenolic compounds against growth, adhesion/invasiveness, biofilm formation ability and virulent gene expression of *C. jejuni*.

Methods: Bacterial growth patterns were determined at various time points. Adhesion and invasiveness assays were performed using chicken fibroblast (DF-1) cells. Biofilm formation ability and virulence genes expression of *C. jejuni* in the presence of individual phenolic components were evaluated. Significance in treatment difference was determined by ANOVA.

Results: In mixed culture conditions, growth of *C. jejuni* was reduced by 0.7 log CFU/ml, 0.6 log CFU/ml and 0.1 log CFU/ml at 48 h (P<0.05) in presence of one mg/ml of GA, 0.4 mg/ml of VA and one mg/ml of PCA, respectively. Interaction of *C. jejuni* with cultured DF-1 cells was altered by the treatments of the individual phenolic compound. The biofilm formation ability of *C. jejuni* was increased significantly by 37.7, 33.15, and 15.4% by pretreatment with GA, VA and PCA, respectively (P<0.05). Further, the treatments altered the expression of multiple virulence genes such as *cdtB, cadF, fldA, sodB* of *C. jejuni* by 2.9 to 0.2 fold and significantly (P<0.05) upregulated the expression of *ciaB* gene 1.9 to-6.6 fold.

Significance: GA, VA and PCA can contribute to the reduction of pathogenic bacterial survival ability, colonization and virulence gene alteration. The basis of action of these compounds may also serve as a foundation for understanding the mechanism of action of berry phenolics against *C. jejuni*.

T4-03 Isolation and Assessment of Poultry-derived Lactic Acid Bacteria for Their Use as Host-specific Probiotics

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❖ ***Developing Scientist Entrant***

Introduction: Lactic acid bacteria (LAB)-based probiotics have been in high demand within the past few years due to their potential positive effects on host health and ability to reduce food-borne pathogens in final products (e.g., poultry meat). Research on probiotics is key to understanding if and how probiotics can increase food safety.

Purpose: The purpose of this study is to identify poultry-derived, host-specific LABs for use as probiotics in the poultry industry.

Methods: Cecal and ileal samples were taken from 15 production broilers from both "Conventional" and "No-Antibiotics-Ever" farms on day zero and 20 of the grow-out cycle (120 birds total). LABs were cultured using two different CO_2 levels (aerobic and elevated/5% CO_2) using MRS media. One LAB isolate was chosen per plate (278 total), Gram stained, and sequenced (16S rRNA and Whole Genome Sequencing). Phylogenetic analyses of our WGS data and publicly available strains were used to test potential host specificity, and our WGS data was also used for identification of genomic elements (e.g. bacteriocins) within strains known to suppress the growth of food-borne pathogens.

Results: 16S rRNA results from our isolates (n= 100) identified 40 *Lactobacilli* (40%), specifically *L. salivarius* (37; 92.5%) and *L. johnsonii* (3; 7.5%) with the remaining 60 identified as *Enterococcus faecalis* (38), *E. faecium* (16), *E. hirae* (three), *E. durans* (two), and *E. villorum* (one). Phylogenetic analysis of five of our *L. salivarius* strains and 88 *L. salivarius* strains in GenBank (n=93) determined that our strains were genetically diverse, and nested in a clade of poultry-associated isolates, suggesting host specificity. Two *L. salivarius* strains contain genes encoding bacteriocin-like peptides.

Significance: These initial results indicate that a small number of *L. salivarius* strains show promising characteristics for poultry-derived, host-specific probiotics.

T4-04 Investigation of the In-feed Reduction of the Antimicrobial Tylosin on Antimicrobial Resistance (AMR) in Enterococci in Feedlot Cattle

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❖ ***Developing Scientist Entrant***

Introduction: Tylosin is frequently administered in-feed to prevent liver abscesses in beef cattle. There is a growing interest in lowering industry reliance on tylosin for liver abscess control because of concerns over antimicrobial resistant (AMR) bacteria entering the food chain.

Purpose: To determine the effect of withdrawing the in-feed administration of tylosin in the first or last 25% of the feeding period on the proportion of erythromycin-resistant enterococci in beef cattle feces.

Methods: A total of 7,500 crossbred yearlings were randomly assigned to one of three treatments: tylosin in-feed for i) the entire feeding period (control), ii) the first 75%, or iii) last 75% of the feeding period, with ten replicate pens per treatment. Fresh fecal samples from the floor of each pen were collected on days zero, 80, and 160 of the finishing period. Appropriate serial dilutions were spread plated onto bile esculin azide (BEA) agar and BEA amended with eight µg/ml erythromycin for determining the proportion of erythromycin-resistant enterococci. A PCR assay was used to speciate isolates.

Results: The proportion of erythromycin-resistant enterococci for control, last 75%, and first 75%, was 27, 17, and 14% upon arrival, and 51, 50, and 21% prior to slaughter, respectively. Although the population of erythromycin-resistant enterococci increased with days on feed (P<0.01), neither the method of tylosin administration (P=0.34) or the tylosin administration×days on feed (P=0.34) interaction were significant. Of the 538 isolates, 97% were confirmed as enterococci, with an increase in *Enterococcus hirae* from 82 to 100% between day zero and days 80 or 160 of the experiment.

Significance: Overall, the administration of tylosin in-feed increased the proportion of erythromycin-resistant enterococci in all three experimental groups over the feeding period.

T4-05 Efficacy of Chlorhexidine Digluconate and Alkyltrimethylammonium Bromide for Carcass Decontamination to Ensure Food Safety

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Introduction: To prevent bacterial cross-contamination of meat is a huge challenge for meat industries. The surface of cattle serves as a host to several enteric pathogens. During the hide removal meat can potentially be cross-contaminated by the pathogens transferred from hides' surface to underlying meat, thus increasing chances of food-related illnesses. To prevent cross-contamination, cattle are expected to be washed with environmentally friendly antimicrobial formulations.

Purpose: This study evaluated the effectiveness of spray washing with an aqueous solution of chlorhexidine-digluconate and alkyltrimethylammonium bromide to reduce pathogens from the haired surface of bovine hide.

Methods: Samples of freshly flayed hides (10 cm^2 each) from processing facilities were inoculated with one ml of a nine log CFU/mL of a three-strain cocktail of a single bacterial species (*Salmonella enterica, Escherichia coli O157:H7 and Listeria monocytogenes*) and allowed to sit for ~one min before spray treatment. Inoculated hide samples were treated individually by spraying five ml of different concentration of chlorhexidine-digluconate or alkyltrimethylammonium bromide and spraying with water was used as the control. Samples for bacterial enumeration were gathered from hide samples at three and five min and plated on the selective medium to allow surviving bacteria to grow. Enumerated bacterial populations were reported as log CFU/cm^2 of hide tested.

Results: With the treatment of chlorhexidine-digluconate the greatest reductions of 2.82 log CFU/cm^2 for *S. enterica*, 2.41 log CFU/cm^2 for *E. coli O157: H7* and 2.18 log CFU/cm^2 for *L. monocytogenes* resulted on inoculated hide surface, relative to a water-only control. Similarly, treatment with alkyltrimethylammonium bromide resulted in maximum reductions of 0.40, 1.77 and 2.05 log CFU/cm^2 for *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* respectively. Results suggest these antimicrobials can reduce pathogens on haired surfaces of hides.

Significance: Carcass pathogen intervention systems have been studied to develop novel pre-slaughter cattle wash formulations for meat safety.

T4-06 Synergistic Effect of Bacteriophages and Buffered Vinegar on *Listeria*-contaminated Ready-to-Eat Products

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Introduction: RTE product producers seek ways to comply with FSIS *Listeria* rules for delivering *Listeria*-free foods. This forces the industry to test and implement new microbial intervention strategies.

Purpose: To assess the use of PhageGuard Listex (a natural bacteriophage solution against *Listeria*) with buffered vinegar as antimicrobial agents of addressing post-lethality *Listeria* contaminations in RTE foods.

Methods: Cooked turkey slices were inoculated with a cocktail of four *Listeria monocytogenes* strains at 10 CFU/g (detection limit 10 CFU/g). Similarly, whole quiches with and without added buffered vinegar were inoculated at two CFU/g (detection limit two CFU/g). Both contaminated products were either surface spray treated with phages (2×10^7 PFU/cm^2) or phages and buffered vinegar. Samples treated with either buffered vinegar or tap water were used as negative controls. Subsequently, samples were vacuum packed and stored at 40°F. During the shelf-life of 21 and 120 days for quiche and turkey slices respectively, samples were retrieved to detect and enumerate *Listeria*. Data presented are the mean value of two individual experiments.

Results: On contaminated turkey slices, phage solution or buffered vinegar alone kept *Listeria* concentration below the detection limit for 10 and 30 days respectively. A clear synergistic effect was observed when combining phages with buffered vinegar, as *Listeria* was below the detection limit for almost 120 days. For contaminated quiche, phages alone kept *Listeria* below a two-log outgrowth level for up to 21 days. A similar, though stronger effect, was observed with phages plus buffered vinegar as this treatment suppressed the growth of *Listeria* throughout the shelf life of quiche.

Significance: These results establish PhageGuard Listex when applied along with buffered vinegar as an effective combination of antimicrobials to control *Listeria* during processing of RTE foods, thus leading to an increase in consumer safety.

T4-07 Use of Medium Chain Fatty Acids to Mitigate *Salmonella* Typhimurium (ATCC 14028) in Dry Pet Food Kibbles

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Introduction: Fats and oils are commonly coated on dry pet food kibbles. This occurs after the process kill step. There is a potential for *Salmonella* contamination in coating materials. Medium chain fatty acids (MCFA) are known to have antimicrobial properties. Therefore, MCFA may be an alternative to reduce *Salmonella* contamination during oil and flavor coating of dry extruded pet foods

Purpose: To determine the effect of MCFAs against *Salmonella* Typhimurium (ATCC 14028) in fats and oils applied to dry dog food kibbles.

Methods: Minimum inhibitory concentration (MIC) of three MCFAs, namely caproic (C6), caprylic C8) and capric (C10) acids were determined using the broth micro and macro-dilution assay technique. Using canola oil as a fat coating, the efficacy of each MCFA was then tested on dry pet food kibbles at 37°C for up to five h. *Salmonella* counts were enumerated at different predetermined time intervals (zero, one, two, three, four, and five h) by plating on trypticase soy agar (TSA) and incubating at 37°C for 24 h. The experiment was conducted in triplicate.

Results: The MICs were found to be 0.3125, 0.3125, and 0.625 for C6, C8 and C10, respectively. When the MCFAs were tested on dry fat coated kibbles, all three reduced (P≤0.05) the *Salmonella* levels by ~five logs after five h when the *Salmonella* recovery from a no treatment control was ~6.4. At each evaluation time point, all three treatments were effective in reducing (P≤0.05) the *Salmonella* load. When reductions were analyzed among different time points, C6 was most effective (P≤0.05) at time zero as compared to other MCFAs.

Significance: This study suggests that the use of medium chain fatty acids during kibble coating may mitigate post-processing *Salmonella* re-contamination in dry dog food kibbles.

T4-08 Cultural and Genetic Characterization of *Escherichia* Phage Osysp and Assessing Its Suitability for Food Safety Applications

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Introduction: Interest in isolating and characterizing new phages for controlling foodborne bacterial pathogens is increasing. This was favored by regulatory approvals of phage applications and researchers' easy access to next-generation sequencing technologies.

Purpose: In this study, we investigated the cultural and genomic traits of the previously isolated *Escherichia* phage OSYSP to assess its suitability for food applications.

Methods: Bacteriophage host range was determined against *Escherichia* and *Salmonella* strains by spot-on-lawn test and double layer plaque assay technique. Pure phage suspension was stored at 4°C for up to 24 months to determine its shelf life stability. To assess pH sensitivity and survival at different incubation temperatures, OSYSP was held at pH values in the range of 2 to 12 and temperatures from 4°C to 47°C for 30 min. The whole genome of *Escherichia* phage OSYSP was sequenced using the combination of Illumina Miseq and Ion Torrent sequencing platforms. Ion Torrent reads along with conventional PCR were used for phage OSYSP genome arrangement confirmation.

Results: Phage OSYSP inactivated pathogenic and non-pathogenic strains of *E. coli* and *S. enterica*. The phage was very effective against all tested O157:H7 strains. Phage stock titers were relatively stable throughout the 24-month storage period at 4°C. Incubation at 4°C to 47°C and pH 4 to 11 had no significant detrimental effect (P>0.05) on phage infectivity. OSYSP showed close relation with T5-like phages; however, confirmed genome arrangements proved the novelty of OSYSP. *In silico* analysis of conventional PCR products and phage, the whole genome showed the presence of lysis-related genes and the absence of undesirable virulence, lysogeny, allergenicity, and antibiotic resistance elements.

Significance: Desirable genomic traits and physiological stability at adverse conditions suggest that bacteriophage OSYSP is a promising biocontrol agent for foods processed and stored under various conditions.

T4-09 Synergistic Antimicrobial Activity between Physical Treatments and Lauric Arginate: Mechanisms Beyond Membrane Damage

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Introduction: The need for more effective antimicrobials is critical for the food industry to improve food safety and reduce spoilage of minimally processed foods. Furthermore, the emergence of bacterial resistance against commonly used sanitizers has also been widely reported.

Purpose: The study was initiated to develop an efficient, applicable and novel antimicrobial approach which combines physical treatments (UV-A or mild heat) and generally recognized as safe lauroyl arginate ethyl (LAE).

Methods: LAE was combined with UV-A or mild heat to treat *Escherichia coli* and *Listeria innocua*. The study was also designed to understand the differences in mode of action between LAE and polymyxin B by comparing minimum inhibitory concentration, liposome model cell membrane, and oxidative stress generation. Synergistic mechanism between LAE and UV-A/mild heat was also evaluated by supplementing with a variety of antioxidants and measurement of cell membrane damage by nucleic acid release.

Results: Synergistic inactivation of bacteria was observed using a combination of LAE and mild physical stresses (heat or UV-A). The synergistic combination resulted in about six-log reduction of target bacteria while individual treatments resulted in less than 1.5-log inactivation under the same set of conditions. Furthermore, the synergistic antimicrobial activity of LAE and mild physical stresses was suppressed by supplementation with antioxidants. Differences in mode of action between LAE and polymyxin B revealed that both compounds differ in their membrane disrupting mechanism, and LAE demonstrated more rapid bactericidal activity which is directly related to oxidative stress generation. Overall, this study illustrates the synergistic antimicrobial activity of LAE with light or mild heat and indicates a novel oxidative stress pathway that enhances the activity of LAE beyond membrane damage.

Significance: Combination of a safe antimicrobial compound and common physical treatments may improve the current sanitation process for the food industry and inactivation of pathogenic strains in biomedical environments.

T4-10 Effect of D-Tryptophan on Psychrotrophic Growth of *Listeria monocytogenes* and Its Application in Milk

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen that is strongly resistant to osmotic stress and can grow at refrigerator temperatures. These two characteristics of *L. monocytogenes* contribute to a particular concern in refrigerated dairy foods and potential risk to public health. Recently, we have reported that exogenously added D-tryptophan (D-Trp) has adverse effects on the growth of some foodborne bacteria under high salt conditions. This amino acid that possesses antibacterial activity may have applications as a novel natural food preservative in controlling the psychrotrophic growth of *L. monocytogenes*.

Purpose: To evaluate the inhibitory effects of D-Trp on the psychrotolerance and osmotolerance of *L. monocytogenes* during long-term refrigerated storage and further examine the feasibility of utilizing D-Trp in controlling *L. monocytogenes* in pasteurized milk.

Methods: We examined the inhibitive effect of D-Trp on *L. monocytogenes* in liquid media via measuring the optical density at 595 nm at 4ºC during a 30-day period. We also investigated the effect of D-Trp on psychrotolerance of *L. monocytogenes* at various sodium chloride (NaCl) concentrations. The viable *L. monocytogenes* cell counts were determined by direct plating on tryptic soy agar plates.

Results: Adding exogenous D-Trp was able to reduce and delay the psychrotrophic growth of *L. monocytogenes* in peptone-yeast extract-glucose medium cultures at 4ºC. A higher level of D-Trp (>30 mM) results in higher and more consistent growth inhibition of *L. monocytogenes*. A concentration of 40 mM D-Trp in combination with high levels of NaCl (>3.0%) caused a greater overall bacteriostatic effect with partial bactericidal effect. In pasteurized milk, 40 mM D-Trp also significantly (P<0.05) inhibited the growth of *L. monocytogenes* during extended refrigeration storage.

Significance: D-Trp appears to be an alternative or complementary strategy to control the psychrotrophic growth of *L. monocytogenes* during long-term refrigeration storage and thus may be appealing to the dairy food industry.

T4-11 Development of Antimicrobial Hydrogel Patches to Control *Vibrio parahaemolyticus* in Raw Fish

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❖ ***Developing Scientist Entrant***

Introduction: In raw fish consumed for sushi and sashimi, non-thermal decontamination technology needs to be applied. Hydrogels composed of edible compounds and antimicrobials should be appropriate as non-thermal decontamination.

Purpose: This study developed an antimicrobial hydrogel patch, composed of edible and non-toxic compounds, to reduce *Vibrio parahaemolyticus* cell counts on slices of raw fish.

Methods: The alginate-based hydrogel was prepared by dissolving five percent sodium alginate powder in 25 ml of distilled water, and mixing with copolymers [1% agar (w/w), 40% glycerol (w/w) and a crosslinker ($CaCl_2$)). The hydrogel was cut into 3.0 by 3.0 cm squares, and they were placed in 10 ml of 0.5% and 1.0% natural antimicrobials (grapefruit seed extract and citrus extract) for two h, followed by drying at room temperature for 30 min. A 100-µl mixture (OD_{620}=0.1) of *V. parahaemolyticus* strains (ATCC17802, ATCC27519, ATCC33844, and ATCC43996) was inoculated on slices of raw fish (halibut). The antimicrobial hydrogels were placed on the inoculated samples and stored at 4ºC for one, 20, 40, and 60 min. After storage, *V. parahaemolyticus* cell counts in the samples were enumerated on thiosulfate-citrate-bile salts-sucrose agar.

Results: *V. parahaemolyticus* cell counts reduced by 1.5 to 2.5 log CFU/cm^2 after the hydrogel application, regardless of antimicrobials. Among the natural antimicrobials, the antimicrobial hydrogel formulated with 0.5% and 1.0% grapefruit seed extract reduced the bacteria by 2.0 and 2.3 log CFU/cm^2 on slices of raw fish, respectively. The antimicrobial hydrogel formulated with 0.5% and 1.0% citrus extract decreased the cell counts by 2.0 and 2.1 log CFU/cm^2, respectively. Thus, the antimicrobial hydrogel composed of 5% alginate, 0.2% $CaCl_2$, 1% agar, and 0.5% grapefruit seed extract was the most appropriate.

Significance: These results indicate that the developed hydrogel can be used to control *V. parahaemolyticus* on slices of raw fish by one-minute application on the surface.

T4-12 Effects of Interventions on Indicator Organism Levels in Beef Slaughter

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Introduction: Beef slaughter establishments employ many different interventions to help minimize the incidence of pathogens in their products.

Purpose: This study explored the relative effectiveness of various common interventions using the answers to questions that FSIS asked as part of the Beef Veal Carcass Baseline Study (2014 to 2015).

Methods: FSIS collected 2,736 swab samples from carcasses at 179 establishments. These included 1,368 samples at post-hide removal (before evisceration) and 1,368 at pre-chill (after evisceration). Samples were tested for pathogens (*Salmonella* and STEC) and indicators (generic *E. coli*, *Enterobacteriaceae*, coliform, and aerobic count) using FSIS-approved methods.

Results: Pathogen positive rates were too low to establish a direct correlation between interventions and pathogens. However, all pathogens correlated with all indicators, suggesting the use of indicators as surrogates. For example, *Salmonella* gave an odds ratio indicating a 57% increase in pathogen prevalence for each log increase in aerobic count. Therefore, we compared indicator levels to assess the effectiveness of interventions. Some interventions such as chlorine wash, correlated with decreased indicator levels, while others, such as trimming alone, correlated with an increase. Most comparisons gave significant trends via ANOVA (P<0.005), and many pairwise correlations were also significant (P<0.05). However, each of the tested intervention strategies generated a wide range of indicator levels.

Significance: This suggests that *how* interventions are applied may be as important as *which* interventions are applied. Although indicator organisms do not provide a comprehensive picture of process control, the correlation between indicators and pathogens provides useful information. Thus, our results can be used by slaughter establishments to help identify the most effective interventions for pathogen reduction.

T5-01 Evaluation of Commercial Molecular Screening Platforms for the Detection of Foodborne Bacterial Pathogens by Food Safety and Inspection Service Field Service Laboratories

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Introduction: The USDA Food Safety and Inspection Service collect approximately 100,000 samples each year to confirm that the HACCP system in Federally inspected commercial establishments is under control. The pathogens evaluated include *Salmonella*, *Listeria monocytogenes*, *Campylobacter*, and Shiga toxin producing *Escherichia Coli*. The methods selected for testing are published in the Agency's Microbiology Laboratory Guidebook.

Purpose: Four commercially available molecular screening platforms were evaluated against the Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) reference cultural methods for *Salmonella* species, *Listeria monocytogenes*, *Campylobacter* species, and Shiga toxin-producing *Escherichia coli* (STEC), including *E. coli* O157 and selected non-O157 STECs.

Methods: A variety of representative food product sample matrices were inoculated with target microorganisms at fractional recovery range of 20-80%. Each molecular screening platform was used by FSIS Field Service Laboratory personnel to analyze a total of 440 *Salmonella*-inoculated samples, 120 *L. monocytogenes*-inoculated samples, 120 *Campylobacter*-inoculated samples, and 180 Shiga toxin-producing *E. coli* (STEC)-inoculated samples. Sixty of the STEC-specific samples were inoculated with *E. coli* O157. Limits of detection and inclusivity/exclusivity panels were examined along with an internal evaluation of the strengths and weaknesses of each technology during use in the FSIS FSL high-throughput microbiology screening environment.

Results: Platforms had a high degree of concordance when compared to the FSIS MLG cultural method. Inoculated samples were identified with a range of 89% to 94% across vendor and matrix. Most technologies erred towards false positives. Specifically, with *L. monocytogenes*, all technologies had agreement with the cultural method, except with smoked fish matrix where all technologies missed about one third of the inoculated samples, except Bax®, that identified all but one . Two technologies were evaluated for *Campylobacter sp*. detection, Bax® and GENE-UP®. Both correctly identified all positive inoculated samples. Detection of non-O157:H7 STECs was evaluated for three vendors. iQ-check® correctly identified a higher proportion, of samples as compared to Bax® or GENE-UP®. Detection of *E. coli* O157:H7 was evaluated on all platforms. Bax® had more false negative results than the other vendors with raw beef matrix. Overall, no single platform was consistently superior for all pathogens and all matrices.

Significance: This evaluation provides evidence of the high consistency offered by the four commercial platforms. Having documented comparable performance, the decision on which to operate depends on the business model and throughput needs of the user. While not identical the technologies are deemed comparable for use as molecular screening tools by FSIS.

T5-02 Untargeted Screening of the United States Food Supply to Detect Novel and Emerging Contaminants

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Introduction: Although the Food and Drug Administration's Total Diet Study is a comprehensive monitoring program for a variety of nutrients and contaminants in the United States food supply, any compounds besides those targeted by the analytical methods will not be detected.

Purpose: The purpose of this work was to develop an untargeted LC-MS screening method that can detect contaminants in foods that are not accounted for by existing methods.

Methods: In this work, a simple QuEChERS extraction was combined with a reverse phase (C18) LC-MS method. Data were collected with a quadrupole-Orbitrap High Resolution MS using nontargeted acquisition. A pooled-sample scheme for a reference sample was used, as sample pooling can lead to a more representative estimation of compounds than the examination of individual samples. The pooled reference sample was used to establish a mass spectrometric signature that is unique to each food matrix. The chemometric software package Compound Discoverer was then used to discern compounds that were present in both spiked and unspiked samples that were not present in the pooled matrix reference. This approach allowed us to eliminate both false positive and false negatives relative to simply using a peak-picking approach.

Results: Composite apple samples were extracted and analyzed. Initially, over 10,000 compounds were identified in apples. Compound Discoverer was used to eliminating false positives and compounds that were identified as being native to apples, as determined by a pooling scheme, with pooled samples used as references. A total of 165 compounds were further identified as belonging to pesticides in a pesticide spike, leaving approximately five to 10 compounds as "suspect" compounds in the apple composites. Compounds native to the apple matrix include sugars, amino acids, flavonoids, and phenolic acids.

Significance: This is a useful method for separating "suspect" compounds from matrix-specific compounds without the need for manual examination of each peak.

T5-03 Non-Targeted Identification of Food Adulterants Using Handheld Near Infrared Spectrometers

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Introduction: Non-targeted methods to identify food ingredients and products that have been accidentally or intentionally contaminated are important to maintain a safe food supply. Miniaturization of near-infrared spectrophotometers, the ability to interface these detectors to smartphones, and cloud-based access to chemometric and artificial intelligence software are increasing the use of hand-held spectrophotometric methods for applications in food safety and traceability.

Purpose: The purpose of this work is to present chemometric methods developed using hand-held near-infrared spectrophotometers for non-targeted identification of adulterants in various food ingredients and products.

Methods: Near-infrared spectral data were collected on five samples with triplicate spectra for each sampling of several herbs and spices. Spectra of common adulterants were also collected including corn starch, monosodium glutamate, brick dust, sawdust, olive leaves, prickly pear seeds and mixtures of spices with these adulterants. Near-infrared spectra were preprocessed and analyzed using principal component analysis to classify the spectra and determine the presence of the contaminants. Calibration matrices were constructed and cross-validated using spectra that were collected independently of those used to construct the calibrations. To evaluate the utility of developing PCA methods for other commodities, additional near-infrared spectral data were collected on ground coffee, chicory, coffee and chicory mixtures, bovine milk and mixtures of milk and water, urea and chalk.

Results: Principal component analysis of near-infrared spectral data were used to detect the presence of 10% or greater of the contaminants tested in turmeric, black pepper, mustard seed, chili powder, cinnamon, coffee, coriander seed, cayenne, oregano, paprika and saffron. Testing of other commodities showed principal component analysis methods were able to differentiate ground coffee from chicory and mixtures of milk and water, urea or chalk.

Significance: The principal component analysis methods that were developed enable rapid non-targeted detection of contaminants in food ingredients and products using inexpensive hand-held near-infrared spectrophotometers interfaced to smartphones.

T5-04 Deep Learning Methods for Classifying Shiga Toxin-producing *E. coli* with Hyperspectral Microscope Images

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Introduction: Foodborne diseases are a serious health issue worldwide. However, the total burden of unsafe food is unknown. Current methods including culture-based, immunological and molecular techniques for identification and detection of foodborne pathogens have limitations, so rapid, accurate and sensitive methods are needed for food safety.

Purpose: The purpose of this study was to evaluate machine-learning algorithms to identify foodborne pathogenic bacteria including Shiga toxin-producing *E. coli* (STEC) to improve classification accuracy.

Methods: Cultures of non-O157 STEC were obtained from the USDA-FSIS Eastern Laboratory in Athens, Georgia. The STEC serogroups for the experiments were O26:H2, O45:H2, O103:H2, O111:H1, O121:H19, and O145:H-. Each of the test strains was positive for the Shiga toxin (*stx1* and/or *stx2*) and intimin (*eae*) genes. A hyperspectral microscope imaging (HMI) system, which consists of an upright microscope, acousto-optic tunable filters (AOTF), an electron-multiplying charge coupled device 16-bit camera, and a dark-field illuminator, was used for image acquisition of STEC samples. Hyperspectral microscopic images were acquired at cellular level with contiguous spectral imagery from 450 to 800 nm using 250 ms integration time and 3.5% gain.

Results: Using spectral information extracted from regions of interests (ROI) of HMI, a deep neural network (DNN) algorithm, which consisted of stacked auto-encoder (SAE) and soft-max regression (SR), performed as a non-linear principal component analysis (PCA) to extract principal features of raw spectral data. The SR classified these features that were learned by SAE with greedy layer-wise training to acquire unsupervised deeper features. A back-propagation fine-tuning method improved classification accuracy with 96.9% compared to linear discriminant analysis (LDA) and support vector machine (SVM) methods whose classification accuracy of 90.6% and 91.3%, respectively.

Significance: Machine learning methods to differentiate foodborne bacteria enable us to improve classification accuracy for big data collected by the hyperspectral microscope imaging technique, which makes near real-time pathogen detection possible with appropriate sample preparation from food matrices.

T5-05 A Label-free QCM Biosensor for Sensitive and Rapid Detection of *E. coli* O157:H7 Based on a Multivalent Aptamer System

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Introduction: *Escherichia coli* O157:H7 is one of the most dangerous foodborne pathogens. The development of a rapid, sensitive and specific method for *E. coli* O157: H7 is needed to improve food safety and security.

Purpose: The objective of this study was to create a multivalent aptamer system based on rolling circle amplification (RCA), which was then integrated into a quartz crystal microbalance (QCM) sensor for rapid detection of *Escherichia coli* O157:H7 in food products.

Methods: First, the DNA template consisting of the complementary sequence of the aptamer and a spacer was circularized and immobilized on the gold surface of the QCM sensor. The same primer used in ligation could then initiate the RCA reaction and produce a very long single-stranded DNA, which contained repetitive aptamer sequences against *E. coli* O157:H7. Finally, the created multivalent aptamer could selectively recognize and capture target *E. coli* O157:H7, resulting in a decrease of the resonant frequency (Hz), which was recorded in real-time by QCM.

Results: The result showed that the created multivalent aptamer system enhanced the sensitivity of the QCM sensor by approximately sevenfold when compared to the monovalent aptamer for the detection of *E. coli* O157:H7. The detection limit and the detection time of the sensor was determined to be 3.4×10^{1} CFU/ml and 50 min, respectively, for pure culture (each test was repeated three times). Non-target bacteria (*Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus, E. coli* K12 and *Listeria innocua*) were tested to investigate the specificity of the developed QCM biosensor (each test was repeated three times), and the result indicated high selectivity for *E. coli* O157:H7.

Significance: This study provides a multivalent aptamer system to improve the sensitivity of a biosensor due to the higher binding efficiency through the repetitive aptamer sequences.

T5-06 Reporter Bacteriophage NRGp4 Utilizes a Novel Nanoluc: CBM Fusion for the Ultrasensitive Detection of *Escherichia coli* in Water

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Introduction: Reporter bacteriophages (phages) are robust biorecognition elements uniquely suited for the rapid and sensitive detection of bacterial species. Upon infection with reporter phages, target bacteria express reporter enzymes encoded within the phage genome. As agricultural water is a common source of contamination in the food industry, we have developed a rapid test to determine the presence of *E. coli* which is used as an indicator of fecal contamination.

Purpose: In this study, the T7 coliphage was engineered to express the newly developed luciferase-NanoLuc (NLuc) fusion enzyme. The reporter phage was then used to test 100 ml water samples for the presence of *E. coli*.

Methods: While several genetic approaches were employed to optimize reporter enzyme expression, the novel achievement of this work was the successful fusion of NanoLuc to a carbohydrate binding module (CBM) with specificity to cellulose. This novel chimeric reporter (*nluc*::*cbm*) bestows the specific and irreversible immobilization of NanoLuc onto a low-cost, widely available cellulosic substrate. Cellulose added to the water sample along with the phage allowed released reporter probe to be collected and concentrated following infection of the *E. coli*. Alternatively, to allow full quantification, the water sample was passed through cellulose filters, trapping the bacteria prior to phage addition. The released reporter enzyme could then bind in vicinity of the CFU effectively marking it's location when substrate was added.

Results: We have shown the possibility of detecting the immobilized fusion protein in a filter plate which resulted from a single CFU of *E. coli*. We then demonstrated that microcrystalline cellulose can be used to concentrate the fusion reporter from 100 ml water samples allowing a limit of detection of <10 CFU mL^{-1} *E. coli* in three hours. Full quantification (zero to 80 CFU/100 ml) in eight hours was achievable using a cellulose filter to trap the bacteria. The methods were compared EPA method 1603 and demonstrated an R^2 of 0.9859 (phage method vs. EPA method) from zero to 80 CFU/100 ml. Therefore, both tests were in agreement on the quantification of spiked samples.

Significance: We conclude that our phage-based detection assay displays significant aptitude as a proof-of-concept drinking water diagnostic assay for the low-cost, rapid and sensitive detection of *E. coli*. Additional improvements in the capture efficiency of the phage-based fusion reporter should allow a limit of detection of <10 CFU per 100 ml.

T5-07 Validation of the Liquid Crystal-based Immunoassay for Rapid Detection of *Salmonella*

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Introduction: *Salmonella* is one of the major foodborne pathogens of great public health concern. A rapid and sensitive detection for this pathogen is critical to ensure food safety. Liquid crystal-based immunoassay uses unique optical characteristics of liquid crystals to perform a rapid and sensitive detection method of pathogens.

Purpose: The aim of this study is to evaluate a novel liquid crystal-based assay for the detection of *Salmonella*.

Methods: The liquid crystal-based immunoassay was developed using immunomagnetic beads (IMB) and liquid crystal. When *Salmonella* is present, the formation of *Salmonella*-IMB aggregates distorts liquid crystal matrix, which creates rapid light transmission, resulting in a detectable signal. The assay was tested for its sensitivity and specificity with various concentrations of *Salmonella* and common pathogens. ELISA was used as a reference method for comparison. All samples were run in triplicate and experiments were repeated at least three times. Results were analyzed by a one-way ANOVA.

Results: The developed immunoassay was able to detect *Salmonella* with detection limits of 10^4 CFU/ml without any enrichment. The total assay was completed within 30 min. With a 12-h enrichment step, *Salmonella* as low as one CFU/ml was detected. The developed assay was highly specific to all tested *Salmonella* serotypes and did not show any cross-reactivity with other common foodborne pathogens. Its sensitivity and specificity were comparable to ELISA, but the positive result was confirmed in a shorter time than ELISA (30 min versus 90 min), and with fewer steps.

Significance: The novel liquid-crystal immunoassay has great potential as a rapid and sensitive detection method for *Salmonella.*

T5-08 ISO 16140-2 Validation of the GeneDisc STEC Method for Analysis of Raw Beef Meat

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Introduction: For a release of raw ground beef products, manufacturers have to guarantee the absence of Shiga toxin-producing *E. coli* (STEC) in 25 g of sample. To limit the cost linked to storage, the time to result (TTR) of the method for STEC detection should be as short as possible. Pall GeneDisc Technologies proposes a flexible protocol with three different enrichment times: five, eight, and 20 h.

Purpose: As part of the ISO 16140-2 (2016) validation of GeneDisc STEC Top5 method, an independent study compared the GeneDisc STEC methods, including a short protocol, to the ISO/TS 13136:2012 reference method.

Methods: The study was based on the requirements of the ISO 16140-2 (2016) standard, including a sensitivity study with 69 samples, an evaluation of the relative limit of detection, a specificity study and an inter-laboratory study.

Results: The results showed that the GeneDisc methods fulfilled all requirements of the ISO 16140-2 technical rules. Regarding the sensitivity study, there was no significant difference observed after five h of enrichment; compared with longer incubation (eight and 20 h).

Significance: This study demonstrated that the GeneDisc STEC method fulfills the EN ISO 16140-2:2016 requirements for raw beef meat samples. Flexibility in enrichment time enables manufacturers to manage the release of their product according to their shelf-life, e.g., to significantly decrease the storage time of fresh products before release.

T5-09 Rapid Detection of Enrofloxacin in Poultry Using a Localized Surface Plasmon Resonance Sensor Based on Polydopamine Surface Imprinted Recognition Polymer

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Introduction: Antibiotic overuse in poultry can lead to the development of resistant bacterial strains, and fluoroquinolones are considered as one of the critically important antimicrobial groups for human health. Current HPLC and ELISA methods have their limitations for in-field detection of antibiotic residues in poultry products. Localized surface plasmon resonance (LSPR) sensors have shown great potential in biodetection, due to its reproducible, label-free and real-time features.

Purpose: The objective of this project is to develop a portable LSPR biosensing system for rapid, specific and sensitive detection of enrofloxacin residue in poultry products, using polydopamine surface imprinted polymer (PDA-SIP) as biological recognition element.

Methods: Enrofloxacin was used as a model target of fluoroquinolones. The PDA-SIP was fabricated by polymerization of dopamine and ENRO in 50 mM Tris buffer (pH=8) on an LSPR sensor chip. After blocking with bovine serum albumin (BSA) and ethanolamine, the enrofloxacin template was removed with acetic acid. The developed sensor chip was used to selectively recognize and capture enrofloxacin in samples. To amply LSPR detection signals of small enrofloxacin molecules, BSA-enrofloxacin conjugates were synthesized, served as competitors, and reacted with the residual binding sites on PDA-SIP.

Results: The proposed method allowed a detection range of enrofloxacin from 10 to 10^5 ng/ml, with a limit of detection of 9.8 ng/ml, below the European Union and China regulations for this antibiotic residue. The results also showed that the PDA-SIP based LSPR sensor could shorten the detection time down to five min, with a flow rate of 20 µl/min.

Significance: The adoption of PDA-SIP not only extended material lifetime but also reduced detection time. The developed LSPR sensor showed high potential for rapid and in-field detection of enrofloxacin in poultry products.

T5-10 AquaSpark, a Novel Chemiluminescent Technology Platform for Dynamic Monitoring of Environmental Bacteria

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Introduction: Fast cleaning validation in food plants is essential for taking timely action to avoid the spread of potentially harmful bacteria. Controls today rely on laboratory endpoint testing which may delay important results by more than a day. Alternatively, fast unspecific testing with limited value, assessing bacterial presence is available. New methods are needed to overcome the hurdle of on-site testing for more effective monitoring programs.

Purpose: We evaluated the novel AquaSpark technology for dynamic monitoring of critical environmental bacteria

Methods: Detection molecules tested were composed of a light emitting part (modified dioxetanes) combined with a tailored enzyme labile groups (ELG). For example, a β-galactoside moiety was used as ELG for coliform detection. Target bacteria were inoculated at logarithmically increasing inoculation levels (from 10 to 10^6 CFU/ml), together with a 10 µM concentration of the analytical molecule. The released luminescence light signal was monitored over the time course of 24 h. Assays were performed in triplicate in 96-well plates.

Results: Depending on the level of initial contamination an elevated luminescence signal could be detected as early as 30 minutes after the start of the experiment. The concept of dynamic monitoring with a variation of the ELG was also shown on specific foodborne pathogens such as *Listeria* and *Salmonella* as well as general hygiene indicators such as *E. coli*/coliforms. Further, we show that the method is suitable for detection of antibiotic-resistant bacteria.

Significance: A fast cleaning validation in food plants is of utmost importance for food safety. Equally, the spread of antibiotic-resistant bacteria is a major challenge in clinical diagnostics. These challenges require fast phenotypic assays to assess the number and kind of active bacteria without unnecessary delay. The enzyme dependent chemiluminescence assay presented here has the potential to mitigate the risks of bacterial dissemination on site.

T5-11 Modifying the Double Layer Plaque Assay for Accurate Phage Titer Determinations: Effect of Solidifying Agent Type and Concentration

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Introduction: If applied at sufficient titer, phages could effectively control pathogens during food or therapeutic applications. Hence, improving the method's accuracy in determining titer improves phage applicability.

Purpose: Investigating how solidifying agent type (agar and agarose) and concentration in the double layer plaque assay affect phage enumerations.

Methods: To test the influence of solidifying agent type and concentration in the top layer of the assay medium on phage titer and plaque diameters, the assay was modified as follows. The top layer was prepared to contain agar or agarose (in LB broth) at 0.2, 0.3, 0.5 or 0.75% final concentrations whereas the bottom layer contained 1.5% agar. For quantification of phages, 100 µl of *Escherichia* phage OSYSP suspension and 200 µl of *Escherichia coli* O157:H7 culture were mixed with the top layer medium and overlaid on a previously prepared bottom layer. After incubation, images of petri plates with phage plaques were taken and analyzed using image analysis software to determine the number of plaques and their diameters.

Results: Decreasing the concentration of a solidifying agent in the top layer from 0.75% to 0.2% significantly (P<0.05) increased the phage titers and the average diameter of the phage plaques. Average phage titer was 0.7 log PFU/ml greater at 0.2% than it was at 0.75% agar. Moreover, average phage plaque diameter increased considerably (P<0.05) from 0.08 cm to 0.74 cm with decreasing agar concentration from 0.75% to 0.2%. When agar was replaced with agarose, similar changes in phage plaque count were observed but plaque diameters were significantly (P<0.05) smaller. The 0.3% agar was determined as the most feasible concentration and solidifying agent type due to quick hardening on the surface of the bottom layer and efficiency in phage recoveries.

Significance: The proposed modification of the assay should minimize phage titer underestimation particularly in dose-dependent phage applications.

T5-12 Deciphering the Antibiotic Resistance Mechanism of *Campylobacter* Using Confocal Micro-Raman Spectroscopy

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne pathogen *Campylobacter jejuni* shows increasing antibiotic resistance (AMR). Identifying the mechanisms of AMR in bacteria is necessary for agricultural management and drug development. Conventional techniques (*e.g.,* mass spectroscopy and DNA microarray) are usually time-consuming and costly.

Purpose: This study aimed to develop a rapid and sensitive approach to characterize the AMR of *Campylobacter* and reveal its mechanism.

Methods: Confocal-micro Raman spectroscopy was applied to monitor the phenotypic responses of *Campylobacter* to antibiotic treatments. Ampicillin and tetracycline were selected because they are the drugs of choice for *Campylobacter* infections. *C. jejuni* cultures were subjected to different concentrations of antibiotics for 5 h. Bacterial cells were harvested and deposited on a gold microarray slide for Raman spectral collection. The chemometric analysis was used to investigate the minimal inhibitory concentrations (MICs) of *C. jejuni* isolates, discriminate sensitive and resistant strains, and identify resistance mechanisms.

Results: MICs of selective *C. jejuni* isolates were determined by analyzing bacterial Raman spectra at different antibiotic concentrations using principal component analysis (PCA). The results were consistent to the reference agar dilution method. PCA and second derivative transformation analysis identified the remarkable spectral differences between sensitive and resistant strains at the standard sensitive breakpoint of a certain antibiotic. For ampicillin treatment, spectral variations between sensitive and resistant *C. jejuni* strains were associated with phospholipids and proteins-related Raman peaks (*e.g.,* 719, 1220, 1580 cm^{-1}), which are the major components of the bacterial cell wall. Higher intensities of proteins-related peaks (*e.g.*, 857, 1030, 1660 cm^{-1}) were observed in tetracycline-resistant strains than that of the sensitive strains, indicating the reduced inhibitory effect of tetracycline on protein synthesis.

Significance: Raman spectroscopy can be used to rapidly predict AMR profiles and mechanisms of *Campylobacter*.

T6-01 Photodynamic Inactivation of Human Norovirus Surrogates in Water

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❖ ***Developing Scientist Entrant***

Introduction: Shellfish and fresh-produce are major sources for human norovirus (HuNoV) infections in the US. These foods can be contaminated following the use of virus-contaminated waters used in shellfish farms or in pre/post-harvest processes of fresh produce. Decontamination of waters used in depuration process of oysters and washing of fresh produce is one of the suggested methods to mitigate this risk. Photodynamic treatment (PDT) using photosensitizers (PS) is extensively used for bacterial inactivation but studies on the use of PDT for viral inactivation in food and water are scarce.

Purpose: To study the inactivation of HuNoV surrogates, in water, by exposure to light in the presence of photosensitizer dyes.

Methods: In triplicate experiments for each treatment, feline calicivirus (FCV) and Tulane virus (TuV) were mixed separately in water containing various concentrations of rose Bengal (RB), Phloxine-B (Ph-B), and chlorophyllin (Ch) as photosensitizers. Then, they were exposed to LED blue light source for different times.. Light exposure time (LET) and photosensitizer concentration required for a 4-log virus reduction were measured by titration of surviving virus after PDT.

Results: *Exposure to blue LED in the presence of RB and Ph-B resulted in inactivation of FCV and TuV. Chlorophyllin was not effective. A 4-log reduction in the titers of of FCV and TuV was seen at 3min-LET/25µM-RB and 10min-LET/50µM-RB, respectively. Similar effect was achieved using 5min-LET/10 µM Ph-B and 5min-LET/50 µM Ph-B, respectively. These results indicate that Ph-B is the most effective dye and that TuV is more resistant to PDT than FCV.*

Significance: *Our results revealed for the first time that PDT using Ph-B, an edible food colorant, is a promising non-thermal treatment for HuNoVs inactivation in water. This cost-effective PDT holds the promise for integration with water used for depuration of oysters and for washing of fresh produce to mitigate the risk of HuNoVs.*

T6-02 Evaluation of Viral Food Safety Risks of Reusing Tailwater for Leafy Green Production

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Introduction: Tailwater is the excess water that runs off a field following furrow or flood irrigation. Due to multi-year drought conditions in California, there is interest in reuse of tail water for preharvest applications such as pre-irrigation and germination. One food safety concern is norovirus, which is known to be highly stable in water long term in varying storage conditions.

Purpose: The purpose of this study was to evaluate the survival of norovirus in tail water In Vitro and determine the risk of tailwater reuse during leafy green cultivation by mimicking the conditions in the Salinas Valley (SV) of California.

Methods: Tailwater and irrigation well water was collected from a representative central coast vegetable production site in the SV. Two surrogate viruses for norovirus, Tulane virus and murine norovirus, were used in this study. Tailwater, well water or MilliQ water were inoculated with Tulane virus and murine norovirus to achieve a titer of 1×10^4 PFU/ml. Samples were stored at 19°C, which is the average temperature measured in the SV for four weeks. Standard plaque assays were carried out to determine the virus titer at zero, one, two, three, seven, 14, 21, 28 days postinoculation (DPI).

Results: The titer of Tulane virus and murine norovirus at 28 DPI was 1.6×10^3 and 5.9×10^3 PFU/ml for tailwater stored at 19°C. There was no significant difference between Tulane virus and murine norovirus titers detected in well water or MilliQ water stored at the same temperatures.

Significance: These results indicate that norovirus is highly stable in tailwater and the high nutrient, basic pH, salinity and turbidity of the tailwater did not significantly impact viral survival compared to other water sources. Therefore, if contaminated with norovirus, this tailwater poses a food safety risk when used for irrigation purposes.

T6-03 The Potential of Pulsed UV Light to Inactivate *Cryptosporidium parvum* Oocysts on High-risk Commodities (Mesclun Lettuce, Spinach, Cilantro, and Tomatoes)

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illness attributed to protozoa continues to increase; the CDC estimates there are 232,549 cases annually and a substantial number of outbreaks have been associated with produce. Improvements in awareness and surveillance of protozoa have emphasized the need for novel interventions to prevent and manage contamination.

Purpose: This study examined the effects of pulsed UV light (PL) on the viability of *Cryptosporidium parvum* oocysts on mesclun lettuce, spinach, cilantro, and tomatoes.

Methods: Oocysts (1.56×10^6/per 0.125 ml of phosphate buffered saline) were applied to glass slides (control, n=24), leafy greens/herbs (0.5 g), or tomatoes (20.0 g) in duplicate (n=6). Samples were air-dried for 60 min and treated with PL for zero, 10, 45, and 90 s. Oocysts were recovered using a modified FDA BAM methodology, resuspended in one ml PBS, and 0.8 ml was added to confluent HCT-8 cells in a six-well plate. After infection (60 min), the inoculum was removed and replaced with fresh media. Following incubation at 37°C for 48 h, DNA was extracted, and *Cryptosporidium* detected by qPCR. Data were analyzed using Tukey's HSD test. Microscopy was used in conjunction with integrated cell culture-qPCR detection.

Results: For controls on the glass, 90 s treatment yielded an average 3.46-log reduction in infectivity of oocysts. Significant differences existed between the infectivity of treated oocysts on the glass for all treatment times compared to the time zero control (P<0.0001). Reduction in infectivity was observed in mesclun (3.45 log), spinach (2.91 log) and tomato (2.73 log) after zero and 90 s of treatment. A 1.12-log reduction in infectivity was detected between oocysts treated on cilantro after zero and 90 s of treatment compared to a 2.03-log reduction between zero and 45s. Overall there was no significant difference in the reduction of infectivity across commodities (P>0.05).

Significance: PL treatment decreases *C. parvum* oocyst viability without major changes in physical appearance and has the potential for commercial application. Studies with *Cryptosporidium* serve as models for other protozoa, including *Cyclospora*.

T6-04 Validation of Industrial Equipment Designed to Apply Peracetic Acid-based Sanitizing Solution on Chia Seeds Using a *Salmonella* Surrogate, *Enterococcus faecium* NRRL B-2354

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Introduction: Previous studies conducted on chia seeds treated with a commercial peracetic acid-based sanitizing solution found: i) *Enterococcus faecium* NRRL B-2354 was a suitable surrogate for *Salmonella* and ii) greater than three-log CFU/g reductions for both *Salmonella* and *E. faecium* were achieved while maintaining seed viability (germination, rancidity and nutrition). Identification of a surrogate and promising efficacy results warranted further optimization testing with the final objective of validating industrial-scale equipment.

Purpose: The objective was to validate an industrial-scale applicator, used to treat chia seeds with the sanitizing solution, and an industrial-scale dryer, used to dry seeds post-treatment.

Methods: Samples (one kg×120) of chia seed were inoculated with 30 ml *E. faecium* (3.6 liters on 120 kg), sifted to remove clumps formed and transferred to a sanitized bin to acclimatize overnight (ambient temperature). The inoculated seeds were loaded into an industrial-scale applicator, treated at a rate of 36 ml/kg, collected into a separate sanitized bin and held for one hour (ambient temperature). An industrial-scale dryer was filled with treated, uninoculated chia seeds to approximately 25 cm from the top and a rubber gasket was installed at this level. Following the post-treatment holding time, the inoculated and treated chia was loaded into the dryer above the gasket and the entire batch was dried at 71.1°C to reach original moisture content (16 min). Samples (20×50 g) were taken for enumeration prior to treatment and after drying, from various locations in the bin or the dryer, respectively.

Results: An average of 0.77±0.27 log CFU/g was recovered from treated and dried seeds compared to an average of 6.41±0.07 log CFU/g from untreated controls, producing an average of 5.63 log CFU/g reduction.

Significance: The industrial-scale system was capable of an average of greater than five-log CFU/g reduction of *Salmonella* surrogate on chia seeds at the treatment parameters tested.

T6-05 Characterization of Bacteriophage T4-Insensitive *Escherichia coli* via Comparative Correlation of Genomic and Phenotypic Microarray Data

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❖ ***Developing Scientist Entrant***

Introduction: Bacteriophages (phages) continue to emerge as antimicrobial agents for control of foodborne pathogens. Bacteriophage insensitive mutants (BIMs) likely differ in ability to metabolize compounds used for growth.

Purpose: A correlative approach combining genomic and phenotypic microarray (PM) data was employed to identify metabolic differences between PM experiments and to correlate them with genetic mutations in an *E. coli* BIM.

Methods: The whole genomes of *E. coli* B and BIM ZZa3, produced by incubating *E. coli* B with phage T4, were sequenced on the Illumina MiSeq platform with 300-bp paired-end libraries and 30X coverage, followed by raw read assembly using the A5 pipeline, and gene annotation with RAST. The metabolic phenotypes of both were determined using PM plates 1-10, on the OmniLog BIOLOG system. Genomic and phenotypic data were characterized using the DuctApe software suite.

Results: Relative to the wildtype, BIM ZZa3 showed decreased respiratory activity in several situations including low or high pH conditions, and also decreased metabolism of several amino acids at pH 4.5 or 9.5. T4 induced mutations also increased metabolism of the C4-dicarboxylic acids m-tartaric acid and bromo-succinic acid and increased sensitivity to osmolytes including four or five percent sodium sulfate and 200 mM sodium phosphate. Genomic analysis indicated that enhanced metabolism of C4-dicarboxylic acids was due to mutations in the *dcuS* gene, which encodes a sensor kinase that regulates the metabolism of exogenous C4-dicarboxylates during cell growth. Amino acid substitutions in GadB, a glutamate decarboxylase that confers acid resistance to *E. coli*, were also observed, which is related to pH based decreased metabolism of amino acids. Mutations in the *waa* operon likely led to increased sensitivity to sodium compounds.

Significance: These results indicate that phage-induced bacterial mutations cause fitness changes that may affect the ability of bacteria to survive and grow in foods.

T6-06 *Ybgc* Regulates Cell Membrane Integrity and Fatty Acid Composition of *Salmonella* Enteritidis in Response to Lysozyme

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Introduction: *Salmonella* Enteritidis has been a major causative agent of foodborne disease (salmonellosis) due to consumption of contaminated eggs and egg products. Our previous study demonstrated that the *ybgC* gene (encoding for acyl-CoA thioesterase) was crucial for *Salmonella* Enteritidis survival in egg white.

Purpose: The aim of this study was to determine whether *ybgC* regulates the resistance of *Salmonella* Enteritidis to lysozyme (a major antibacterial component of egg white) by survival ability test and cell membrane characterization.

Methods: The survival of *Salmonella* Enteritidis wild type, Δ*ybgC* mutant and Δ*ybgC*-C complementary strain was tested by the plate count method after incubation for 24 h in egg white filtrate supplemented with lysozyme, ovotransferrin, ovalbumin, avidin, and ovomucoid, and in egg white filtrate (as a control). Cell outer membrane permeability, cellular morphology and fatty acid composition of the three strains were further determined after exposure to lysozyme for 6 h by *N*-phenyl-1-naphthylamine assay, scanning electron microscopy and gas chromatography-mass spectrometer, respectively. The experiment was repeated twice with three replicates per treatment.

Results: A six-log reduction was observed in Δ*ybgC* mutant after incubation for 24 h in filtrate-lysozyme, whereas no significant ($P>0.05$) difference was found among the three strains in filtrate and filtrate-ovotransferrin, ovalbumin, avidin, and ovomucoid, respectively. Additionally, a significant increase of outer membrane permeability and apparent alterations in cellular morphology were found in Δ*ybgC* mutant after exposure to lysozyme. Moreover, the relative ratio of total unsaturated fatty acids to total saturated fatty acids in Δ*ybgC* mutant was significantly ($P<0.05$) higher than that in wild type and Δ*ybgC*-C complementary strain.

Significance: These results provide some insights into the underlying survival mechanisms of *Salmonella* Enteritidis in egg white. Moreover, the *ybgC* gene has potential as a novel molecular target for controlling *Salmonella* Enteritidis in eggs.

T6-07 Prevalent Terpenes and Their Inhibitory Effects on *Escherichia coli* O157:H7 in Fresh Cheese Made with Oregano and Rosemary Essential Oils during Storage

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Introduction: Outbreaks involving fresh cheese contaminated with *Escherichia coli* O157:H7 have been reported in several countries. The combined application of essential oils from *Origanum vulgare* L. (oregano; OVEO) and *Rosmarinus officinalis* L. (rosemary; ROEO) in synergism is cited as a strategy to control *E. coli* O157:H7 growth in cheese. However, there is a lack of information regarding the active compounds of these essential oils (EOs) during refrigerated storage.

Purpose: This study evaluates the effects of OVEO and ROEO in combination on viable counts of *E. coli* O157:H7 in fresh cheese and determines their prevalent terpenes during refrigerated storage.

Methods: Fresh cheese manufactured with OVEO (0.03 µl/g) and ROEO (1.32 µl/g) in synergistic concentrations was inoculated with *E. coli* O157:H7 (10^5 CFU/g), homogenized using a stomacher, packaged and stored at 7±0.5°C under refrigeration. Viable cells were counted at zero, three, six, nine, 12, 15, 18, and 21 days of storage by serial dilution and plating on MacConkey-sorbitol agar. At the same intervals, the prevalent terpenes of OVEO and ROEO were monitored in fresh cheese by GC-MS. Pearson's linear correlations coefficients (r) between the amounts of terpenes and *E. coli* O157:H7 counts were calculated.

Results: Counts of *E. coli* O15:H7 decreased by 1.5 and three log CFU/g in cheese with EOs after six and 15 d of storage, respectively. No further changes were observed after this period. In cheese without EOs *E. coli* O157:H7 counts increased ~2.8 log CFU/g over the 21 d. The amounts of α-pinene, β-pinene, eucalyptol, camphor, camphene, ρ-cymene, borneol caryophyllene, myrcene and linalool, detected only in cheese with EOs, showed a positive correlation ($r>0.8$) with decreases in counts of *E. coli* O157:H7 during storage.

Significance: Results show the efficacy of OVEO and ROEO terpenes for controlling *E. coli* O157: H7 growth in fresh cheese during refrigerated storage.

T6-08 Directed Evolution of *Bacillus cereus* Endospores with UV-C Stress Resulted in an Increased UV-C Resistance of Spores But Limited Effect on Their Vegetative Cells

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Introduction: Bacterial endospores are exposed to a variety of sublethal and lethal stresses in the food production chain. Generally, these stresses will not completely eliminate the existing spore populations, and thus constitute a selection pressure on the spores. However, the impact of this selection pressure on the evolution and properties of the surviving cells have not yet been addressed. Previously, UV-C directed evolution yielded *Bacillus cereus* endospores with increased resistance against UV-C.

Purpose: To evaluate the impact of acquired endospore-level UV-C resistance on growth at 30°C, UV-C resistance and toxin production of *B. cereus* in the vegetative state.

Methods: Three *B. cereus* (ATCC 14579) UV-C resistant spore populations that are >30-fold more resistant at 164 J/cm² were isolated. Subsequently, their vegetative cells were tested for growth at 30°C, UV-C resistance, and toxin production (Duopath kit, Merck-millipore). UV-C inactivation was tested on cells in an exponential growth phase with a maximum dosage of 0.016 J/cm². Growth curves were fitted using DMFit (Combase, Baranyi model). Sample size was three. Statistical analysis (Student's *t*-test) was performed with SPSS (IBM).

Results: Average growth speed ($hour^{-1}$, ±SD, $n=3$) of the three UV-C resistant isolates is 0.895±0.025, 0.774±0.024 and 0.840±0.027, while for controls it was 0.841±0.035. The lag-phase of UV-C cycled and non-stress cycled controls is comparable to wild-type, and UV-C inactivation of the vegetative cells is not statistically different ($n=3$, $P>0.05$). There are no quanitative differences in enterotoxin production between wild-type and UV-C resistant isolates.

Significance: While previous work demonstrated that endospore-level UV-C resistance in *B. cereus* can be easily acquired, and can negatively affect other endospore properties such as germination and heat resistance, current work suggests that it does not affect relevant characteristics at a vegetative cell level.

T6-09 Investigation of a Lytic *Bacillus cereus* Phage with High Specificity and High Stability Under Various Stressed Conditions

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❖ ***Developing Scientist Entrant***

Introduction: *B. cereus* is ubiquitous in nature due to its capacity to form a heat-resistant endospore and grow at a broad range of pH and temperature. Therefore, an onsite and rapid method is required to detect *B. cereus* specifically at any stage of food production as a bio-recognition element in a biosensor method.

Purpose: The purpose of this study is to isolate, purify and characterize a *B. cereus*-specific phage as a novel, efficient bio-recognition element and bio-control agent.

Methods: Samples were collected from wastewater and pig liver from slaughterhouses and raw milk and soil from a farm. Isolation of *B. cereus*-specific phage was performed by incubating the sample with *B. cereus*. The isolated *B. cereus*-specific phage was then propagated and purified using CsCl gradient ultracentrifugation and dialysis. The specificity study of purified phage infecting *B. cereus* (KFS-BC1) was conducted using a dot assay against 88 foodborne pathogens. The stability of KFS-BC1 was investigated using a plaque assay after exposing it (10^8 PFU/ml) to various pHs (one through 12) and temperatures (-20, 4, 22, 37, 50, 60 and 70°C) for one hour.

Results: Twenty-nine phages infecting *B. cereus* were isolated from samples. Among them, six phages infecting *B. cereus* isolated from soil were used for the further isolation of a single phage. Finally, KFS-BC1 was then propagated due to great lytic activity and the final concentration of KFS-BC1 was determined to be 5×10^{11} PFU/mL. KFS-BC1 exhibited excellent specificity against 15 strains of *B. cereus* only among 88 foodborne pathogens. In addition, KFS-BC1 demonstrated excellent stability in the pH range of three to 11 and temperature range of -20-60°C.

Technical

Significance: Lytic KFS-BC1 was successfully isolated from soil with a high concentration, specificity, and stability as a potential bio-recognition element.

T6-10 *Salmonella* Serotype Fitness in Various Water Types and Habitat Transition from Water to Tomato Fruit

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* is endemic to Mid-Atlantic surface waters. However, questions on serotype dynamics remain. It is unclear how serotypes compete in this niche and whether certain strains have a higher proclivity to transfer from water to crops.

Purpose: To investigate the ability of *Salmonella* to persist in water and transfer to tomato fruit.

Methods: Filter-sterilized non-tidal river, tidal river, pond and reclaimed water samples were singly inoculated with environmental isolates of *Salmonella* Heidelberg, Javiana, Typhimurium, or Newport and assayed at days one, 30 and 60 for persistence. Sample aliquots were directly plated on tryptic soy agar for culture-dependent enumeration. Aliquots were also treated with 25 µM propidium monoazide (PMA) followed by total DNA extraction and quantitative PCR of the RNA polymerase $\sigma^{D/70}$ factor gene *rpoD* for culture-independent measurements. To assess the transfer capacity of *Salmonella*, various strains were incubated in different water types for 30 days before inoculation onto tomato fruit cv. 'Heinz-1706' and subsequent retrieval 14 hours later.

Results: *Salmonella* counts of all serotypes declined in all water types over 60 days. Reclaimed water exhibited the slowest while non-tidal fresh water the most decline ($P<0.05$). After 60 days non-tidal freshwater samples displayed significantly different rates of decline between qPCR and direct plating methods ($P<0.05$). The number of days in water impacted *Salmonella* transfer to tomatoes. Pond water supported less transfer compared to other water types ($P<0.05$) across all serotypes tested. Incubation time in water significantly influenced transfer in one non-tidal fresh water ($P<0.05$). *Salmonella* Heidelberg exhibited the poorest transfer from water to crops regardless of water type or incubation time in water ($P<0.05$).

Significance: *Salmonella* persistence in surface and reclaimed water varied by serotype. Serotypes differences exist in capacity for habitat transition from water to tomato. These data are important to assess the safety and adequacy of irrigation water sources.

T6-11 Evaluation of a Typing Scheme Based on Deep Amplicon Sequencing to Aid Epidemiological Linkage of Cyclosporiasis Cases.

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Introduction: *Cyclospora cayetanensis* causes cyclosporiasis, a foodborne illness responsible for seasonal outbreaks in the United States. Past outbreaks have been linked to fresh produce imported from endemic countries. Epidemiologic investigations often fail to identify contaminated food items, making it difficult to prevent outbreaks. A robust genotyping tool to aid in linking cases has yet to be developed.

Purpose: We evaluated a novel typing strategy based on eight genetic markers and two similarity-based algorithms that assess the relatedness of infections based on their haplotype composition. The algorithms generate pairwise distances for each specimen pair that reflect the likelihood that the pair is related, helping discriminate between cases from the same or different sources.

Methods: PCR and deep sequencing were used to identify the haplotypes in specimens containing *C. cayetanensis* for the eight markers. This data was used as input for the algorithms. We tested 1056 specimens collected during 2018, including 342 epidemiologically assigned to one of five groups; specimens linked to one of two major outbreaks (120 and 122 specimens), specimens linked to two minor clusters (two and three specimens), and those linked to international travel (39 specimens). A dendrogram was generated from the pairwise distances, allowing visualization of tightly grouped specimens, indicative of possible linkage to a common source. Genetic links were compared to epidemiological information to assess the concordance between the two approaches.

Results: Of 1056 specimens, 740 were successfully genotyped. Five major genetic clusters were apparent. Nearly all, 233 (94%) of 247, samples from the epidemiologic clusters were grouped into comparable clusters based on their genotype. Samples from international travelers were of various genotypes, distributed amongst the five major genetic clusters.

Significance: We observed high concordance between the epidemiological data and the clusters assigned using our typing strategy which shows great promise as a tool for aiding investigations of cyclosporiasis outbreaks.

T6-12 Safety Status of Some Traditionally Fermented Foods in Nigeria

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Introduction: Traditional fermented foods from cereals and legumes contribute significantly to the energy and protein demand of many households in Nigeria but their production is still a household art which in most cases compromise their safety.

Purpose: To determine the occurrence of bacteria, fungi and toxins in these selected fermented foods in order to access their safety.

Methods: Within the last two years, fermented foods within various locations in South-west Nigeria were sampled and analyzed to establish their safety such as *ogi (65), ogi baba (65), ugba (50), iru (50), and ogiri (50).* During this period, a descriptive cross-sectional study was carried out amongst 86 fermented food sellers using open and close-ended questionnaires to establish their perceived attitude, practice, and knowledge of bacterial and fungal colonization of foodstuffs. The samples were analyzed for *Escherichia coli*, *Salmonella spp*., *Shigella spp*., *Pseudomonas spp*., fungi and their toxins.

Results: There was a wide knowledge gap amongst those under study, as 98% could not link fungi to mycotoxin contamination and associated health risks. A total of 442 bacterial isolates were obtained from the samples: *ogi* =45 isolates, *ogi baba* =11 isolates, *ogiri* =100 isolates, *iru* = 191, *ugba* = 95 isolates. *E. coli* was detected only in *iru* (15%) and *ogiri* (33%). Additionally, 67% of all *ugba* samples were positive for *B. subtilis* but only 3 bacteria genera (*Sphingomonas, Pantoea* and *Bacillus)* were found in *ogi baba.* Thirteen (13) genera were detected in those samples most of which belonged to the *Enterobacteriaceae* family. Among the selected samples (275), 82% of them had mycotoxins occurring singly or in combination.

Significance: Although low levels of pathogenic bacteria and fungi with their toxins were noted in these fermented foods which are undesirable, there is a need for calls for the development and enactment of adaptable food safety measures.

T7-01 Validation of Abiotic Bacterial Surrogates for Surface Sanitation in Food Processing Facilities

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Introduction: Effective sanitation of equipment is a critical step in an integrated food safety system. Existing sanitation verification methods have significant shortcomings that decrease their effectiveness and create an opportunity for novel approaches. We are presenting a novel efficient, effective, and low-cost sanitation verification method.

Purpose: This study validated abiotic bacterial surrogates (SaniTracers) for the verification of processing plant sanitation in real-world applications.

Methods: Abiotic bacterial surrogates were manufactured by encapsulating short, naturally occurring DNA sequences (derived from marine algae) within food-grade material particles. The particles were designed to match the response of bacteria to chlorine-based sanitizers. Experiments were conducted in a protein-processing pilot plant, where multiple locations were inoculated with the surrogates and non-pathogenic *E. coli*; and an active tree fruit packing plant, which was only inoculated with the surrogates. The response of the surrogates to the sanitation process was compared to that of *E. coli* in the protein plant and environmental bacteria in the fruit packing plant. Bacteria were measured using traditional microbiological methods, and the surrogates were quantified by quantitative PCR.

Results: In the protein processing pilot plant, the surrogates showed an average log reduction of 4.3 and SD=1.3. The *E. coli* showed an average log reduction of 4.32 and SD=1.48, however, the *E. coli* post-sanitation recovery was low. In the fruit packing plant, the APC log reduction was significantly less when the surrogates indicated that the cleaning was ineffective. The surrogates were detected and quantified in approximately 20 minutes, while the bacterial tests took more than 24 hours.

Significance: The behavior of these abiotic bacterial surrogates under sanitation in real-world applications can predict the lethality of the sanitation process on contaminating bacteria. The surrogates are a sanitation verification solution that is specific, rapid and overcomes sampling errors.

T7-02 Synergistic Effects of Ultrasound and Natural Antimicrobials Against *Listeria innocua* and *Escherichia coli* K12

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Introduction: Fresh produce washed with chlorine-based sanitizers is often not effectively disinfected, causing serious foodborne disease outbreaks. Chlorine is not stable in wash water and it can form carcinogenic by-products by reacting with organic matter.

Purpose: The objective of this study is to investigate the potential of using ultrasound and food grade natural antimicrobials to improve fresh produce sanitation processes.

Methods: Inactivation capacities of a 20 kHz ultrasound probe device (US20) or a one MHz ultrasound therapy device (US1) combined with nine different natural compounds were tested against *Listeria innocua* and *E. coli* K12. Test organisms were in the stationary phase at 10^6 to 10^7 CFU/ml). The natural compounds were in water containing two to five percent ethanol. Experiments were run at a power density of 200 W/liter and 22±2°C. After each treatment, bacteria were enumerated on tryptic soy agar and compared to control samples treated by ultrasound alone or antimicrobials alone. Intracellular oxidative stress, intracellular pH, and morphology of treated bacteria were also measured by chemical assays and transmission electron microscopy. All data were an average value obtained from triplicate experiments.

Results: Results showed that both ultrasound treatments alone did not reduce *Listeria* ($P>0.05$). However, the 20 kHz probe combined with carvacrol (two mM), citral (10 mM) or ceraniol (five mM) resulted in an additional 2.5 to 3.0-log reduction of *L. innocua* in 15 min compared to the reduction from the 20 kHz probe with any of these antimicrobials alone. Similarly, the one kHz probe with carvacrol (two mM) induced an additional one-log reduction. No synergistic effects were observed after treating *E. coli* K12. Additionally, the enhanced antimicrobial effect was evidenced by increased intracellular oxidative stress. For example, for cavacrol, relative fluorescent value increased from 0.85±0.08 to 2.02±0.47 ($P<0.05$), intracellular pH decreased from 7.1 to 4.8 ($P<0.05$), and there was damage to the cell wall and membrane structures after treatments.

Significance: Findings showed that ultrasound in combination with natural antimicrobial compounds is a potential safe approach to reduce microbial contamination during fresh produce washing.

T7-03 Inactivation of *Listeria* and *E. coli* Using UV-C LED: Effect of Substrate on Inactivation Kinetics

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Introduction: Environmentally persistent pathogens such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 are the leading cause of foodborne illness, with dire economic and public health consequences. UV-C LEDs were recently developed as a chemical-free, energy-efficient approach to mitigate contamination by pathogens, but their effectiveness has not been fully explored.

Purpose: The effectiveness of UV-C LED on inactivation of *Listeria innocua* and *E. coli*, and the effect of the substrate on inactivation kinetics were investigated.

Methods: *L. innocua* FSL C2-0008 and *E. coli* ATCC 25922 were used as nonpathogenic surrogates for *L. monocytogenes* and *E. coli* O157:H7, respectively. Early stationary phase (16 h) colonies were: i) streaked on tryptic soy agar; ii) inoculated in liquid films (0.6 mm to 2.4 mm thick); and iii) spot-inoculated on stainless steel surfaces, with or without air drying, to mimic various scenarios encountered in food handling environments. These substrates were exposed to a custom-made UV-C LED array (λ=275 nm, fluence rate=40 µW/cm^2) for 10 s to 1500 s. Untreated controls were prepared under similar conditions. Survivors were recovered and enumerated, and log-reduction calculated. All experiments were performed in triplicate, with independently grown cultures. Data was statistically analyzed.

Results: Inactivation levels for both *E. coli* and *L. innocua* increased with UV-C LED exposure time. UV-C resulted in over a five-log reduction of both bacteria after 600 s in thin liquid films (thickness ≤0.6 mm), and on all stainless steel surfaces. Inactivation curves were non-linear, and inactivation kinetics was significantly ($P<0.05$, ANOVA) affected by the substrate. Weibull model was able to successfully model inactivation kinetics ($0.99 \geq R^2 \geq 0.87$). The fastest initial inactivation was achieved on dry stainless steel for both bacteria, but maximum inactivation was similar for the dry and wet conditions.

Significance: UV-C LEDs are a promising alternative to mercury UV lamps for inactivating foodborne pathogens, but disinfection success depends on the substrate and environmental conditions.

T7-04 Impact of Co-Culturing with *Pseudomonas aeruginosa* on *Listeria monocytogenes* Biofilm Physiochemical Properties and Sanitizer Tolerance

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❖ ***Developing Scientist Entrant***

Introduction: Biofilm formation facilitates microbial persistence in food processing environments and increases the likelihood of product flow contamination.

Purpose: Investigate how the biofilm physical structure, matrix composition, and sanitizer tolerance of *Listeria monocytogenes* is affected by coculturing with *Pseudomonas aeruginosa* in a CDC Biofilm Reactor.

Methods: Biofilms were produced on 304 stainless steel carriers at 21°C using a CDC Biofilm Reactor. Biofilms were treated with a LIVE/DEAD stain and a Concanavalin A lectin and imaged using a laser scanning confocal microscope. Biofilm three-dimensional projections were assembled and quantified using IMARIS 7.0 software (n=3). Cell counts were performed in order to assess biofilm sensitivity to a commercial quaternary ammonium compound sanitizer at 200 ppm for five minutes (n=3).

Results: *L. monocytogenes* produced weak biofilms with cell counts of 6.3±0.2 log CFU/coupon. Biofilms presented flat, microcolony architecture with biovolume and maximum thickness values of 4.74±2.6×10^5 mm^3 and 10±1.4 mm, respectively. *L. monocytogenes* levels increased to 8.1±0.36 log$_1$ CFU/coupon after coculturing with *P. aeruginosa* (P<0.05). Biovolume and maximum thickness values also increased to 36.3±24.2×10^5 mm^3 and 41.0±9.8 mm, respectively (P<0.05) following coculture with *P. aeruginosa*. Chemical sanitizers produced a 4.2±0.5 log$_{10}$ CFU/coupon reduction against *L. monocytogenes* biofilms and a <0.5 log CFU/coupon reduction for both organisms in dual-species biofilms (P<0.05). Lectin staining revealed extracellular polymeric substance (EPS) residues were only detected in dual-species biofilms.

Significance: Biofilm organismal complexity significantly impacts biofilm 3-D architecture, matrix composition, and sensitivity to chemical sanitizers. Future studies investigating *L. monocytogenes* biofilms should incorporate additional organisms into biofilms.

T7-05 Impact of Residential Bacteria on Product Quality: The Cold-smoked Salmon Case Study

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Introduction: In the food processing environment, bacteria are able to remain on surfaces after sanitization procedures. These surface residential bacteria are a major source of product contamination and spoilage and play an essential role in food safety and quality. In order to manage food safety in the food processing plant, surface residential bacterial community characterization is an important concern.

Purpose: This study aimed to characterize the bacterial communities of the processing environment and to evaluate the impact on cold-smoked salmon (CSS) quality. A polyphasic approach based on culture-dependent method, 16S metabarcoding, chemical and sensory analysis was used.

Methods: Samples from surfaces of a CSS processing plant and from products during the process and storage all along the shelf life were collected. Bacteria were plate counted and identified by MALDI-TOF MS or full 16S rDNA sequencing. DNA was extracted directly from samples and analyzed by V3-V4 16S metabarcoding. Chemical and sensory analyses were performed on end-products.

Results: The residential bacterial surface community was identified and characterized as homogeneously spread within the processing plant. This "house microbiota" was mainly composed of spoilage bacteria such as *Brochothrix thermosphacta, Carnobacterium maltaromaticum, Serratia liquefaciens, Psychrobacter* spp., and *Pseudomonas* spp. Beta-diversity and network analysis allowed to highlight environmental bacterial source hotspots and to identify contamination routes. These results were consistent with the sensory analysis.

Significance: Microbial ecology knowledge and the use of polyphasic approaches in a complex ecosystem such as a food processing plant could be useful to characterize microbial reservoirs, improve targeted hygiene procedures, and lead to a better product quality all along the shelf life.

T7-06 Isolation and Serotyping of *Vibrio vulnificus* and *Vibrio cholerae* in Seafood in Korea

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Introduction: *Vibrio* species are abundant in seafood and pose a risk to human health. Shellfish and mollusks have potential to accumulate *Vibrio* species in gills and digestive glands, but there are only a few studies for enumerating and characterizing *Vibrio vulnificus* and *Vibrio cholerae* in shellfish and mollusk.

Purpose: The purpose of this study was to investigate the contamination levels of *V. vulnificus* and *V. cholerae* in shellfish and mollusk, and to serotype the isolates.

Methods: Ninety-six seafood samples (66 shellfish samples and 30 mollusk samples) were collected from seafood markets in South Korea from July to December in 2018. To use the most probable number (MPN) method, 10 ml, one ml and 0.1 ml of sample were inoculated in 10 ml alkaline peptone water (APW). The tube was incubated at 35°C for 14 h, and one-ml aliquots of the cultures were used to extract the DNA for the identification and the serotyping by PCR. The amplified DNA products were electrophoresed and visualized under UV light.

Results: *V. vulnificus* were detected in six samples (6.3%), and *V. cholerae* was detected in two samples (2.1%). The highest prevalence month of *V. vulnificus* contamination was September (6.3%), and the highest contamination level was 530 MPN/g detected from cuttlefish and salted oyster. *V. cholerae* was detected only in November from date mussel and common orient clam, and both samples showed the same contamination level (36 MPN/g). The *V. cholerae* isolates were further analyzed for serotyping, and the results showed that all isolates were negative for both *V. cholerae* O1 and *V. cholerae* O139.

Significance: This result indicates that *V. vulnificus* and *V. cholerae* contaminated some shellfish and mollusk samples, and the highest prevalence month was September in S. Korea.

T7-07 Evaluation of Commercially Available Protective Cultures to Control *Listeria monocytogenes* and Shiga Toxin-producing *Escherichia coli* in Soft, Surface Mold-ripened Raw Milk Cheese

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) are pathogens of concern in the production of cheese, including those manufactured from unpasteurized milk. The use of protective cultures (PCs) capable of producing antimicrobial compounds In Situ represents a potential biological control strategy compliant with current regulation.

Purpose: The purpose of this study was to evaluate two commercially available PCs of *Lactococcus lactis* and *Hafnia alvei* for the control of *L. monocytogenes* and STEC, respectively, in a surface mold-ripened soft cheese.

Methods: Lab scale soft-ripened cheeses were manufactured using unpasteurized milk inoculated with cocktails of either *L. monocytogenes* or STEC alone (control; two batches per pathogen) at ~two log CFU/mL or with one of two PCs at ~six log CFU/mL (two batches per treatment). Cheese composition targets and culture selection allowed for an atypically long aging period without ripening prematurely. After de-hooping and dry salting, cheeses were ripened at 12°C and 93% relative humidity prior to cold storage. Samples were collected throughout cheesemaking, ripening, and storage. Pathogens in subsamples (two per cheese) were enumerated on CHROMagar *Listeria* or STEC.

Results: During manufacture *L. monocytogenes* counts did not increase during milk ripening, however, STEC counts increased by ~one log CFU/g. At day 21 mean counts in both STEC treatment and control cheeses decreased by ~one log CFU/g. *L. monocytogenes* counts in cheese increased 1.43 and 0.65 log CFU/g in control and PC treated cheeses, respectively, from day one to 77; however, the change in counts over time was not significant (P=0.98).

Significance: These results identify the survival and growth of pertinent pathogens during the manufacture and aging of a soft-ripened cheese manufactured from unpasteurized milk. The data suggest that some protective cultures available to producers may not provide an adequate level of pathogen control in this cheese type. Further work to validate the effectiveness of PCs in other cheeses of the differing composition is needed.

T7-08 Effect of Commercial Bacterial Fermentates and Protective Cultures on *Listeria monocytogenes* Growth in a Refrigerated Model High-moisture Cheese

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❖ ***Developing Scientist Entrant***

Introduction: High-moisture, low-acid cheeses have been shown to support *Listeria monocytogenes* growth during refrigerated storage. Commercial bacterial fermentates and protective cultures represent clean-label options for inhibiting *L. monocytogenes* in RTE foods.

Purpose: The purpose of this study was to determine the efficacy of three commercial bacterial fermentates and three commercial protective cultures on the growth of *L. monocytogenes* in a model soft cheese.

Methods: Cream, micellar casein, water, salt, lactose, and lactic acid were combined to make a model soft cheese of pH 6.0, 56% moisture, and 1.25% salt. In one set of experiments, the cheese was supplemented with one of three commercial fermentates (CM-1 or CM-2 cultured milk; or CSV-1 cultured sugar-vinegar blend, 0.5% or 1.0%). In a separate set of experiments, model cheese was combined with one of three commercial protective cultures (PC-1, PC-2, or PC-3, six log CFU/g). Control cheeses without commercial ingredients were included in experiments. Formulations were inoculated with three log CFU/g *L. monocytogenes* (five-strain mixture). Twenty-five-g samples were vacuum-sealed and stored at 4°C for eight weeks, with triplicate samples enumerated on modified Oxford agar weekly. Formulations were tested in duplicate trials and time-to-growth determined from average growth curves.

Results: Cheeses formulated with one percent fermentate CM-1 or CM-2 delayed growth (greater than or equal to one-log increase) of *L. monocytogenes* until 6.5 to 7.5 weeks, whereas fermentate CSV-1 inhibited pathogen growth throughout the eight-week study (compared to growth in one week in the control). Supplementing the model cheese with 0.5% fermentate (CM-1, CM-2, or CSV-1) supported growth within 2.5 to 3 weeks. Cheeses formulated with six log CFU/g protective culture (PC-1, PC-2, or PC-3) supported the growth of *L. monocytogenes* within one to 1.5 weeks, showing little to no inhibition regardless of the application (incorporated as ingredients or co-inocula).

Significance: These data suggest that certain fermentates have greater anti-listerial activity than protective cultures in soft cheeses stored at refrigeration temperatures.

T7-09 Identification of Key Environmental Sites to Help Small-scale Raw Milk Cheesemakers Improve Sanitation

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Introduction: Maintaining a sanitary environment for cheesemaking is critical to reducing the risk of foodborne illness, especially for cheeses made from unpasteurized milk. Small-scale cheesemaking operations often employ fewer than five people, which poses challenges to developing and maintaining documented sanitation controls.

Purpose: To help cheesemakers improve sanitation controls through the identification of key environmental problem areas and gaps in practices.

Methods: Five small-scale raw milk cheesemaking facilities were visited four to six times over 13 months. Approximately 25 sites per facility were selected by observation in receiving, cheesemaking, aging, packaging, cleaning, laboratory, and transition areas. Environmental *Listeria* species (EL), coliforms, *E. coli* (EC), and aerobic plate counts (AC) were collected using prehydrated sponge sticks and enumerated on 3M Petrifilm count plates. Documented sanitation procedures and records were reviewed. Cheesemakers received results and feedback after each visit.

Results: A total of 892 environmental samples were evaluated, some using multiple indicators. EL tested positive at 87 (38%) of 226 sites total, EC tested positive at 44 (18%) of 245 sites, and AC and ATP were tested at 520 and 514 sites, respectively. Overall positive rates did not change significantly over time. The most common sites with sanitation problems were drains/drain covers (41% of EL positives), floor squeegees/brushes (18% of EC positives), and floors/floor-wall junctures (29% of EL positives). These sites also had consistently high AC counts. Beginning the study, one cheesemaker had documented sanitation procedures; the others had one logbook to document all activities. By the end, all cheesemakers showed progress in developing written sanitation procedures, schedules, and logs.

Significance: Identification of key sanitation problem areas and gaps in sanitation practices helped cheesemakers improve their GMPs and develop sanitation controls. This aided in the development of resources for all small-scale cheesemakers to help reduce environmental food safety risks.

T7-10 The Microbial Ecology and Resistome of Raw and Pasteurized Retail Milk

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Introduction: Raw milk is advertised as having multiple benefits for human health, but the microbiome and resistome differences between raw and pasteurized milk remain elusive.

Purpose: The study aimed to assess the population of viable bacteria and profile the microbiome and resistome in various types of retail cow's milk sold for human consumption.

Methods: A total of 1,920 milk samples were collected from eight milk brands including ultra-pasteurized milk (*n*=2), HTST-pasteurized milk (*n*=3), vat-pasteurized milk (*n*=1) and raw milk (*n*=2). Sampling occurred between March and August 2017 at Davis, CA through eight independent purchases for all brands of milk. After each purchase, samples were aliquoted into three tubes and incubated for zero, two, four, six, 12, and 24 h at both 4°C and 23°C. All milk samples were used to quantify the total aerobic counts, coliforms and *Escherichia coli* using standardized methods. Concurrently, DNA was extracted from all milk for 16S-rRNA sequencing. In addition, 24 milk DNA samples, which includes both raw and HTST milk before and after 24 h incubation at 23°C were subjected to shotgun metagenomic sequencing.

Results: Different types of milk possess distinct microbiome structure (*P*=0.04), and raw milk has significantly more viable bacteria than other retail milk (*P*<0.05). Remarkably, the raw milk microbiota was dominated with *Pseudomonadaceae* and *Enterobacteriaceae* with minimal to no detection of probiotic groups. Raw milk carries more antimicrobial resistance genes (ARGs) than pasteurized milk (*P*<0.001). Specifically, 138 individual ARGs conferring resistance to 11 classes of antibiotics were observed in raw milk compared with 25 ARGs found in HTST milk. Incubation at 23°C drives the bloom of viable bacteria in milk which also significantly enriches the population of ARGs (P<0.001).

Significance: Raw milk harbors more viable bacteria and ARGs than pasteurized milk, and incubation of raw milk at 23°C dramatically increases such risk.

T7-11 Transcriptome Sequencing of *Listeria monocytogenes* during Co-Cultivation with Cheese Rind Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: The survival of the pathogen *Listeria monocytogenes* in foods and food production environments is dependent on several genes for combating environmental stressors; this includes competing with other resident bacteria.

Purpose: We aimed to uncover what genes are expressed in the transcriptional response of the *L. monocytogenes* sequence type (ST) 121 strain 6179 in co-culture with common cheese rind bacteria.

Methods: *L. monocytogenes* 6179 was cultured in brain heart infusion broth or plates with either gram-negative (*Psychrobacter* L7) or gram-positive (*Brevibacterium* S111) cheese rind bacteria. For broth and plate cultures, RNA was isolated after two and 12, or 24 and 72 h of co-cultivation. RNA samples (*n*=24, in duplicate for each strain and condition) were sequenced using Illumina NextSeq. Mapping of reads to the *L. monocytogenes* 6179 genomes was conducted using BWA. DESeq2 was used to identify differentially expressed genes in *L. monocytogenes* 6179 comparing co-cultivation to *L. monocytogenes* 6179 monoculture. For this, q-values lower than 0.05 were considered significant.

Results: Transcriptome sequencing resulted in 4.1 to 12.2 million reads per sample. For 12 h co-cultivation in the broth of *L. monocytogenes* 6179 with *Brevibacterium* and *Psychrobacter,* 387 and 597 differentially expressed genes were identified with log twofold changes up to 6.2. After 72 h co-cultivation on plates, 190 (*Brevibacterium*) and 489 (*Psychrobacter*) *L. monocytogenes* 6179 differentially expressed genes were identified with up to 8.4 log twofold changes. Significantly upregulated genes included genes involved in the biosynthesis of cobalamin (*n*=42) and the utilization of ethanolamine (*n*=13) and propanediol (*n*=18). Interestingly, 78 genes of three highly conserved *L. monocy*togenes 6179 prophages were significantly upregulated in co-culture with *Psychrobacter* (but not with *Brevibacterium*) after 72 hours.

Significance: Uncovering genes involved in the competitive fitness of *L. monocytogenes* might contribute to the development of additional targets against *L. monocytogenes* in foods and food production environments.

T7-12 Using Machine Learning to Predict Pasteurized Fluid Milk Spoilage Based on Quality Management Practices

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❖ ***Developing Scientist Entrant***

Introduction: Pasteurized fluid milk is susceptible to contamination with spoilage bacteria, resulting in a reduced shelf life. Of particular concern, psychrotolerant Gram-negative bacteria that enter the product post-pasteurization can lead to bacterial levels above 20,000 CFU/ml within seven to 10 days of processing.

Purpose: The aim of this study was to identify top plant-level predictors of pasteurized fluid milk spoilage occurring within 10 days of processing.

Methods: Pasteurized fluid white milk samples were collected from 24 milk plants three to five times between July 2015 and September 2017 and tested for standard plate count (SPC) after seven and 10 days of storage at 6°C. A survey was administered at the completion of milk sampling for each plant; this survey included questions regarding practices in processing and production, cleaning and sanitation, maintenance, training, and general quality management. Conditional inference random forest models were constructed to predict whether a milk sample exceeds 20,000 CFU/ml SPC within 10 days of processing and relative importance was used to select top predictors that were included in subsequent models. Model performance and marginal effects of features on the predicted outcome were assessed.

Results: Among all milk samples tested, 180 (36%) of 506 exceeded 20,000 CFU/ml SPC within 10 days of processing. Based on the model including all features, top predictors included plant, type of filler CIP sanitizer, type of filler CIP detergent, and in-house testing program. The model including only the top predictors had better performance than the model including all features. Assessment of marginal effects indicates an interaction between filler CIP sanitizer and detergent.

Significance: Our findings suggest that cleaning and sanitation as well as finished product quality monitoring are important predictors of milk spoilage within 10 days of processing and should be targeted for developing and implementing practices aimed at improving milk quality.

T8-01 Observational Assessment of Food Safety Behaviors at Farmers' Markets in Ontario, Canada

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Introduction: Farmers' markets face numerous food safety challenges due to their operational characteristics, and they have occasionally been identified as a source of foodborne disease outbreaks.

Purpose: The purpose of this study was to evaluate the food safety behaviors of farmers' market vendors in Toronto and two surrounding regions (Peel and York) of Ontario, Canada, to identify possible food safety risks.

Methods: All markets in the study region (n=60) were identified via publicly available databases and were visited between May and September 2018. The food safety behaviors of vendors selling foods requiring time-temperature control were observed discretely and results were recorded on a standardized and pre-tested form via a smartphone.

Results: A total of 454 vendors were observed, with prepared foods (50%) being the most common food type sold. While general cleanliness and sanitation of vendors were very high, 27% did not provide cross-contamination protection methods for at least some of their foods. Similarly, 66% and 48% of vendors who sold foods requiring cold and hot holding (*n*=410 and 162, respectively) did not provide temperature control for at least some of their foods. Vendors' use of cold-holding temperature control was more commonly observed among those selling raw meats, poultry, and fish (odds ratio=5.36; 95% confidence interval=2.82 to 10.12), and less commonly observed among vendors selling RTE meats, fresh-cut leafy green vegetables, tomatoes, and melons, and cheese and dairy products. A mean of 4.55 (SD=2.62) behaviors requiring handwashing were observed per vendor across 1356 customer transactions, but handwashing was observed by only 13 vendors for one percent of these transactions. Most vendors (79%) handled food with their bare hands on at least one occasion.

Significance: Results of this study have identified targeted areas for future food safety education, training, and outreach with farmers' markets vendors in Ontario.

T8-02 A Sequential Mixed-Methods Approach in Assessing Consumers' Self-Identified At-home Beef Storage, Handling, and Preparation Knowledge and Behaviors

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Introduction: The World Health Organization, Centers for Disease Control and Prevention, and the Food and Drug Administration promote consistent messages for reducing foodborne illness risks. However, consumer food handling beliefs and behaviors vary between American sociocultural demographics.

Purpose: The purpose of this exploratory sequential mixed-methods study was to identify consumer beliefs, attitudes, and behaviors for in-home beef storage and handling through focus groups and a national survey.

Methods: Thirteen semi-structured focus groups discussing beef purchasing, storage, and preparation were conducted in Virginia and North Carolina. Results guided the development and implementation of a 69-question nationwide online Qualtrics survey about consumer food storage and handling behaviors. Survey results (*n*=500) were analyzed with Chi-square goodness-of-fit tests (*P*<0.05; Cramer's V≥0.15). Focus group transcripts and open-survey responses were coded and also analyzed with NVIVO 12Pro with a secondary coder (Cohen's-kappa=0.92).

Results: Participants frequently engaged in inadvisable beef product storage and preparation behaviors: improperly cold storing and defrosting, washing meat products, using subjective, non-temperature indicators to determine doneness. Ethnicity and race were significantly associated with behaviors outside of recommended guidelines; minoritized populations frequently practiced risky behaviors: improperly defrosting meat, washing meat products, and not using a thermometer to measure beef doneness despite knowing safe beef endpoint cooking temperatures (*P*<0.05). Citing personal experience and perceived control, individuals with higher educations and incomes were more likely to engage in riskier behaviors. Income, gender, age, and education also affected thermometer knowledge and usage. Older individuals were more likely to own but less likely to use thermometers (*P*<0.05); males were more cognizant of safe food preparation temperatures than females.

Significance: Culturally competent, linguistically compatible, and targeted interventions for different sociocultural demographics are needed. Alternative messaging and delivery strategies to accommodate consumers' current food safety behaviors may increase behavior change and adoption.

T8-03 Online Professional Training, Consumer Training and Student Training: Symbiosis for Learning Material for Different Target Groups

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Introduction: Food safety training is crucial for consumers, students, and professionals. Online modules can increase the dissemination, efficiency, and multiple-use of the material developed, but may need to be tailored to different audiences.

Purpose: Online learning material in the domain of food microbiology has been developed for very diverse settings. Certain parts of these materials can be used in multiple settings, hence creating a symbiosis between the development of the materials and the dissemination.

Methods: Learning material was developed directed at students for an online BSc course and an online MSc course in the form of small private online courses (SPOC). For industry and government professionals, courses were developed in the form of online food safety modules and YouTube videos. For consumers, a massive open online course (MOOC) was established using the edX-platform.

Results: The MOOC was fully automated (with only moderation) and several times attracted thousands of registered people with diverse background levels. The SPOCs were followed by smaller groups (five to 50) but did involve (live) video contact with lecturers. The professional courses with year-round open access were followed by around 100 students and 50 professionals per year.

Significance: Much of the materials developed for distance learning can be used in all kind of other settings, such as for blended learning, thereby making optimal use of the various building blocks and fine-tuning the education for a specific audience or situation, either as basic content or background material. The materials give great opportunities for mutual enrichment by learning from each other by multiple users, but also by multiple uses of parts.

T8-04 Designing Food Safety Training Using the Integrated Behavior Model

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❖ ***Developing Scientist Entrant***

Introduction: Effective training enables participants to turn knowledge into actual skills and behaviors on the job. Therefore, it is important to design the training in a way that will make an impact on trainee's behaviors. Previous researchers, investigating the effects of food safety training on large- and small-scale dairy processors, have used the Integrated Behavior Model (IBM) to predict which components of intentions to perform behaviors have the greatest effect on workers actual behaviors. Instrumental attitudes, perceived norms, and personal agency were all found to be significant predictors of behaviors.

Purpose: The purpose of this study was to analyze the training target audience, specifically North Carolina small dairy processors, to determine which components of the IBM are significant predictors of intentions to perform safe food handling behaviors.

Methods: The IBM was used as the framework for the survey. Linear regression was used to determine which components of the IBM were predictors of intentions to perform behaviors.

Results: Perceived behavioral control (R^2=0.2381; P=0.0340; n=19) and perceived norms (R^2=0.1633; P=0.0862; n=19) were predictors of intentions to perform food-safe behaviors. After breaking the components down into sub-components, it was found that instrumental norms (R^2=0.2650; P=0.0241; n=19) and self-efficacy (R^2=0.4483; P=0.0017; n=19) were the greatest predictors of intentions to perform food safe behaviors.

Significance: Training can be maximized by designing it to make an impact on the specific components that drive the target audience's behaviors. Designing training to meet the needs of the target audience will not only be good for businesses, by making training more feasible, but it also has the ability to make the food system safer.

T8-05 Food Safety Modernization Act Foreign Supplier Verification Rule: Three Years of Data about the Impact on the United States Food Import Chain Under FDA Jurisdiction.

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Introduction: We have assessed the implementation of the new FSMA Foreign Supplier Verification Program (FSVP) requirements for United States imports over the last three years .

Purpose: Understand the current status of compliance with the new FSVP import requirements.

Methods: We interviewed 500 professionals, including 100 importers of record, 300 United States agents and 100 customs brokers. We then repeated the questionnaire on a sample of 50 United States FSVP importers during FSVP classes offered by the Food Safety Preventive Controls Alliance and 50 during informational seminars. We then compared the results.

Results: Sixty percent of importers of record interviewed did not know the rules, and 30% of them are not ready to become FSVP importers. Only 10% are working to be in compliance. Also 98% of United States agents are not aware of this topic, and only two percent are fully in compliance; 80% of customs brokers know something about FSVP but only 20% of them can clearly explain the new requirements. Food import chains in direct contact with third countries have a lack of knowledge about new FSVP requirements. That could have an impact on foreign food companies' approach to FSMA requirements.

Significance: For effective implementation of FSVP and real prevention of misunderstandings and noncompliance, communication strategies must be updated to effectively achieve public health goals linked to FSMA.

T8-06 Building a Competitive Advantage through the Safe Quality Food Certification in Food Manufacturing: Leveraging a Global Food Safety Initiative Scheme

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Introduction: In maintaining consumer trust and confidence, the food industry is plagued by many challenges. Incidents of food safety and substandard quality continue to rise. Consumers are beginning to mistrust and have lower confidence in the food supply chain. Regulators continue to report increased recalls and withdrawals of food items from commerce. Studies have therefore proved that third-party audits are more expedient to fight food processing failures.

Purpose: The purpose of this presentation is to understand how the Safe Quality Food (SQF) certification as a Global Food Safety Initiative scheme contributes to competitive advantage in producing safe and quality food products.

Methods: Methodology approach for this presentation discusses interview responses, experiences, and responses of current participants in the SQF scheme on how the program has enhanced their food manufacturing operations.

Results: Majority of participants had a positive perception of the value SQF provides to improving the overall safety and quality of food products; 77.14% of respondents demonstrated this viewpoint by identifying SQF as a framework to meet customer expectation and requirements; 85.71% of respondents described SQF certification as a credible and robust GFSI scheme that provides effective guidelines for food production; 94.29% of respondents also noted that SQF has been used to identify and provide ways to eliminate the inherent risks in the food-production process through continuous improvement.

Significance: With the various cases of food-production operations being compromised, presentations such as this is important to assure consumers that efforts are in place to address the potential failure points in the food supply chain. Secondly, the SQF certification allows food safety and quality systems to be verified and validated throughout the food chain, increasing brand protection, consumer confidence, and loyalty. Hence, this presentation is aimed at exposing the benefits of this program as a GFSI scheme and encouraging other food producers to adopt this program.

T8-07 The Use of Matrix-adapted Bacterial Isolates of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in Validation of High-pressure Treated Juices

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❖ ***Developing Scientist Entrant***

Introduction: High pressure processed (HPP) juice manufacturers are required to demonstrate a five-log reduction of the pertinent microorganism to comply with FDA juice HACCP. However, there is no consensus on validation study approaches for bacterial strain selection or preparation and no agreement on which HPP process parameters affect overall efficacy.

Purpose: To compare HPP inactivation of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* isolates in buffer and apple juice.

Methods: Individual bacterial strains were grown using three different growth conditions: neutral, cold-adapted, and acid-adapted. Approximately six log CFU/ml of the matrix-adapted bacterial strains were inoculated into buffered peptone water at pH 3.50±0.10 (HCl-adjusted) and apple juice at pH 3.50±0.10 (store-purchased) and treated at sublethal pressures of 500 MPa for *E. coli* O157:H7 and 200 MPa for *Salmonella* and *L. monocytogenes* (180 s, 4°C initial). Analyses were conducted at zero, 24 and 48 h (4°C storage) post-HPP on non-selective media.

Results: *E. coli* O157:H7 exhibited greater barotolerance than *Salmonella* spp. and *L. monocytogenes*. In buffered peptone water neutral growth conditions, *E. coli* O157:H7 strain TW14359 demonstrated greatest resistance (2.94±0.64-log reduction) and *E. coli* O157:H7 strain SEA13B88 was significantly more sensitive (P<0.05). Acid-adapted *L. monocytogenes* strain MAD328 had <1.00±0.23-log reduction while acid-adapted *L. monocytogenes* strains CDC and ScottA were significantly more sensitive with 2.13±0.48 and 3.43±0.50-log reductions, respectively. Cold-adapted *Salmonella* Cubana and *Salmonella* Montevideo showed significantly greater resistance (P<0.05) compared to other cold-adapted strains. *Salmonella* isolates, neutral and acid-adapted, expressed similar barotolerance to one another in buffered peptone water. In apple juice, acid-adapted *Salmonella* Cubana was the only strain which did not demonstrate significant increases in barotolerance when tested under all conditions. The time-dependent loss in viability occurred in all post-HPP storage samples.

Significance: These results suggest under the conditions tested, matrix composition and bacterial strain and preparation methods influence HPP efficacy and should be considered when conducting validation studies.

T8-08 Effect of Sublethal Food Processing and Associated Environmental Conditions on *Salmonella* Mutation

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Introduction: Whole genome sequencing (WGS) is a powerful tool which can be used for pathogen source tracking as part of a microbial root cause investigation of a contamination event in the food industry. Determination of similarity between strains is largely based on reported mutation rates and genetic differences (e.g., single nucleotide polymorphisms, SNP) observed mostly from outbreak-related isolates. However, the impact of food-processing/associated environmental conditions on *Salmonella* mutation is not considered and may potentially impact WGS data interpretation.

Purpose: To test the hypothesis that *Salmonella* can accumulate mutations when exposed to sublethal food processing and associated environmental conditions.

Methods: *Salmonella* Agona was exposed to dry and heat stress to mimic sublethal food processing and associated environmental conditions expected in a low moisture food manufacturing facility. *Salmonella* Agona was inoculated into a model matrix, dry animal digest powder. After acclimatization, the inoculated dry matrix was exposed to 90°C for five min. Following the heat treatment, *Salmonella* Agona was recovered and regrown and used as an inoculum for the next round of heat treatment in the matrix. This cycle of heat treatment and regrowth was repeated seven times. Illumina HiSeq sequencing was done on isolates derived from the inocula and the heat treated dry matrix for the seven iterations. Genomes were compared by wgMLST and SNP analysis.

Results: A maximum of six allelic/SNP differences was observed after one heat treatment in the dry matrix. The range of allelic/SNP differences increased following each subsequent iteration, reaching a maximum of 23 SNPs after seven cycles of heat and dry exposure conditions. Conservative SNPs and their location in the genome were identified.

Significance: Food processing and associated environmental conditions can induce mutations in *Salmonella*. Laboratory simulation experiments can help the interpretation of WGS results for *Salmonella* source tracking.

T8-09 Effect of Location and Design of Refrigerated Display Cases on Temperature Control in Retail Stores

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❖ ***Developing Scientist Entrant***

Introduction: Produce temperature control reduces food safety risks and maintains quality. Retrofitting display cases with doors reduces temperature variation. Previous studies regarding the overall benefits of retrofitting display cases are varied. This has caused confusion and skepticism regarding its benefits.

Purpose: Operational temperatures in display cases from different retailers across the United States were measured before and after retrofitting with doors. Display case design, ease of cleaning, and differences in energy consumption were evaluated.

Methods: Temperature and humidity were recorded in 25 refrigerated display cases in ten retail stores in five states. Vertical refrigerated multi-deck self-service open and closed display cases were monitored at eight positions: front and back position for each top, middle, bottom, and under the bottom rack. The sensors recorded data every two minutes for nine months with data uploaded to the cloud using Bluetooth connection and web-based application (Delta Trak; Pleasanton, CA) for statistical analysis (SAS 9.4; Cary, NC).

Results: There was a significant difference between stores, position in the display case, and cases with and without doors (P<0.0001) for temperature and relative humidity. Cases with doors held temperatures significantly lower (<5°C). The top front position of the cases in all stores and all cases had the highest temperature abuse (>5°C) most frequently and for the longest duration, reaching up to 100% of total evaluation time. Differences in design suggest variation in temperature abuse and energy efficiency in open and closed cases related to air circulation. Increased heat load on the compressor may be caused by high relative humidity. Observations evidenced design limitations for cleaning procedures that could lead to possible safety risks.

Significance: This study provides retail stores and manufacturers with data for comparison of abuse conditions in open and closed display cases to reduce food waste while increasing refrigeration efficiency and improving food safety.

T8-10 Evaluation of Cantaloupe Contact Surfaces in Retail Stores

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Introduction: Limited data exist on the likelihood of contamination from contact surfaces for whole produce displays at retail.

Purpose: Contact surfaces in retail display of the whole cantaloupe were evaluated by environmental monitoring and interviews. Cantaloupe was selected as a model due to recent foodborne outbreaks.

Methods: A cross-sectional study was performed in five retail stores. Contact surfaces associated with the whole cantaloupe were sampled in each retail store at five time points (6 am, 10 am, 2 pm, 6 pm, and 10 pm). Swabbed surfaces included: plastic carts used to transport cantaloupe; reusable plastic containers (RPCs) used for display; wood display structures; mesh, styrofoam, and plasticor-sheeting used in displays. Samples were tested for *Listeria* species, *L.*

monocytogenes, coliforms, *Escherichia coli*, and aerobic plate counts (APCs) using standard methods. Interviews with produce managers were also performed to capture cleaning and sanitizing schedules.

Results: Two hundred swabs were collected; none tested positive for *E. coli* or *L. monocytogenes.* Average APCs and coliforms across all samples were 5.3±1.3 and 3.9±1.6 log CFU/swab, respectively. While no significant difference was observed between surfaces and time-points, data showed higher APCs for samples collected on food contact surfaces and at later time-points. Approximately 65 (33%) of 200 samples were positive for *Listeria* species. Each surface type was *Listeria* spp. positive at least once (six out of six). Non-food contact surfaces (e.g., plastic carts; 8%, wood displays; 24%) had the lowest *Listeria* spp. prevalence, while food contact surfaces (e.g., styrofoam; 46%, plasticor; 54%) had higher *Listeria* spp. prevalence. Surfaces that were cleaned/sanitized daily had significantly lower *Listeria* spp. prevalence than those surfaces that were cleaned/sanitized on a variable, or as needed schedule (8 vs. 36%).

Significance: *Listeria* spp. were detected on contact surfaces in retail whole cantaloupe displays; emphasizing the importance of sanitation programs. Whole produce contact surfaces at retail should be evaluated for microbial safety, including cleanability, in addition to aesthetics and other factors.

T8-11 Rapid and Synergistic Antimicrobial Processing for Fresh-cut Vegetables in Fast Food Restaurants

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Introduction: Recent foodborne disease outbreaks in fast-food restaurants emphasize the critical need for rapid and effective antimicrobial processing for fresh-cut vegetables before serving to customers.

Purpose: The study was initiated to develop a rapid, effective and cheap antimicrobial processing which combines mild thermal treatment and generally recognized as safe (GRAS) food additives.

Methods: Lactic acid (LA), gallic acid (GA) and propyl gallate (PG) were selected based on our screening study to evaluate the synergistic combination of GRAS compounds and mild heat treatment. Mild thermal treatment was conducted in a metal heating block maintained at 55°C to minimize the come-up time. Briefly, peas or carrots were inoculated with *Escherichia coli* at refrigeration temperature for four hours and treated using a pre-warmed 55°C antimicrobial solution (PA+GA+PG). The survivor population was enumerated after treatment for four min. In comparison, chlorine at 20 ppm was chosen as a positive control to treat inoculated peas or carrots, mimicking current fresh produce sanitation procedure.

Results: Peas model: a combination of mild thermal processing and GRAS additives yields additional 2.7-log and 1.8-log reduction of *E. coli* compared with mild thermal processing alone, and chlorine positive control, respectively. Carrots model: a combination of mild thermal processing and GRAS additives yields 1.5-log and 2.8-log reduction of *E. coli* compared to mild thermal processing alone and positive control, respectively. No significant changes in texture and color were observed for the treated vegetables.

Significance: The study demonstrated a rapid (within four min) and effective (1.5-log to 3-log reduction) antimicrobial approach to realistic food items. The new approach may be readily transferred to a fast-food restaurant to achieve rapid sanitation of fresh-cut vegetables before serving to consumers.

T8-12 Restaurant Food Consumption and Diarrheal Illness: What is the Relationship?

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Introduction: Although outbreak data suggests that contaminated food resulting in foodborne illnesses is most commonly prepared in restaurants, a comprehensive examination of the relationship between restaurant consumption and diarrheal illness has not been completed.

Purpose: In this study, I examine the relationship between restaurant patronage and diarrheal illness using individual-level data from the National Health and Nutrition Examination Survey (NHANES).

Methods: Common econometric models and analyses (e.g., OLS, probit) are used to examine the relationship between individuals' restaurant consumption patterns and their likelihood of suffering from diarrheal illness. Controls for other common causes of diarrheal illness (e.g., health conditions and alcohol consumption) and demographics are included. NHANES observations from surveys fielded between 2001 and 2016 are used in the analyses. Observations that do not include valid responses for the dependent variable (diarrheal illness) and the core independent variables (restaurant consumption and controls for other causes of diarrheal illness) are omitted. A resulting total of 41,430 observations are used in the analysis.

Results: Between 2001 and 2016 restaurant consumption was associated with 59% of outbreaks and 44% of illnesses from CDC reported foodborne illness outbreaks (n=15,935). Nevertheless, my results show little support for the proposition that restaurants are a relatively risky source of illness. Controlling for confounding factors, I find no statistical association between diarrheal illness and the amount of food consumed at restaurants or the portion of the food dollar spent eating out. Diarrheal illness is marginally less common for those who eat more meals away from home (~0.1%, P<0.10), though illnesses are more common for those consuming more meals from restaurants serving fast food and pizza (~0.1%, P<0.01). Though statistically significant, these results are not statistically meaningful.

Significance: This research suggests that restaurants, as a whole, may not be a relatively riskier source of food for consumers.

T9-01 WITHDRAWN

T9-02 Prevalence of Top Seven Shiga Toxin-producing *Escherichia coli* in Microbial Populations through Slaughter in Australian Beef Export Abattoirs

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Introduction: Australian beef processors must ensure that manufacturing beef being exported to North American markets are deemed free of Top 7 Shiga toxin-producing *Escherichia coli* (STEC) serogroups. A better understanding of the microbiota of beef carcases, the abattoir environment and the dissemination of STEC in the abattoir could assist in determining the sites of cross-contamination of STEC through slaughter.

Purpose: Investigate changes in the microbiota and STEC presence throughout slaughter in an integrated and a fragmented Australian beef export abattoir.

Methods: Abattoir A (integrated) and B (fragmented) were each visited twice. At each visit 90 samples consisting of 10 faecal samples, 15 hides, 15 post-hide pull carcases, 15 post-evisceration carcases, 15 pre-chill carcases and 10 environmental samples were collected. Samples were assessed for total viable count (TVC), *E. coli*/coliforms, and traditional (*stx*, *eae* and O-antigens) and novel (*espK* and/or *espV*) STEC markers. Culture confirmation was conducted on potential positive (PP) samples (*stx*$^+$, *eae*$^+$ and O-antigen$^+$). In addition, samples were processed and analysed for 16S rRNA metagenomics.

Results: Potential positives were identified in 64 to 81% of samples across all visits. Additional novel STEC markers did not significantly (P<0.05) reduce the number of PP's. STEC were isolated from both abattoirs and were typically from hide and faecal samples. However, greater frequency and diversity of STEC was observed in abattoir B. In abattoir B, TVC in post-hide pull carcases was significantly higher (P<0.05), though TVC in pre-chill carcases was significantly lower (P<0.05). The microbiota of carcases shared greatest similarity with hide samples, however, carcases from abattoir A were notably influenced by environmental contaminants.

Significance: The microbiota and associated Top 7 STEC prevalence are linked to the hide contamination of incoming animals. Reducing the level of contamination on hides and the transfer onto carcases will result in safety and quality improvements.

T9-03 Effect of Dry Aging of Beef on the Survival of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Dry-aged beef is a traditional product of high quality that receives increasing interest.

Purpose: To evaluate the effect of different process conditions during the dry-aging of beef on the survival rate of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on the meat surface.

Methods: Beef sirloins (n=2) were submitted to various dry-aging conditions of temperature and relative humidity (RH): Trial 1: 6°C, 75% RH; Trial 2: 2°C, 75% RH; Trial 3: 6°C, 85% RH; Trial 4: 2°C, 85% RH) for 42 days. The meat surfaces were inoculated with a mixture of the three pathogens (*E. coli* O157:H7, *Salmonella* and *L. monocytogenes*) at four to five log CFU/cm^2. Samples were taken at different time points. The limit of detection (LOD) of the counting method was one CFU/cm^2. The log reduction values were calculated and compared using ANOVA.

Results: There were statistically significant differences (P<0.05) in the log reduction values at different time points between the different trials, with *L. monocytogenes* having highest survival rate. For instance, the log reduction value at the end of 42 days of dry-aging process for *E. coli* O157:H7 was <LOD, 3.70±0.28, 4.03±0.21, <LOD in trials one, two, three, and four. For *Salmonella* it was 3.20±0,00, 3.15±0.07, 2.86±0.11 and <LOD, and for *L. monocytogenes* 2.13±1.65, 2.50±0.00, 2.09±0.07 and <LOD.

Significance: The survival rate of all three pathogens is less than the detection limit (one CFU/cm^2) in trial four (2°C, 85% RH) at the end of the 42nd day of incubation. So, 2°C and 85% RH is the ideal process condition to dry age the beef to increase the safety of the product.

T9-04 Evaluating the Efficacy of Trim Interventions Against High and Low Levels of *Escherichia coli* O157:H7 and Their Impact on Ground Beef Color

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks and recalls associated with ground beef, have increased over the past two decades. *Escherichia coli* O157:H7 is a common pathogen implicated in these outbreaks. Ground beef, which consists of carcass trims, has a higher potential for contamination during processing. Furthermore, temperature abuse (>5°C) during retail storage could also influence pathogen growth, making conventional controls insufficient. Trim interventions, such as organic acid and biocontrols, could be an effective way to reduce contamination in ground beef.

Purpose: Evaluate the efficacy of trim interventions in reducing *E. coli* O157:H7 in ground beef and their impact on ground beef color.

Methods: Lactic-acid (5%), peracetic-acid (400 ppm), water or *E. coli* O157:H7-specific bacteriophage cocktail (nine log PFU/ml) were tested. A five-strain *E. coli* O157:H7 cocktail, at high (six log CFU/g) and low (two log CFU/g) inocula was used to inoculate beef-trims (n=24; four by four inches). Inoculated beef trims were sprayed (20 ml) with the antimicrobials, ground in a meat grinder, and stored in retail packages at four and 8°C. Surviving pathogen populations and color values (L-lightness, a-redness and b-yellowness) were obtained over a four-day period. Data were analyzed using one-way ANOVA (P<0.05).

Results: All the treatments, except water, showed significant reductions in pathogen populations compared to the control. In ground beef inoculated with high pathogen levels, treatments were equally effective in reducing populations on day zero, at both temperatures. However, only bacteriophage treatment was able to maintain this reduction over four-day storage. In ground beef inoculated with low levels of the pathogen, phage treatment was the most effective. At both storage temperatures, phage treatment significantly reduced pathogen populations on day zero (0.6 to 0.7 log CFU/g) and day two (0.7 to 0.9 log CFU/g), compared to control and other treatments. Instant color blooming (significant increase in redness) was observed for phage, lactic-acid, and water treatments at both storage temperatures.

Significance: Trim interventions could be effective in reducing ground beef contamination without affecting color.

T9-05 Validation of Immersion Versus Electrostatic Spraying with Commercial Antimicrobials Against Unstressed and Acid-, Starvation-, or Cold-stress Adapted *Campylobacter jejuni* on Broiler Wings, and Related Cost Effectiveness Analysis

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Introduction: New performance standards require that *Campylobacter*-positive poultry meat samples should be <10.4%. Electrostatic spraying can improve the coverage of antimicrobials on poultry meat product surfaces and save water use.

Purpose: This study aims to compare the efficacy of commercial antimicrobials to inactivate unstressed and stress-adapted *Campylobacter jejuni* on broiler wings using immersion and electrostatic-sprayer, and to compare the economic feasibility of the two methods.

Methods: Three strains of overnight cultured (18 h) *C. jejuni* strains were unstressed or acid shocked in pH 5.0 Bolton's broth (two h), sub-cultured in 0.9% saline solution (two h), and stored in Bolton's broth (4°C, five days) to prepare acid-, starvation-, and cold-stress-adapted cells, respectively. Fresh wings inoculated with unstressed or stress-adapted *C. jejuni* were untreated or treated. Treated wings were immersed in or electrostatic-sprayed with antimicrobials for 30 s. These were peroxyacetic acid (PAA, 1,000 ppm), lactic acid (LA, 5%), lactic and citric acid blend (LCA, 2.5%), sodium hypochlorite (SH, 70 ppm), or Sanidate 5.0, a mixture of PAA and H_2O_2 (SD, 0.25%). Surviving bacteria were recovered onto Brucella agar under microaerophilic conditions. Cost-effectiveness was analyzed based on the assumption of daily wings production of poultry plants. Data (two repeats with three to four samples per repeat) were analyzed using the Mixed Model in SAS.

Results: No difference (P>0.05) was seen between immersion vs electrostatic-sprayer on the lethality of *C. jejuni* cells. Compared to the unstressed cells (1.53 to 2.54 log CFU/ml), reductions of acid-, starvation- and cold-stress–adapted cells indicated cross-protection (0.12 to 0.60 log CFU/ml), cross-protection (0.83 to 1.81 log CFU/ml), and no effect (1.52 to 2.37 log CFU/ml), respectively, of pathogens during subsequent exposure to antimicrobials by immersion or

electrostatic-sprayer. Among all stressed cultures, PAA, LA, LCA, SH, and SD reduced (P<0.05) *C. jejuni* population by 0.34 to 2.54, 0.75 to 2.34, 0.59 to 2.37, 0.29 to 2.21, 0.12 to 2.28 log CFU/ml, respectively, over the control. The economic benefits of the electrostatic-spraying become more evident as the scale of production increases, although the immersion method is more economical for small producers.

Significance: Applying post-chilling antimicrobial treatments in an electrostatic-sprayer could effectively reduce *Campylobacter* on broiler parts. Challenge studies should include stress-adapted cells.

T9-06 *Lactobacillus curvatus*: A Natural Food Safety Hurdle for *Listeria monocytogenes* Inhibition on RTE Chicken Strips

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Introduction: contamination with *Listeria monocytogenes* is a hazard reasonably likely to occur in all RTE chicken products exposed to the post-lethality processing environment. A GRAS approved lactic acid culture (LAC) has shown its efficacy as a natural antimicrobial to suppress the growth of *L. monocytogenes* on RTE chicken products.

Purpose: the purpose was to evaluate if the addition of a specific *Lactobacillus curvatus* strain can inhibit the growth of *L. monocytogenes* on three recipes of chicken strips without negatively impacting on their sensory properties.

Methods: Nine challenge tests (three recipes with two batches of LAC and one control per recipe) were performed. The LAC was applied after cooking by spray on application. Chicken strips were modified atmosphere-packed and stored until their use-by-date considering part of the storage at abuse temperature. Concentrations of artificially inoculated *L. monocytogenes* were measured at D_0, $D_{1/3}$ and $D_{\text{end of shelf life}}$. Simultaneously, lactic acid bacteria concentrations and the bacteriocin production of LAC were measured to better evaluate the robustness of this antimicrobial. Sensory evaluation was also performed by the chicken processors on samples that were not inoculated with *L. mononocytogenes*.

Results: The growth of LAC was significant regardless of the chicken strips recipe tested, and both tested LAC batches had significant bacteriocin production. In addition, the behavior of *L. monocytogenes* was significantly influenced by the chicken recipes. *L. curvatus* inhibits the *L. monocytogenes* growth in two out of the three tested recipes while in the third one no growth of *Listeria* was observed independently from the addition of the LAC. No taste deviation was reported (triangular test, six panelists).

Significance: L. *curvatus* is an efficient natural antimicrobial agent to control *L. monocytogenes* in post-lethality exposed chicken products.

T9-07 Multispectral Imaging as a Rapid Method to Detect Adulteration of Fresh and Frozen-Thawed Minced Chicken and Pork

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Introduction: Minced meat adulteration via partial substitution with meat from different (than the one claimed) animal species/tissue is rather common. In this context, the rapid and facile detection of this fraud type is expected to be of significant value both for surveillance and mitigation purposes.

Purpose: The aim of this study was to assess the efficacy of multispectral imaging in detecting adulteration of minced chicken and pork meat, both in fresh and frozen-thawed meat samples.

Methods: Chicken and pork meat, purchased from four different butcher shops, were minced and appropriate portions of the two meat types were mixed in order for three adulteration levels to be attained: 25, 50 and 75%; these adulteration levels were studied along with two levels of pure meat (100% chicken, 100% pork). Six different samples were prepared for each one of the abovementioned levels. In total, 120 multispectral images (five levels with six samples in four batches) were acquired. The meat samples were then freezer stored (-20°C for four months), thawed (six to eight h incubation at 4°C), and multispectral images were acquired once again. The collected data corresponding to fresh and frozen-thawed samples was distinctively subjected to partial least-squares discriminant analysis (PLSDA) for the samples' classification in three groups (pure chicken, pure pork and adulterated meat). Three of the tested meat batches were used for model calibration and one batch for external validation (i.e. prediction).

Results: Overall correct classification for calibration and prediction of the fresh samples was 100% in both cases, while the corresponding values for the frozen-thawed samples were 100 and 80%, respectively.

Significance: Multispectral imaging appears to be very promising for the rapid detection of minced chicken meat adulteration with pork (and vice versa), not only for fresh but also for frozen-thawed meat samples.

This work has been supported by the project "PhasmaFOOD".

T9-08 Isolation and Characterization of Native Lactic Acid Bacteria Toward Their Selection as Poultry Probiotics

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❖ ***Developing Scientist Entrant***

Introduction: Probiotics, known to be a live, microbial supplement which confers health benefits to the host, are now accepted as suitable alternatives to antibiotics in animal production. Lactic acid bacteria (LAB) with remarkable functional properties have been considered as excellent probiotics.

Purpose: The purpose of this study was to isolate and evaluate LAB with probiotic potentials from the gastrointestinal tract of a broiler.

Methods: LAB strains were isolated and screened for antagonistic activity against *Escherichia coli*, *E. coli* O157:H7, *Enterococcus faecalis*, *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Listeria monocytogenes* and their co-aggregation using standard techniques. Other probiotic properties including survival of LAB strains in simulated gastric juice, tolerance to phenol, bile salts and NaCl, adhesion to ileum epithelial cells, auto-aggregation, hydrophobicity, and antibiotic susceptibility were also evaluated. Most promising LAB strains were identified by API 50 CHL, 16S rRNA, and bacteriocin genes sequencing.

Results: Out of the 58 LAB strains isolated, 15 inhibited all the pathogens tested, with zones of inhibition ranging from 12.5±0.71 to 20±0 mm. Only six LAB strains were able to survive in simulated gastric juice (at pH 2.0), tolerate 0.4% phenol, 0.3% bile salt and 6.5% NaCl. The selected LAB strains significantly decreased (P<0.05) the ability of the tested pathogens to adhere to ileum epithelial cells with viable counts of three to five log CFU/ml more than the pathogens. Selected LAB strains identified as *Lactobacillus paracasei* subsp. *paracasei, Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lactococcus lactis* subsp. *lactis*, and *Lactobacillus pentosus* showed significant (P<0.005) auto and co-aggregation abilities. The antibiotic susceptibility test showed 100.00% resistance of the LAB strains to oxacillin, and 83.33% resistance to oxacillin, erythromycin, vancomycin, ciprofloxacin, streptomycin, and tetracycline with multiple antibiotic resistance indexes above 0.5.

Significance: The selected LAB strains are ideal probiotic candidates, and can be evaluated In Vivo in poultry.

T9-09 Prevalence and Biofilm Formation of *Staphylococcus aureus* Isolated from Animal Food in Shanghai, China

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❖ ***Developing Scientist Entrant***

Introduction: Biofilm is one of the important factors affecting the antimicrobial resistance of bacteria. *Staphylococcus aureus* usually has strong biofilm formation ability, and it is widely found in animal food.

Purpose: The purpose of this study was to determine the prevalence and biofilm formation ability of *S. aureus* in animal food, and to find an effective inhibitor to the biofilm-forming of *S. aureus*.

Methods: Separation and identification of *S. aureus* was performed according to China's National food Safety Standard. The ability of biofilm formation was quantitatively evaluated by microtiter plate assay and crystal violet staining. Naftifine and its 26 derivatives were used to inhibit the biofilm producers at the concentrations from 50 to 1000 μM.

Results: A total of 349 samples of animal food were collected from randomly selected locations (local supermarkets and wet markets). The overall separation rate of *S. aureus* was 87 (25%) of 349. For each food category, the separation rate was 23 (32%) of 72 for chicken, 42 (29%) of 145 for pork, nine (24%) of 37 for beef, three (23%) of 13 for duck, eight (22%) of 37 for aquatic products, and two (10%) of 21 for egg. No isolation was found from lamb (n=13) and pasteurized milk (n=11). And 85% of the all 87 isolates had strong biofilm formation ability and the rest were moderate. Remarkably, significant difference was found in biofilm formation ability between those isolates from supermarkets and wet markets (P<0.01). In the biofilm formation inhibition test, a series of naftifine derivatives could significantly inhibit all the strong biofilm producers when the concentration was no less than 600 μM. And the strongest inhibitor was JX08806 at 50 μM.

Significance: The contamination rate of *S. aureus* from animal food was pretty high. Wet markets had more serious contamination of *S. aureus.* Most of these *S. aureus* isolates were strong biofilm producers. Naftifine derivatives can be potential inhibitor to control the *S. aureus* biofilm.

T9-10 Comparison of Methods for Detection of Total *Vibrionaceae* as an Indicator of Pathogenic *Vibrio* Species in Oysters and Seawater

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Introduction: *Vibrio parahaemolyticus* and *V. vulnificus* infect humans through shellfish and seawater. Detection methods are tedious, expensive and time-consuming.

Purpose: We evaluated the rapid, simple and inexpensive colony overlay procedure for peptidases (COPP) assay, for the identification of total *Vibrionaceae* in oysters and seawater to determine whether the COPP assay can be used as an indicator of pathogenic vibrios.

Methods: A total of 330 oyster and 330 seawater samples were collected from five sites in the Chesapeake Bay, Maryland and three sites in the Delaware Inland Bays, Delaware, from May to October 2016 and 2017. Comparisons were performed with the COPP assay for total *Vibrionaceae*, direct plating on CHROMagar *Vibrio* and MPN-real-time PCR (MPN-PCR) for *V. parahaemolyticus* and *V. vulnificus*. MPN-PCR involved assays for *tlh*, *tdh* and *trh* genes for *V. parahaemolyticus,* and *vvh* and *vcg*C genes for *V. vulnificus*.

Results: All oyster and seawater samples were positive for total *Vibrionaceae*. Positive *V. parahaemolyticus* for seawater and oysters by MPN-PCR were 89% and 92%, respectively in Maryland; 100% for both in Delaware; and by direct plating was 32% and 76% in Maryland; 71% and 87% in Delaware. Positive *V. vulnificus* by MPN-PCR were 99% and 100% in Maryland; 100% for both in Delaware; and by direct plating was 47% and 86% in Maryland and 58% and 77% in Delaware. The total *Vibrionaceae* was positively correlated (r=0.5 to 0.7) with MPN-PCR *V. vulnificus* in oysters and seawater and direct plating in seawater and oysters. Total *Vibrionaceae* was significantly correlated (r=0.63 to 0.65; P<0.05) with MPN-PCR or direct plating for *V. parahaemolyticus* in seawater but not in oysters.

Significance: The COPP assay is a viable alternative to direct plating or MPN-PCR for the detection of *V. vulnificus* and *V. parahaemolyticus* in seawater and *V. vulnificus* in oysters, and is currently under further evaluation for its ability to serve as an indicator for *V. parahaemolyticus* in oysters.

T9-11 Characterization of a Novel Enzyme from *Photobacterium phosphoreum* with Histidine Decarboxylase Activity

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Introduction: Research has shown that *Photobacterium phosphoreum* can produce histamine levels >200 ppm, which causes the human illness scombrotoxin poisoning. However, whole genome sequencing (WGS) and real-time PCR data have not been able to identify the histidine decarboxylase gene in these bacteria. Therefore, the mechanisms of histamine production in these bacteria are uncertain.

Purpose: The objective of this study was to identify mechanisms of histamine production by *Photobacterium phosphoreum*.

Methods: A gene flanking two commonly known histamine production regulatory genes (histidyl tRNA synthetase and the histidine-histamine antiporter) was identified from WGS data as a candidate for histamine production by *P.* phosphoreum. This gene was cloned, expressed, and purified using the Champion pET Directional TOPO Expression System, pET100 cloning vector, and HisPur Cobalt resin. The histidine decarboxylase (HDC) activity of the enzyme was determined after four h at 20°C in reaction buffer and compared to HDC from *Morganella morganii* and *Photobacterium kishitanii*. Additionally, the activity of this novel enzyme was determined using conditions ranging from 50 to 60°C, pH 3 to 10, and zero to eight percent NaCl in reaction buffer.

Results: The novel HDC from *P. phosphoreum* produced 4570.7 ppm histamine in reaction buffer compared to 4485.6, 3733.2, and 2.2 ppm for the HDC from *P. kishitanii*, *M. morganii*, and the negative control, respectively. This novel HDC is annotated as arginine decarboxylase at NCBI and is 2,310 bp long compared to the 1,143 bp hdc gene in *P. kishitanii* and there is only 12% similarity in the protein sequences. Optimal activity for the enzyme was at 20 to 35°C and pH 3 to 6. There was no significant difference (P=0.115) in the enzyme activity at NaCl concentrations between zero to eight percent.

Significance: We identified a novel enzyme with significant histamine production capabilities in *P. phosporeum*. Understanding the mechanism of histamine production is important to develop proper guidance and control strategies for histamine production in scombrotoxin-forming fish.

T9-12 Effectiveness of a Novel, Rechargeable, Non-leaching Polycationic *N*-Halamine Antibacterial Coating on *Listeria monocytogenes* Survival in Food Processing Environments

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Introduction: Outbreaks and recalls due to *Listeria monocytogenes* are often associated with RTE products.

Purpose: To evaluate the bactericidal efficacy of a novel *N*-chloramine coating (SymbiCoat) on stainless steel surface to reduce *L. monocytogenes* transfer to RTE beef products

Methods: Chlorinated *N*-halamine coating was applied onto stainless steel surfaces (from 25 to 225 cm^2; n=306) under the following surface conditions: no coating (control), coated intact and coated scratched. Individual *L. monocytogenes* strains and an *L. monocytogenes* cocktail (four strains; 10^6 CFU/ml) were suspended in PBS or meat purge and then applied on stainless steel surfaces for 45 min. For PBS, bacterial reductions were calculated. For deli meat three beef wieners and three roast beef slices were placed on each surface separately for 20 min. Meat products were removed, packed individually in easy open tab bags, stored at 2°C and tested at zero, 48 and 72 h. For *L. monocytogenes* enumeration, tenfold dilutions were prepared and plated onto Oxford agar overlaid with trypticase soy agar (thin agar layer method). All experiments were replicated three times.

Results: Overall for PBS, *L. monocytogenes* reductions ranged from 4.8 to 5.2 log CFU/cm^2. Regarding deli meat, for sausages, the coated intact and coated scratched showed similar antimicrobial capacity (P=0.50) and resulted in less than one log CFU/cm^2 of *L. monocytogenes* transfer (90.0% *L. monocytogenes* reduction) compared to control surface. In contrast, for the roast beef product, coated scratched showed the lowest *L. monocytogenes* transfer, followed by coated intact and then the control group (P<0.01). In this case, coated intact reduced *L. monocytogenes* transfer by 67.0%, while the reduction by coated scratched was 79.5%. Regardless of the coating conditions, in both sausage and roast beef, overtime *N*-halamine coating showed lower antimicrobial *L. monocytogenes* transfer from 0 to 72 h post exposure (<3.5 log CFU/cm^2) without any decline in its antimicrobial effect.

Significance: Due to the high antimicrobial capacity of the *N*-halamine coating, it can be used by the food industry to reduce the likelihood of *L. monocytogenes* cross-contamination and persistence.

T10-01 Development of a User-friendly Software Tool for Validation of Predictive Models

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Introduction: Proper validation of models that predict the growth of pathogens in food will increase confidence in using them to make important food safety decisions.

Purpose: The current study was undertaken to develop a user-friendly, validation software tool (ValT) for predictive models that are based on the test data and model performance criteria of the acceptable prediction zone method developed by the United States Department of Agriculture.

Methods: The new software tool was developed in Excel and was demonstrated using a published model for growth of *Salmonella* Typhimurium definitive phage type 104 on chicken skin. A model prediction was considered acceptable when its residual (observed minus predicted) was in an acceptable prediction zone from -1 log (fail-safe) to 0.5 log (fail-dangerous). A model was considered to provide predictions with acceptable accuracy and bias when the proportion of residuals in the acceptable prediction zone (pAPZ) was ≥0.700 and there were no local prediction problems. A local prediction problem occurred when pAPZ was <0.700 for a single level of an independent variable (time or temperature).

Results: The overall pAPZ were 0.823 for dependent data (n=384), 0.826 for interpolation data (n=178), and 0.786 for extrapolation data (n=196) to another food matrix (kosher chicken skin). Although overall pAPZ were acceptable, the model failed validation for dependent data, interpolation, and extrapolation because the dependent data and interpolation data did not satisfy all criteria for test data and because of local prediction problems for all sets of test data.

Significance: A new software tool called "vault" (ValT) was developed that will make it easier for model developers to properly validate their models using a comprehensive set of criteria developed by the USDA.

T10-02 WITHDRAWN

T10-03 Using Predictive Pre-processing Risk Scores to Reduce Foodborne Disease

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Introduction: Most food processing plants operate statically: pathogen mitigation interventions are run at the same parameters regardless of the risk posed by incoming products. Often, pre-processing pathogen testing alone is insufficient to differentiate risk. However, combinations of pre-processing testing, historical performance data, and location-based metadata can be used in machine learning (ML) algorithms to generate accurate pre-processing risk scores.

Purpose: To reduce the risk of foodborne pathogen transmission by providing risk scores that allow producers to differentiate pathogen risk in incoming products.

Methods: We develop a suite of ML algorithms that takes available pre-processing data including pathogen tests, historical performance data from growing locations, and location-based metadata, and generates a predictive risk score identifying which product batches are most likely to have positive pathogen tests on a final product. Risk scores can be used to aid decision-making in processing plants – whether to alter intervention parameters, divert certain product batches (e.g. from raw to cooked), or other available techniques depending on the product, with the goal of reducing the final pathogen prevalence and ultimately foodborne disease.

Results: Utilizing data from over 2000 commercial broiler chicken placements, and combining live operations *Salmonella* results, house equipment (e.g., types of heaters, feeders, drinkers, etc.), and early health metrics with processing plant final product tests, we employ an ensemble ML algorithm to assign risk scores based on processing plant outcomes. We test the algorithm on 20% of the data that was initially set aside; it was able to identify with 80% accuracy which days the processing plant would have a positive *Salmonella* test in the outgoing product.

Significance: Predictive algorithms can accurately assign risk scores prior to processing, allowing for producers to make intervention-based and diversionary decisions that reduce the prevalence of pathogens on the outgoing product, lowering the public risk of foodborne disease.

T10-04 Risk Categorization of Federally Registered Meat Establishments in Canada Using the Canadian Food Inspection Agency's Establishment-based Risk Assessment Model

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed a quantitative risk assessment model to help inform inspection resources' allocation for food establishments. In 2014, a pilot assessed the model's performance with 49 meat/poultry establishments resulting in a Spearman correlation coefficient of 0.64 (P<0.001) between the model outputs (annual number of DALYs) and the assessment done by senior inspectors.

Purpose: To assess the food safety risk of federally registered meat/poultry establishments across Canada, to subsequently integrate the model outputs in the Agency's work planning for risk-informed oversight.

Methods: From April to October 2017, 679 meat/poultry establishments and their assigned inspectors attended WebEx information sessions and provided inputs on the inherent/mitigation factors associated with their facilities, using an Excel questionnaire. This was analyzed by the model algorithm along with up to five years' compliance data from CFIA's systems.

Results: Nine establishments were not considered in the analysis because they refused to participate (0.15%), were not operating (0.74%), or were not processing/storing meat products (0.44%) at the time of data collection. Establishments reported processing multiple meat species (50%), distributing products directly to a vulnerable population (24%), and applying several additional treatments (e.g., antimicrobials) (13%) and controls for incoming supplies (77%) to further reduce their risk. Ten establishments (distributing 25% of the total Canadian domestic meat volume) were responsible for 35% of the total risk, while 85% of the establishments represented only 10% of the total food safety risk related to this sector.

Significance: This model permitted categorization of meat establishments in four groups considering its individual contribution to the overall food safety risk in this sector (3, 89, 227, and 351 for category 1 to 4 respectively, where 1 represents the highest risk and 4 the lowest, as of January 2019). These findings will be used to proportionally allocate inspection resources for risk management based on the establishment risk contribution.

T10-05 WITHDRAWN

T10-06 WITHDRAWN

T10-07 Predicting the Food Sources of Sporadic Cases of *Listeria* Infection Using Whole Genome Multilocus Sequence Typing

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Introduction: Whole genome sequencing (WGS) has substantially refined the ability to subtype *Listeria monocytogenes* isolates. PulseNet maintains the metadata of sequenced isolates from patients, food, and food production environments.

Purpose: We used these data to predict food sources of sporadic listeriosis cases by comparing genetic features between isolates from humans with those from food sources.

Methods: We developed a random forest model using whole genome multilocus sequence typing (wgMLST; >4,000 genes) on a training dataset of 484 isolates from known sources of 5 categories: dairy, fruit, meat, seafood, and vegetable (65% were from 2012 to 2016). We used the model to predict the probable food sources for human isolates from the corresponding years. Alleles of human isolates that were not present in the training data were treated as missing and imputed using an unsupervised random forest approach. Uncertainties in the attribution proportions were estimated from multinomial bootstrap of the predicted probabilities from the model.

Results: The model attributed the 3,424 human isolates to the following food categories, in decreasing order: dairy (28%, 95%CI 28 to 30%), vegetable (26%, 95% CI 25 to 27%), meat (24%, 95% CI 23 to 25%), fruit (13%, 95% CI 12 to 14%), and seafood (8%, 95% CI 7-9%). The estimated proportions of sporadic illnesses attributed to each food category were different from 2016 outbreak-based estimates by the Interagency Food Safety Analytics Collaboration (71% of illnesses included in their model were from 2012 to 2016) which estimated 45% (95% CI 27 to 67%) for dairy, 31% (95% CI 10 to 53%) for fruit, and 19% (95% CI 4 to 46%) for vegetable. This suggests causal pathways may be different between detected outbreaks and sporadic illnesses.

Significance: This model may assist with investigations by providing hypotheses about the most probable vehicles based on wgMLST of the outbreak strain. An increase in the available number of sequenced isolates from known food sources will likely help to enhance the reliability of the model's predictions.

T10-08 Evaluating the Prevalence of *Salmonella* Virulence Gene Expression in Chicken to Incorporate into a Risk Assessment Framework

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Introduction: *Salmonella* is responsible for 11% of all foodborne infections in the United States. It covers a diverse genetic landscape, with more than 2,600 named serovars with varied pathogenicity profiles being identified for *Salmonella enterica* subsp. *enterica* alone. Despite this, current quantitative microbial risk assessment (QMRA) models, which utilize relevant food safety information to understand and evaluate the risk of foodborne illness, do not account for this intraspecies variability in microbial behavior.

Purpose: The purpose of this study was to determine the applicability of whole-genome sequencing in revising prevalence and risk estimates for *Salmonella* in chicken.

Methods: Whole-genome sequences of 150 *Salmonella* samples (serovars including Enteritidis, Heidelberg, Typhimurium, and Kentucky) isolated from chicken by the US Food and Drug Administration (FDA) GenomeTrakr project were analyzed in this study. Virulence, stress-response, and antimicrobial resistance phenotypes were predicted using multiple sequence alignment and logistic regression. The sequences were clustered based on the average expression of virulence factors using 3-means clustering. Cluster-specific phenotypic expression was correlated with spatiotemporal factors obtained from the National Oceanic and Atmospheric Administration (NOAA) using a logistic regression model and prevalence re-estimation was performed using a Poisson regression model with R software (version 3.5.2).

Results: Phenotypic closeness for the selected virulence genes was estimated using a conservative guide (inclusion criteria ≤20 single nucleotide polymorphism match) and the regression was validated using a receiver operating curve (area under the curve=79%). Considerable inter- and intra-strain variation was observed. A significantly higher number of virulence genes were expressed in Cluster 3 (22.8%) compared to 1 (27.8%) and 2 (49.28%) (P<0.05, Fisher's exact test). Poisson regression analysis indicated a significant correlation between temperature (X^2=84.2130; P<0.048) and seasonality (X^2=71.3710; P<0.0001) of bacterial isolation and prevalence of *Salmonella* Cluster 3.

Significance: This study demonstrates a means to apply whole-genome sequencing to a quantitative risk assessment framework.

T10-09 Stochastic and Dynamic Predictive Modeling Using a Monte Carlo Simulation to Estimate the Behavior and Survival Probability of Bacterial Spores

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❖ ***Developing Scientist Entrant***

Introduction: Conventional kinetic models describing bacterial inactivation do not account for variability and uncertainty. In contrast, stochastic models describe bacterial death with probability distributions which is taking individual cell heterogeneity into account. In addition, actual food thermal processing is non-isothermal although almost all of the stochastic models of previous studies describe bacterial reductions in isothermal processing. Appropriate design of minimal food processing requires stochastically accurate estimations of bacterial survival behavior in non-isothermal processing.

Purpose: The objective was to develop a second-order Monte Carlo (2DMC) simulation model describing variability and uncertainty in the spore survival behavior using the bootstrap method. In addition, the simulation results were verified with experimentally observed data.

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, 85°C and 90°C in a thermal cycler; the survival spore counts were determined on tryptic soy agar after 24 h incubation at 37°C. The survival kinetics in each condition were fitted to the Weibull model; distributions of fitted Weibull parameters were described by linear regression with the bootstrap method. The changes in the survival spore behavior during non-isothermal heating were estimated with 2DMC using randomly selected parameters from the distributions. The developed 2DMC was verified with experimentally observed survival spore counts and the survival probability of the bacterial population.

Results: In all of three thermal histories, the variances in the survival spore counts were successfully described with the developed 2DMC simulation model (Student's t-test: P>0.05). The survival probability of *B. simplex* during heating showed a sigmoidal decrease. In addition, the estimations of 2DMC successfully corresponded with the observed changes in survival probability.

Significance: With the 2DMC simulation using the bootstrap method, stochastic estimations for bacterial survival in non-isothermal process conditions will be successfully derived from conventional kinetic models.

T10-10 Thermal Inactivation of *Salmonella enterica* and Non-pathogenic Bacterial Surrogates in Wheat Flour by Baking in a Household Oven

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Introduction: Wheat flour has been implicated in recalls and outbreaks linked to *Salmonella* and pathogenic *Escherichia coli*. An instructional online video posted on a popular YouTube channel with over 12 million subscribers claimed that safe raw cookie dough could be made from flour baked in a household oven at 177°C (350°F) for five min but no evidence in support of that claim was provided.

Purpose: This study was conducted to assess thermal inactivation of two *Salmonella* strains, as well as *Enterobacter aerogenes* and *Pantoea dispersa* in wheat flour during home-oven style baking.

Methods: Wheat flour was inoculated with *Salmonella* Enteritidis PT 30, *Salmonella* Typhimurium PT 42 or their potential surrogates at ~six log CFU/g before baking in household oven at 149, 177, and 204°C (300, 350, or 400°F) for up to seven min. Flour was heated in aluminum tray in a ~two cm layer. Baked wheat flour samples (five g each) were enumerated in triplicate, and microbial concentration was expressed in log CFU/g. Thermal profiles of wheat flour and air in the oven during the baking were recorded. Water activity of wheat flour samples were also measured before and after baking.

Results: Water activity of wheat flour decreased from ~0.65 as baking time increased. Water activity ranged from 0.4 to 0.2 after five min as oven temperature increased from 149 to 204°C. Inactivation kinetics were linear until counts approached the detection limit for all microorganisms. Inactivation rates ranged from 0.51 to 0.55 log CFU/min at 149°C, 0.65 to 0.72 log CFU/min at 177°C, and 1.03 to 1.14 log CFU/min at 204°C. Both *Salmonella* strains and surrogates showed similar inactivation patterns.

Significance: Baking of wheat flour in household toaster ovens has potential as an inactivation treatment of pathogenic bacteria in consumers homes, despite its low water activity.

T10-11 WITHDRAWN

T10-12 Evaluating Uncertainty and Variability Associated with *Toxoplasma gondii* Survival While Cooking and Freezing Fresh Cut Meats

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❖ ***Developing Scientist Entrant***

Introduction: *Toxoplasma gondii* is a widespread zoonotic parasite with high seroprevalence in the human population. More than 40 million people carry this parasite in the United States. Consumption of raw or undercooked meat containing *T. gondii* tissue cysts is a major source of infection in humans. Cooking and freezing processes are the most important steps to inactivate *T. gondii* in meat.

Purpose: Current available data are not sufficient to suggest safe cooking or storage temperatures and holding time for fresh cut meats. Consumer preferences of cooking and storing meats introduce variability. The main objective of this study was to analyze uncertainty and variability in the survival pattern of *T. gondii* tissue cysts in fresh meat after cooking and freezing processes.

Methods: In the United States, there has been only two studies done on heat inactivation of *T. gondii* tissue cysts and one on its inactivation by freezing. Data regarding survival time of tissue cysts and treatment temperature were collected from those studies and were re-sampled with a bootstrapping method in R software. Monte Carlo simulation was used to quantitatively simulate uncertainty and variability associated with parameters.

Results: The results showed a negative correlation (r=- 0.992) between cooking temperature and survival time for *T. gondii* whereas positive correlation (r=0.9989) was observed between freezing temperature and time. All correlations were highly significant at a P-value of 0.05. Regression models were established with correlation coefficients and confidence intervals. The uncertainty level was further decreased by the jackknife-after-bootstrap method which identified outliers and narrowed down the bootstrap confidence interval by 16.7% for the cooking process.

Significance: This study could be helpful in validating the current USDA recommended minimum cooking temperature for fresh cut meats and establishing a survival module for *T. gondii* under a temperature gradient.

T11-01 Desiccation in Oil Protects Bacteria in Thermal Processing

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❖ ***Developing Scientist Entrant***

Introduction: The protective effect of lipid oil on bacteria in thermal treatments was first reported in 1913. Since then, researchers attempted to understand its mechanism without reaching a conclusive explanation. Recent studies on low-moisture foods have shown a strong correlation between increased heat tolerance of vegetative bacteria and low water activity of food matrices in thermal treatments. Our recent research also verified that the a_w in plant oil decreases sharply with increasing temperature. Thus, we hypothesize that the thermal dynamic equilibration of water vapor between vegetative bacterial cells and oil causes desiccation of the bacteria in thermal treatments, which may explain the oil protection phenomenon.

Purpose: To prove the hypothesis that desiccation in oil protects bacteria against thermal treatment.

Methods: Peanut oil inoculated with *Enterococcus faecium* NRRL B-2354 was preconditioned under controlled humidities, the bacterial thermal death times at 80°C were tested using two methods: in closed-system (capillary tube) and humidity-controlled environment (TAC), at 14 a_w levels each with three biological independent replications. Thermal death curves in all tests were first-order; D-values were calculated and plotted logarithmically against high-temperature a_w of peanut oil in capillary tubes or that of the head-space relative humidity in TACs.

Results: The log D-values from the two methods are in good agreement, showing a linear relationship with a_w (at 80°C) between 0.1 and 0.6. Our results are quite comparable to a previous study on the relative humidity dependent heat resistance of *E. faecium* inoculated on sand, indicating that water activity is the key factor that determines the bacterial thermal resistance, regardless of the carrier.

Significance: The results support the hypothesis that oil protection is caused by desiccation of bacterial cells in oil at high temperatures.

T11-02 Key Factors Influencing Thermal Resistance of Bacterial Pathogens in Low-moisture Foods

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❖ ***Developing Scientist Entrant***

Introduction: Microbial safety associated with low-moisture foods (LMFs) is an emerging issue. The food industry is experiencing challenges in seeking solutions to control *Salmonella* due to its high tolerance to thermal treatments. The heat resistance of bacterial pathogens in different LMFs is also difficult to predict, because of several confounding factors. Water activity is commonly reported in studies on thermal resistance of bacterial pathogens in LMF. But water activities of foods change with temperature, the degree of the changes depends on compositions.

Purpose: This presentation summarizes results from our recent research to elucidate the important role of water activity of food matrices measured at treatment temperatures (not room temperature) on thermal inactivation of *Salmonella* and a surrogate, *E. faecium,* in LMF.

Methods: Our research consisted of two major components: i) determining water activity of different food matrices (powders with different composition and peanut oil) to establish relationships between product moisture content, water activity, and temperature (from 20 to 80℃) in sealed containers; ii) correlating thermal resistance (D-values) of *Salmonella* and *E. faecium* in LMFs with relative humidity (water activity) at high temperatures.

Results: Water activity of foods rich in protein and starch increased sharply with increasing temperature, whereas foods with high oil contents did not increase much or even decreased with temperature. But regardless of the food matrices, D-value for *Salmonella* and *E. faecium* increased exponentially, by up to 100-fold, with reduction of water activity (measured at treatment temperature) from 0.7 to 0.2. The findings suggest that water activity of food matrices at treatment temperatures is a determinant factor on thermal resistance of bacterial pathogens.

Significance: Our results explain the difficulty in thermal inactivation of bacterial pathogens in oil-rich products and suggest that relative humidity at treatment temperature should be considered as a control parameter in designing effective thermal treatment operations for pathogen control in LMFs.

T11-03 Decontamination of *Salmonella enterica* in Low-moisture Foods by Cold Atmospheric Plasma

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❖ ***Developing Scientist Entrant***

Introduction: Cold atmospheric plasma (CAP) offers a dry, non-thermal, and rapid process for surface decontamination of food products.

Purpose: The purpose of this study was to evaluate the effects of treatment time, treatment distance from the plasma actuator, and pre-conditioning of the product on the inactivation efficiency of CAP on *Salmonella enterica* on low-moisture foods.

Methods: In-shell pecans (in triplicate) and black peppercorns (one g in triplicate) were spot-inoculated with a mixture of five strains of *S. enterica* (10^7 CFU/ml), and air dried. The inoculated pecans and black peppercorns were treated by CAP for two, five, and 10 minutes at one, two, and five centimeters from the plasma actuator. Similarly, inoculated pecans and black peppercorns moistened after air-drying were treated by CAP for two and five minutes at two cm from the actuator. Experiments were repeated at least two times. Mean values of log reduction of *S. enterica* cells after treatments were compared using ANOVA.

Results: Treatment time had a significant effect on the reduction of the pathogen on both pecans and black peppercorns. With 10-minute CAP treatment, 4.04 and 3.63-log CFU reductions of *S. enterica* were observed at all distances on pecans and black peppercorns, respectively. Moistening of inoculated pecans or black peppercorns prior to treatment achieved an additional one-log reduction of the pathogen compared to the treatment without moistening.

Significance: These results show that CAP can be a viable and flexible technology for inactivation of foodborne pathogens on low-moisture foods such as tree nuts and spices.

Technical

T11-04 Microbiological Profile, Incidence and Behavior of *Salmonella* in Seeds Commercialized in Mexican Markets

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Introduction: Seed consumption has been increased in recent years due to the high nutrient content. Unfortunately, the number of outbreaks caused by *Salmonella* associated with the consumption of low water activity food items has also increased although they do not support microbial growth.

Purpose: The main goal in this study was to quantify microbial indicators and to determine the prevalence and behavior of *Salmonella* in chia, amaranth and sesame seeds obtained from Mexican markets.

Methods: Sixty-five samples of each product (chia, amaranth and sesame seeds) were collected from Querétaro city markets. Aerobic plate count (APC), coliforms and *Escherichia coli* were quantified, and the presence of *Salmonella* spp. was determined according to the methods proposed by the Food and Drug Administration Bacteriological Analytical Manual. Chia, amaranth and sesame seeds (one kg) were inoculated with a cocktail of five *Salmonella* strains (five log CFU/ml) and stored at ambient temperature. The population of *Salmonella* was quantified for up to 70 days.

Results: The median content of APC in chia, amaranth, and sesame seeds were 2.3, 2.5, and 3.9 log CFU/g, respectively. The content of coliforms oscillated from 0.5 to 1.4 log MPN/g. *E. coli* positivity was 3% in chia, 6.1% in amaranth and 9.2% in sesame seeds. *Salmonella* was detected in 18.5, 10.8, and 13.9% of chia, amaranth, and sesame seeds, respectively. After 70 days of storage, the population of *Salmonella* reduced 1.1 log CFU/g in chia, 1.3 log CFU/g in amaranth, and 1.5 log CFU/g in sesame seeds.

Significance: The incidence of *Salmonella* in the seeds was high, which could represent a risk for consumers, specifically when the food is consumed without treatments that guarantee the pathogen inactivation. Is necessary to develop technologies to control foodborne pathogens in low water activity food such as seeds.

T11-05 Survival of *Salmonella* and Surrogate Microorganisms in Whole Wheat and All Purpose Flour during Long-term Storage

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Introduction: Flour may become contaminated at numerous points during the wheat harvesting and flour production processes. There have been several recalls and outbreaks associated with wheat flour and wheat products, but little is known about microbial behavior in wheat flour during long-term storage.

Purpose: This study assessed survival of *Salmonella enterica* and two nonpathogenic surrogates (*Enterobacter aerogenes* and *Pantoea dispersa*) in both whole wheat and all-purpose flour.

Methods: Whole wheat or all-purpose flour was inoculated with nalidixic acid resistant *Salmonella* Enteritidis PT 30, *Salmonella* Typhimurium PT 42 or their potential nonpathogenic surrogates (~five log CFU/g). Inoculated flour samples were stored at 21°C for 27 weeks in tightly closed containers, and five-g samples were enumerated on tryptic soy agar supplemented with nalidixic acid at appropriate time intervals. All experiments were conducted in triplicate and microbial populations were expressed in log CFU/g.

Results: *Salmonella* showed better survival than *E. aerogenes* and *P. dispersa* in both whole wheat and all-purpose flour over the storage period. *Salmonella* in flour could be enumerated for at least 17 weeks, with average declines of 0.13 to 0.19 log CFU/week depending on *Salmonella* strain and flour type. Non-pathogenic surrogate populations could be enumerated for at least nine weeks of storage, with average declines of 0.23 to 0.38 log CFU/week depending on surrogate strain and flour type. Native microflora (2.7 to 3.7 log CFU/g) and mold (2.2 to 3.0 log CFU/g) concentrations in both whole wheat and all-purpose flour remained consistent during storage. There was no significant difference between native microbial populations in whole wheat and all-purpose flour.

Significance: This study shows *Salmonella* can survive for prolonged periods in the wheat flour. A suitable surrogate for *Salmonella* survival in flour was not identified. These findings will be useful for future flour microbial risk assessments and will add to the growing literature on *Salmonella* behavior in low-moisture foods.

T11-06 Studies of Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus* on Ground Flax Seeds

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Introduction: Flax seed has gained popularity as a human food supplement and animal feed because of its richness in protein, dietary fiber and omega-3 fatty acids. It has been reported that flax seed supports fungal growth and mycotoxin production during storage.

Purpose: This study investigated the effect of water activity and temperature on growth and aflatoxin production by *Aspergillus flavus and A. parasiticus* on ground flax seeds.

Methods: Sterilized ground flax seeds (2.5 g) adjusted to 0.82, 0.86, 0.90, 0.94, and 0.98 water activity were placed in separate triplicate plates. Each plate was spot inoculated with 10 µl spore suspension (10^6 to 10^7 conidia/ml) at 20, 27 or 35°C for 30 days in closed glass jars. Fungal growth was measured and levels of aflatoxins in seeds were determined after incubation for five, 10, 15, 20 and 30 days. Total aflatoxins (B1, B2, G1 and G2) were determined using high-performance liquid chromatography after each sample was purified by an immunoaffinity column.

Results: Highest level of total aflatoxin production by *A. flavus* was 298 µg/kg at 35°C with 0.90 a_w while *A. parasiticus* produced 364 µg/kg at 35°C with 0.98 a_w. Both fungal strains didn't grow at 0.82 a_w. Linear regression models showed that 35°C and water activity levels of 0.90 to 0.94 a_w were optimal for *A. flavus* growth and its total aflatoxin production while *A. parasiticus* had the same optimal temperature for growth (35°C) as *A flavus* but 0.94 a_w was the least favored water activity for its aflatoxin production.

Significance: Ground flax seed supports the growth of *A. flavus* and *A. parasiticus* as well as the production of aflatoxins in a narrow range of water activity and temperature. The data collected from this study can be used to establish storage standards of ground flax seed to minimize the production of aflatoxin.

T11-07 Influence of the Germination Time on Aflatoxins Production during Malting of Wheat for Use in Craft Beer

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Introduction: Mycotoxins are fungal secondary metabolites with harmful effects on humans and animals. They can be produced in contaminated grains if toxigenic fungi are present and conditions are favorable. When wheat is malted for use in craft beer, grains are immersed in water and kept moist for extended times before kilning. The ability of fungi to produce mycotoxins during wheat malting is unknown.

Purpose: This study evaluates the production of aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) during wheat malting under typical conditions used by craft beer producers.

Methods: Malting was performed according to Central European Commission for Brewing Analysis procedures. Steeping processes includes extended periods of alternating water immersion and aeration. Aflatoxin free, toxigenic fungi-free wheat grains (600 g) were inoculated in the first water immersion step by immersing in a suspension of *Aspergillus flavus* (WG06-A, five log spores/ml), known to produce AFB_1 and AFB_2 originally isolated from wheat grains. Grains were germinated at 15°C for 48, 72, 96 and 120 h. Germinated grains were kilned (16 h at 50°C, one h at 60°C, one h at 70°C and five h at 80°C) and the rootlets removed. Duplicate samples of wheat malt obtained from each time were collected for determination of AFB_1 and AFB_2 (dry basis) by HPLC (detection limit 0.03 µg/kg AFB_1; 0.02 µg/kg AFB_2; quantitation limit 0.09 µg/kg AFB_1; 0.07 µg/kg AFB_2).

Results: The highest levels of both AFB_1 (160.07±0.99 µg/kg) and AFB_2 (4.52±0.21 µg/kg) were detected in grains germinated for 96 h. The lowest levels of AFB_1 (103.67±11.97 µg/kg) and AFB_2 (0.48±0.21 µg/kg) were detected in grains germinated for 48 h.

Significance: Malting germination time influences the amounts of AFB_1 and AFB_2 produced in the wheat malt, and the levels aflatoxin produced exceed Codex Alimentarius recommendations for a variety of foods. This should be considered by craft beer manufacturers using malted wheat.

T11-08 Rapid Identification of Lineage Types and Phylogenetic Relationships of *Clostridium botulinum* strains by Whole Genome Sequencing

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Introduction: Whole Genome Sequencing (WGS) allow studying the phylogenetic relationship of clostridia strains. Recent developments with WGS are moving towards the metagenomic level of characterization and may be a viable tool to perform source track investigations for botulism outbreaks as well.

Purpose: We sequenced the whole genome of 13 unknown *C. botulinum* strains to study their phylogenetic relatedness and lineage types through SNP analysis.

Methods: *C. botulinum* strains were grown and genomic DNA was isolated using DNeasy blood and tissue kit. Identification of the strains belonging to *C. botulinum* Group I (proteolytic) or Group II (non-proteolytic) was performed by a PCR assay. Strains were sequenced using a MiSeq (Illumina) with the 250 bp pair-end reads chemistry according to manufacturer's instructions at 140X coverage. CLC Genomics Workbench software version 8.0.2 (QIAGEN) was used for *de novo* assembly and genome sequence analysis. The phylogenetic analysis was performed using a core genome multilocus sequence typing approach (cgMLST) using Ridom SeqSphere+ v. 2.4.0 software.

Results: In Silico MLST of the 13 sequenced genomes revealed that three of them belonged to non-proteolytic *C. botulinum* group II. The other 10 strains belonged to six different STs. One new ST was identified (ST88) for five of the strains. These five strains have an identical profile than Langeland strain type F and carried a single BoNT cluster (BoNTF). The remaining five strains were toxin bivalent strains and belonged to known STs (6, 10, 22, 7, and 4). An SNP analysis (using 21 additional *C. botulinum* group I genomes available at NCBI) showed that these 10 strains belonged to different *C. botulinum* group I lineages, with the majority (five) belonging to Lineage 3, and matched closely with strains 230613 and Langeland, both BoNTF strains.

Significance: Our results showed that the use of WGS in combination with the proper use of bioinformatic tools allowed for fast identification, phylogeny, and toxin cluster determination for unknown *C. botulinum* samples.

T12-01 Maternal Dietary Risk Factors for Neural Tube Defects in Guatemala

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Introduction: Mycotoxins are important foodborne pathogens involved in the parthenogenesis of various human and animal health outcomes. In particular, the consumption of foods contaminated with fumonisins has been associated with liver cancer and congenital malformations, such as neural tube defects (NTDs). Fumonisins are found in the diets of a large proportion of the world's population where maize is a dietary staple; low- and middle-income countries are disproportionately and continually exposed to high quantities.

Purpose: The objective of this case-control study was to assess the relationship between maternal exposure to fumonisin via intake of contaminated foods, particularly maize-based food, and NTDs in newborns in Guatemala.

Methods: A total of 545 infants (109 NTD cases, 436 controls) were enrolled at birth in two Guatemalan departments (Guatemala and Alta Verapaz) from January to October 2014. Data were collected about maternal demographics, socioeconomic status, prior birth history, and gestational age. Types and amounts of foods consumed in the previous week were estimated using a food questionnaire. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated through multivariate conditional logistic regression to assess the relationship between NTD and dietary intake.

Results: Maternal stillbirth history and consumption of tamales, tamalitos and onions were significantly different between cases and controls. After adjusting for covariates, consumption of boiled corn (OR 1.22; 95% CI: 1.01 to 1.47) and beef (OR 1.10; 95% CI: 1.02 to 1.17) significantly increased odds of NTDs. Each additional food item consumed (OR 0.93; 95% CI: 0.88 to 0.97) decreased odds of NTDs.

Significance: The inverse association of diet diversity and positive association of maize and beef consumption with risk for NTDs suggests further research is needed to more accurately examine the association of maternal diet with NTDs. In particular, studies that measure fumonisin biomarkers in mothers and test for fumonisins in foods are needed.

T12-02 A Summary of Foodborne Illness Outbreaks Investigated by FDA's Coordinated Outbreak Response and Evaluation Network from January 2011 to December 2018

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Introduction: The Coordinated Outbreak Response and Evaluation Network (CORE) of the Food and Drug Administration (FDA), formed in August 2011, is a multidisciplinary team that evaluates, investigates, and guides prevention efforts of foodborne illness outbreaks. Working with federal, state, and local colleagues, CORE has responded to 170 outbreaks attributed to FDA-regulated products.

Purpose: The purpose of this analysis is to display and summarize FDA CORE's data while providing transparency on the work FDA CORE is doing for public health as well as to highlight CORE's accomplishments.

Methods: The 170 outbreaks, occurring between January 2011 to December 2018, were analyzed by product category, year, pathogen, number of illnesses, hospitalizations, and deaths using data from the FDA CORE database.

Results: The analysis established that between January 2011 and December 2018, CORE has responded to 170 U.S. foodborne illness outbreaks resulting in an estimated total of 10,484 illnesses, 2,385 hospitalizations, and 100 deaths. Most of the outbreaks were attributed to produce (n=69), followed by seafood (n=25) and processed food (n=22). Illnesses were also predominantly caused by outbreaks associated with produce (n= 6270), followed by seafood (n=970) and processed foods (n=940). Sixty-three deaths were attributed to outbreaks associated with produce, followed by dairy (n=20). These deaths are primarily caused by foods contaminated with *Listeria monocytogenes* (72%), whereas most of the illnesses are attributed to *Salmonella* infections (54%). In response to these outbreaks, CORE has worked in partnership with the CDC, USDA, all five FDA regional offices, and 20 FDA district offices. CORE also allied with at least 75 State public health/agriculture agencies, as well as international partners.

Significance: A coordinated effort to respond to foodborne illness outbreaks enables FDA CORE to streamline and quickly identify, respond to, and prevent outbreaks. CORE continues to collaborate with its federal, state, and international partners to refine its processes to efficiently detect, investigate, and prevent outbreaks to secure a safer food supply.

T12-03 When Unregulated Food Sales Go Wrong: *Clostridium perfringens* from a Church Fundraiser in North Carolina

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Introduction: Unregulated food sales in North Carolina are regularly carried out by non-profit groups, and although legal exemptions within the NC General Statutes allow food sales once per month without food service permit requirements, foodborne illness outbreaks do occur because of challenges such as inadequate equipment and untrained volunteer employees.

Purpose: A foodborne illness outbreak investigation was conducted after an initial report of illnesses from a church barbecue fundraiser in November 2018 by a multi-jurisdictional team including state and local communicable disease nurses, epidemiologists, and environmental health specialists.

Methods: The case findings were obtained through a press release by the local health department, with all persons experiencing diarrhea or vomiting identified as a case. A cohort study was performed to determine the likely cause of the outbreak using an online survey protocol. Environmental assessments and laboratory testing of both food and stool samples were also conducted to complete the investigation.

Results: A total of 123 illnesses were reported from the online surveys, but 109 met the case definition. Foodservice took place between 6:00 am and 7:00 pm on November 1st, with 83% (90) illnesses beginning between 6:00 pm on November 1st and 6:00 am on November 2nd. The cohort study revealed that individuals who ate Brunswick stew were 7.2 times more likely to develop an illness. Laboratory testing on leftover Brunswick stew and stool samples from ill patrons showed *Clostridium perfringens* to be the organism which caused illness. Food samples had *C. perfringens* levels of greater than 106 CFU/g.

Significance: This outbreak illustrates potential risks associated with food exempt from evaluation and prepared by untrained volunteers; it highlights the need for a multi-jurisdictional approach to outbreak investigations. Educational interventions targeting non-profit groups holding food sale fundraisers are necessary to help prevent future outbreaks.

T12-04 Impact of Prospective Whole Genome Sequencing on Multi-jurisdictional *Salmonella* Outbreaks Associated with Frozen Raw Breaded Chicken Products in Canada

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Introduction: Frozen raw breaded chicken products (FRBCP) are a risk factor for salmonellosis, however, traditional laboratory sub-typing methods lack sufficient discriminatory power to identify outbreaks of common *Salmonella* serotypes associated with FRBCP and to confirm a microbiological association between clinical and food isolates. Whole genome sequencing (WGS) of *Salmonella* isolates was implemented by the National Microbiology Laboratory (NML) in Canada for outbreak detection in May 2017.

Purpose: To describe the impact of WGS on the investigation of FRBCP-associated outbreaks in Canada, public health action, and its contribution towards policy change.

Methods: Multi-jurisdictional outbreaks of *Salmonella* isolates within zero to 10 allele differences were identified using whole genome multilocus sequence typing (wgMLST). Epidemiologic data were assessed and analyzed to identify FRBCP as a likely vehicle of infection. WGS was used to establish a microbiological link between clinical cases and FRBCP isolates. Risk-based criteria were developed and applied to guide risk management determinations (e.g., product recall).

Results: Between May 2017 and December 2018, 15 multi-jurisdictional *Salmonella* outbreaks associated with poultry were investigated. Of the 12 (80%) outbreaks associated with FRBCP, there were 378 cases, 72 hospitalizations, and two deaths with an estimated burden of illness of 10,101. A link between clinical and FRBCP isolates was established in 10 (83.3%) investigations and 10 product recalls were issued for various FRBCPs.

Significance: WGS has improved the detection of *Salmonella* outbreak investigations associated with FRBCP. These investigations have led to public communication, food recall warnings, and a statement from the Canadian Council of Chief Medical Officers of Health emphasizing the importance of handling and preparing FRBCPs with caution. Furthermore, the accumulated evidence led to a new requirement that manufacturers of FRBCPS in Canada must reduce *Salmonella* to below detectable levels starting April 1, 2019. A reduction of *Salmonella* illnesses and outbreaks associated with FRBCPs is expected.

T12-05 Presence and Identification of *Campylobacter* spp. in East Tennessee Rivers

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter* spp. are one of the main causes of bacterial gastroenteritis in the United States. Campylobacteriosis is commonly associated with the consumption of poultry, meat, and raw milk; however, following a previous study, researchers quantified populations of *Campylobacter* spp. within one location of East Tennessee river water.

Purpose: The purpose of this study was to better understand the spatiotemporal distribution of *Campylobacter* spp. and further, elucidate surface water as a possible vehicle for campylobacteriosis by sampling multiple sites of the Tennessee River.

Methods: Samples (*n*=267) were collected from four public access river sites within East Tennessee for one calendar year (October 2017 to November 2018). Water samples (10 ml and 100 ml) were vacuum filtered through a 0.45 µm membrane filter. Filters were plated onto Campy CHROMagar and incubated at 37˚C for four h then 42°C for 44 h in a microaerophilic environment (5% O_2, 10% CO_2). All presumptive positives (*n*=244) were confirmed using PCR with targets for *Campylobacter* spp., *C. jejuni*, *C. coli*, and *C. lari*.

Results: From the 244 presumptive positive samples, 168 were confirmed as *Campylobacter* spp. and five as *C. coli*. All collection sites were associated with positive *Campylobacter* spp. Positives were 30 (79%) of 38 at site 1, 29 (76%) of 38 at site 2, 32 (89%) of 36 at site 3, and 77 (58%) of 132 at site 4. Five water samples were confirmed as *C. coli* from site 4, which were obtained from January to March.

Significance: These data indicate that *Campylobacter* spp. are present within the surface waters of the Tennessee River throughout East Tennessee. Further testing of positive samples will clarify if antibiotic resistance and commonly associated virulence factors exist and what these implications mean for food safety, prevention education, and future treatment options.

T12-06 Fate of Antibiotic Resistance in the Environment: From Beef Cattle Production through Manure Storage and Land Application

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❖ ***Developing Scientist Entrant***

Introduction: Knowledge gaps exist regarding the transfer of antibiotic-resistant bacteria from livestock to humans via environmental pathways, which hinders a systems assessment of the impact of antibiotic uses during food-producing animal husbandry on public health.

Purpose: The purpose of this study was to evaluate the survival and transfer of antibiotic-resistant bacteria and genes in the environment in a continuum of beef cattle primary production, cattle manure storage and land application.

Methods: Different in-feed antibiotic treatments (control, tylan and chlortetracycline) were introduced to beef cattle on feedlot (32 animals per treatment). Samples of rectal feces, hides and pen surface on feedlot (five months); manure during stockpiling (three months; 12 samples per pile per sampling); and amended the soil at land application sites (three months; 16 samples per site per sampling) were collected. Changes in prevalence and concentration of generic and macrolide- and tetracycline-resistant *E.coli*, *Salmonella* and *Enterococcus* were determined over the project lifespan. Antibiotic-resistant genes were characterized and quantified following shotgun metagenomics sequencing using the Illumina HiSeq platform.

Results: No statistically significant difference in antibiotic-resistant bacteria load and gene abundance was detected across antibiotic treatments throughout the study from beef cattle production to manure application. During the three-month period of manure storage as stockpiles, the concentration of generic *E.coli* and *Enterococcus* dropped from ~five log CFU/g to a maximum of two to three log CFU/g. Manure storage as static piles significantly reduced antibiotic-resistant bacteria and genes in three months.

Significance: Our results indicate antibiotic use during beef cattle production might not be associated with extra risk of contamination of antibiotic-resistant bacteria and genes in animal wastes and the following manure and amended soil. Stockpiling with sufficient time can effectively eliminate resistant bacteria and genes in manure before land application, highlighting the importance of manure management in controlling the transfer of antibiotic resistance through the environment.

T12-07 The Fecal Resistome of Dairy Cattle is Associated with Diet during Nursing and Weaning

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Introduction: Antibiotic-resistant bacteria display an age-dependent distribution in dairy cattle, in which younger calves harbor a higher abundance of resistance. The mechanism responsible for this resistome distribution is yet unclear.

Purpose: The study aimed to examine the hypothesis that the assembly of gut microbiome of dairy calves promotes the establishment of bovine-associated antimicrobial resistance genes (ARGs) and is driven, in part, by diet.

Methods: Fresh feces (*n*=484) were obtained from 22 dairy calves, from at birth to week 10 between April and September of 2015 at UC Davis Dairy Facility. Two colostrum samples were also collected per calf prior to feeding (*n*=44). DNAs were extracted from all samples for 16S-rRNA sequencing. In addition, selected fecal samples (*n*=12) from dairy calves (*n*=3) at four time-points per calf (day two, day five, week three and week seven), as well as the corresponding colostrum samples (*n*=6; two of each calf), were subjected to shotgun metagenomic sequencing.

Results: The intestinal microbiome and resistome assembled rapidly in newborn calves. A total of 329 ARGs conferring resistance to 17 classes of antibiotics were observed in dairy calves. The abundance of total ARGs declined markedly during nursing, however, some clinically-relevant ARGs encoding resistance to macrolides-lincosamides-streptogramins and tetracyclines increased throughout this period. ARGs in dairy calves was found to co-occur with antibacterial biocide/metal resistance genes. Colostrum was predicted to be the source of over 90% of ARGs observed in dairy calves on day two. The early succession of resistome in dairy calves is a result of gut microbiome assembly associated with the dietary transition, in particular, from colostrum to milk replacer and increased intake of calf starter.

Significance: The assembly of gut resistome, in dairy cattle, is associated with diet during early life. Identifying the drivers influencing resistome assembly may inspire targeted interventions of the gut microbiome through food modifications to reduce the overall prevalence of antibiotic resistance.

T12-08 Viability-linked Metagenomic Analysis of the Disposable Glove Microbiome

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Introduction: Previous studies on new unused disposable gloves (DG) employed in healthcare have shown considerable microbial contamination. Metagenomic analysis of gloves utilized in food processing facilities has shown cross-contamination potential with various foodborne pathogen indicators including *Listeria* spp. and *Yersinia spp*.

Purpose: Limited microbial information is available on DG utilized in food processing/service when new or in production environments. An Eagle Protect PBC risk reduction program required establishment of baseline information in order to create food relevant test methods & standards.

Methods: Appropriate methods were surveyed and trialed with three DG types (two nitrile and one vinyl) sold for food use. DGs were sampled in pools of 50 each (*n*=8), concentrated with aerobic (APC) and anaerobic (AnPC) plate counts performed including culture for *Staphylococcus*, mesophilic spore-formers, yeast and mold (Y&M) prior to metagenomic analysis. Using the NSF method in parallel total viable counts (TVC) were defined as APC plus Y&M counts.

Results: A total of eight different genera were found in the metagenomic analysis of the three different gloves associated with both inside and outside (food contact surfaces). Using pooled metagenomic data it was determined that microbial profiles were different for inside and outside surfaces for each glove sampled, reflecting manufacturing steps. Geometric mean TVC for outside surfaces were one log CFU/glove while inside surfaces were 1.2 log CFU/glove. This contrasts with extremes of greater than three CFU/glove in published reports. It was determined that the optimal DG profile of non-detect for all of the microbial groups tested, including both inside and outside of gloves is possible but unless carefully controlled, could represent a safety hazard.

Significance: This is the first known report of its kind with test methods trialed that examines the glove microbiome indicative of DG manufacturing that can allow survival of potential microbial contaminants on both inside of glove (user relevant) and on outside food contact surfaces.

Poster Abstracts

P1-01 Sterilization of Food Contact Surfaces Using Chlorine Disinfectants to Control Planktonic Cells and Biofilms of *Salmonella* spp.

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Introduction: *Salmonella* infection is a frequently occurring foodborne illness worldwide. Contamination on equipment in production lines by *Salmonella* jeopardizes the microbiological quality of finished products. Chlorine disinfectants such as chlorine dioxide (ClO_2) and sodium hypochlorite (NaOCl) are frequently used for reducing foodborne pathogens effectively. Therefore, these disinfectants can be applied to inhibit *Salmonella* spp. on food-contact surfaces.

Purpose: The current study examined the efficacy of two disinfectants (ClO_2 and NaOCl) against *Salmonella* spp. for the reduction of biofilm formation on food contact surfaces.

Methods: First, three isolates each of *Salmonella* Enteritidis, Kentucky, and Typhimurium were selected and incubated for 24 h. We tested planktonic cells using ClO_2 (30 to 40 and 30 to 180 ppm for clean and dirty conditions, respectively) or NaOCl (50 to 150 and 75 to 300 ppm for clean and dirty conditions, respectively) for one or five min. After forming biofilms, food-contact surfaces (stainless steel, silicon rubber, and plastic coupon) were treated with disinfectant (ClO_2 at 10 to 100 ppm or NaOCl at 50 to 150 ppm) for one or five min.

Results: When these disinfectants were applied to *Salmonella* spp., there were no differences among bacteria. In planktonic cells, there was different sterilizing power between clean and dirty conditions at same concentration, and by treatment time as well. The biofilm of *Salmonella* on food-contact surfaces was decreased rapidly when ClO_2 concentration (four and five-log reductions for one and five min at 100 ppm, respectively) or NaOCl concentration (three and 3.5-log reductions for one and five min at 300 ppm, respectively) increased.

Significance: *Salmonella* was effectively reduced at lower concentrations of ClO_2 than NaOCl in both planktonic and biofilm cells. This result suggests that ClO_2 is more effective for inhibition of *Salmonella* contamination thorough food manufacturing processes than NaOCl.

P1-02 Validation of the Rapidchek Select *Salmonella* Test Method for the Detection of *Salmonella* species on 12” by 12” Stainless Steel Environmental Surfaces

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Introduction: Raw meat and poultry are important vehicles of transmission of *Salmonella*. Control measures, like environmental monitoring, implemented along food production lines in meat and poultry abattoirs help reduce the levels of *Salmonella* in these matrices; however, microbiological testing retains a key role in preventing food borne salmonellosis.

Purpose: To validate test method stainless steel surfaces by demonstrating equivalent performance to the FDA BAM reference method for the detection of *Salmonella* spp. in environmental surfaces.

Methods: Stainless steel surfaces (12” x 12” (test method) and 4” x 4” (FDA BAM method)) were inoculated with *Salmonella derby* both with and without *Citrobacter freundii*. For each matrix and method, 20 low-level inoculated, 5 high-level inoculated, and 5 negative control samples were tested. After swabbing, sponges were held for 2 hours before enriching. Test method samples were enriched in 225mL primary media, transferred to secondary media, evaluated with test strips and plated on selective agar. FDA BAM reference method samples were enriched in 225mL lactose broth, transferred to TT and RV media, and plated on selective agar following the FDA BAM method Chapter 5.

Results: Without *C. freundii*, the test method resulted in 18 confirmed positives and the reference method resulted in 15 confirmed positives. With *C. freundii*, the test method yielded 19 positives while the reference method yielded 10 positives. For both methods, non-spiked surfaces were negative for *Salmonella* and all high-spiked samples were positive. Probability of Detection (POD) analysis demonstrated a statistically significant difference at the 5% level in the number of positive samples detected by the test method and the FDA reference method on stainless steel surfaces spiked with *Salmonella* Derby and *C. freundii*.

Significance: The RapidChek® SELECT *Salmonella* method offers a rapid and reliable tool for testing 12” x 12” stainless steel surfaces for *Salmonella* species.

P1-03 One Mississippi, Two Mississippi: Phylogenetic Analysis Supports That *Salmonella enterica* subsp. e*nterica* Serovar Mississippi is Polyphyletic

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Introduction: *Salmonella enterica* subsp. e*nterica* serovar Mississippi is the 14th most commonly isolated serovar from human clinical cases of salmonellosis in the United States (CDC, 2016). Initial analyses using seven-gene MLST data suggested that this serovar was polyphyletic.

Purpose: The goal of this study was to characterize the phylogenetic structure of *Salmonella* Mississippi, and to identify the presence of antimicrobial resistance and virulence-associated genes in a collection of *Salmonella* Mississippi isolated from human clinical infections.

Methods: Whole genome sequence (WGS) data for 65 *Salmonella* Mississippi isolates were either downloaded from NCBI or were sequenced for this study. Raw sequences were trimmed with trimmomatic, assembled with SPAdes, and single nucleotide polymorphisms (SNPs) were called with kSNP3. Serotype information was confirmed using sistr. RAxML was used to construct a phylogenetic tree of core SNPs. BLAST searches were performed to identify target genes including antimicrobial resistance (AMR) and virulence-associated genes.

Results: WGS data support the presence of two phylogenetic clades of *Salmonella* Mississippi. Typhoid toxin genes were unique to *Salmonella* Mississippi isolates in clade A, which clusters with other NTS serovars *Salmonella* Javiana, Montevideo, Schwarzengrund, and Cubana, all of which are also typhoid toxin-positive. Clade B strains, which clustered with serovar *Salmonella* Typhi, were all missing *avrA,* encoding a SPI-1 effector associated with intracellular survival. AMR genes were present in eight (12%) of 65 isolates , with eight isolates encoding β-lactamase resistance genes, two isolates encoding genes associated with resistance to sulphonamides and aminoglycosides, and one isolate encoding rifampicin resistance genes. There was no relationship between the presence of an AMR gene and the clade that the isolate was in.

Significance: Using *Salmonella* Mississippi as an example, our study highlights the public health importance of characterizing polyphyletic serovars, as the distribution of virulence-associated genes may enable select clades within a polyphyletic serovar to cause a more severe illness.

P1-04 Heat Inactivation of *Listeria monocytogenes* on Pecans, Macadamia Nuts, and Sunflower Seeds

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Introduction: *Listeria monocytogenes* is a major concern for the food industry in RTE foods. In the last two years, large-scale recalls occurred with contaminated sunflower seeds and macadamia nuts that triggered product withdrawals. These recent events stress the importance of understanding *Listeria's* ability to survive heat treatments in these low water activity foods.

Purpose: This project was undertaken to determine the kinetic parameters of thermal inactivation of *L. monocytogenes* on pecans, macadamia nuts and sunflower seeds subjected to heat treatments simulating industry processes.

Methods: Five *L. monocytogenes* strains (serovar 4b) were grown overnight in liquid or agar non-selective media, mixed and re-suspended before inoculating macadamia nuts, pecans and sunflower seeds (6 to 9 log CFU/g). Re-dried inoculated pecans and macadamia nuts were heated in an oven at a temperature range of 90 to 140ºC. Unshelled sunflower seeds were heated in sunflower seed oil. The thermal inactivation was determined by measuring viable cell count using standard microbiological methods. Average count data were fit to log-linear model and thermal death kinetics were calculated.

Results: On pecans, the count was reduced three and 3.5 log CFU/g after 40 min at 110ºC and eight min at 140ºC, respectively. On macadamia nuts, the *L. monocytogenes* population was reduced by five log CFU/g after 20 min at 120ºC. Unshelled sunflower seeds were subjected to heat treatment via a hot oil bath. On sunflower seeds reductions greater than 7 log CFU/g were observed after 15 minutes at 120ºC. The *D*-value of inactivation on pecans at 140ºC was 3.1 min and on macadamia nuts at 120ºC was 4.4 min. Inactivation of *L. monocytogenes* was influenced by the kind of the nut or seed.

Significance: The findings from this study will contribute to assess the effectiveness of heat treatment for the control of *Listeria monocytogenes* on nuts and seeds.

P1-05 Culture Supernatants of *Lactobacillus plantarum* Reduces Sporulation, and Biofilm Formation, of *Clostridium perfringens* by Downregulating Transcription of Agr-like Quorum Sensing Genes

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❖ ***Developing Scientist Entrant***

Introduction: Bacteria form biofilms and spores to protect themselves in the environment. In the Gram-positive bacterium *Clostridium perfringens*, an Agr-like Quorum Sensing (QS) system (CpAL) regulates transcription of these virulence factors. Compounds from probiotics have been used as antimicrobial agents and to interfere with expression of virulence factors.

Purpose: Evaluate the effect of supernatants from lactic acid bacteria on growth, biofilm/spore formation and transcription of CpAL genes of *C. perfringens*.

Methods: The minimal bactericidal concentration (MBC) of supernatants (freeze-dried cell-free supernatant) from cultures of *L. plantarum* (SLP) was determined by microdilution method. The effect of a sublethal concentration (SC) of SLP on biofilm formation index (BFI), and sporulation, was assessed by a colorimetric method, and on Duncan Strong medium, respectively. RNA from biofilms, or sporulating cultures, was extracted using the Trizol-Chloroform protocol and cDNA generated with the iScript cDNA synthesis kit (BioRad). For qPCR reactions iQ™ SYBR Green Supermix (BioRad) and were run in PikoReal system and the $2^{-\Delta\Delta CT}$ was determined. For data analysis, the Number Cruncher Statistical System version 6.0 software (NCSS, LLC) was used.

Results: The MBCs of SLP against two strains of *C. perfringens* was 121µg/ml. SC of SLP (24 mg/ml) reduced the BFI between 60% to 40% for FD-1041, and FD-1, when compared to the untreated control respectively. In general, SC of SLP delayed spore formation by 2 h of strain FD-1041, and slightly reduced the amount of spores in *C. perfringens* (0.7 log at 24 h). Transcription of *agrB*, *agrD* and *Spo0A* gene was downregulated during the first 4 h post-inoculation. In conclusion, SLF killed vegetative cultures of *C. perfringens*. A sublethal dose of SLP reduced biofilm and spore formation, in part, by downregulating transcription of *agrB*, *agrD* and *Spo0A*.

Significance: SLP could be used to control *C. perfringens* and improve the safety of foods.

P1-06 A Pilot Study Evaluating Oxford Nanopore Sequencing Technology for *Salmonella* Serotype Prediction

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Introduction: Whole genome sequencing (WGS) has been shown to provide reliable results for predicting *Salmonella* serotypes, which are critical to incident investigation in the food industry. An emerging sequencing platform developed by Oxford Nanopore Technologies (ONT) provides an alternative method of WGS via Illumina. Advantages of the ONT system include portability and real-time long reads, which could meet the need for effective and efficient *Salmonella* identification.

Purpose: This pilot study aimed to initiatively explore the potential of *Salmonella* serotype prediction using WGS data generated by the ONT sequencing system.

Methods: Genomic DNA were extracted from pure cultures of 24 *Salmonella enterica* strains (17 serotypes) using Qiagen DNeasy Blood & Tissue Kit. Rapid kit were used for library preparation. DNA sequencing was performed using the ONT sequencer and R9.4 flow cell for 12 to 48 hrs. Data analysis was performed by NanoPlot, Porechop and Canu sequentially, using fastq files generated by MinKNOW. The same isolates were also sequenced by Illumina Hiseq (200 coverage) for comparison. SeqSero2 and SISTR were used for serotype prediction.

Results: The quality score and high-quality data percentage were found to decline over the sequencing time and sequences generated within the first two hours were determined to be sufficient for serotype prediction. The size of two hours data ranged from 201 to 1072 MB (42 to 203 coverage), and the mean read lengths ranged from 3,589 to 11,721 bp. After assembly, consensus serotype predictions were obtained from SeqSero2 and SISTR for all 24 isolates using the ONT sequencing data. All predictions were identical to the corresponding results generated by Illumina.

Significance: This pilot study indicated that the prediction using sequencing data generated by the ONT system is comparable to results obtained from Illumina. The procedure created in this study can be used as a reference in future systematic validation of identification methods.

Poster

P1-07 *Enterococcus faecium* NRRL B-2354 as a *Salmonella* Surrogate in Validating Thermal Treatment of Dairy Powders with Different Lactose and Milk Protein Compositions

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* outbreaks associated with dairy powders are a global concern since there is generally no post-drying kill step. However, the development and validation of thermal treatments for dairy powders using a nonpathogenic strain could help to reduce the risk of foodborne illness.

Purpose: This study compared the thermal resistance of *Enterococcus faecium* and a *Salmonella* cocktail *(Salmonella* Agona*,* Reading, Tennessee, Montevideo, and Mbandaka) in skim milk powder (SMP), lactose-free skim milk powder (LFSMP), lactose powder (LP), and milk protein isolate (MPI) at 0.25 water activity (a_w).

Methods: Dairy powders (100 g) were inoculated with one ml of either an *E. faecium* or *Salmonella* cocktail, hand-massaged in a Whirl-Pak bag for three min, and equilibrated to 0.25 a_w (24 h). Using a pestle, visible clumps were ground to obtain a homogenous powder that was reequilibrated for one to two days. After confirming the homogeneity of the inoculum, one-g samples were packed into aluminum test cells and subjected to three time-temperature treatments in a water bath at 65 to 90°C to achieve reductions of three to five log. Survivors were then enumerated on a non-selective differential medium after 24 h of incubation.

Results: *E. faecium and Salmonella* demonstrated similar (P>0.05) thermal resistance in LFSMP ($D_{70°C\text{-}Ef}$ 14.5±0.4 min; $D_{70°C\text{-}Sal}$ 15.6±0.4 min). Based on a $D_{T\text{-}80°C}$, *E. faecium* was more resistant (P<0.05) in LP (33.1±4.8 min) than in LFSMP (2.6±0.1 min), however no difference in z-values ($z_{T\text{-}LP}$ = 15.3±1.4°C; $z_{T\text{-}LFSMP}$ = 13.7±0.3°C) was observed.

Significance: This study demonstrates that *E. faecium* can be used as a *Salmonella* surrogate for validating thermal treatment of dairy powders, taking into account that thermal resistance of both microorganisms can be affected by lactose and milk protein levels.

P1-08 Investigation of Relationship between Desiccation Tolerance of *Salmonella* spp. and Glass Transition Temperature

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Introduction: Outbreaks of foodborne diseases have been increasing in low water activity (a_w) foods. *Salmonella* spp. are the most important bacterium causing outbreaks in low a_w foods. We hypothesized that the desiccation tolerance of *Salmonella* may be related to the physical properties of the bacterial cells such as glass transition. Moisture content or a_w of biochemical materials would highly be correlated with a glass transition temperature (T_g).

Purpose: The purpose of this study was to investigate the relationship between T_g of *Salmonella* and a_w level. In addition, we examined the survival kinetics of *Salmonella* under heat treatment to clarify the relationship between T_g and thermal tolerance of *Salmonella*.

Methods: *Salmonella enterica* serovar Oranienburg and *Salmonella* Enteritidis were grown in tryptic soy broth at 37°C for 48 h. The grown cells were collected by centrifugation, and freeze-dried. The dried cells were placed in an airtight container at the desired relative humidity (43, 65, 75, and 87% RH). The T_g was determined by thermal rheological analysis procedure that is equivalent in principle to a thermal mechanical compression test. To examine heat tolerance of *Salmonella* under low a_w, the freeze-dried samples were sealed into small plastic films with thin-layer and submerged in the water bath set 60°C for varying durations (10, 20, 30, and 40 min). Then, the heated samples were diluted with peptone water and incubated on tryptic soy agar plates at 37°C for 24 h.

Results: The T_g of both the *Salmonella* spp. significantly increased as the a_w decreased. For example, the T_g of *Salmonella* Enteritidis was 40.7°C and 77.1°C at a_w=0.87 and 0.43, respectively. As the T_g increased, which means a_w decreased, the survival ratio of *Salmonella* under heat treatment increased.

Significance: These results suggested a possible mechanism of desiccation tolerance of *Salmonella* under low a_w conditions in which higher T_g was shown.

P1-09 Performance of an Improved Thermal Death Time Sandwich System for Determining the Thermal Death Kinetics of *Salmonella*

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❖ ***Developing Scientist Entrant***

Introduction: A novel system called the thermal death time (TDT) sandwich was previously developed and tested to address the shortcomings of traditional methods for determining the thermal death kinetics of microorganisms. Improvements have since been made to the system such as enhanced temperature uniformity and elimination of temperature overshoot during initial heating, and now needs to be tested against traditional methods such as water baths.

Purpose: To compare the thermal resistance of *Salmonella* spp. in whole and non-fat dry milk powders measured using TDT sandwiches and a water bath.

Methods: Whole and non-fat dry milk powder samples were dry-inoculated in triplicates with a cocktail of *Salmonella* to initial populations of at least 6.5 log CFU/g and equilibrated to water activity of 0.23±0.01 and 0.18±0.01, respectively. Samples were packed into aluminized plastic pouches with low moisture permeability (3.875×10^{-4} g·m^{-2}·h^{-1}) or aluminum test cells. Subsequently, they were heated in TDT sandwiches or water bath to 85, 90, or 95°C. Random samples were taken out at selected time points to achieve at least three-log reduction, submerged in ice, and plated in triplicates on tryptic soy agar supplemented with 0.05% (w/v) ammonium iron citrate and 0.03% (w/v) sodium thiosulfate.

Results: Preliminary results on the TDT sandwich determined the D-values for *Salmonella* spp. in whole milk powder at 85, 90, and 95°C to be 30.6, 13.6, and 9.4 min, respectively. The D-values at the same temperatures for non-fat dry milk powder were found to be 76.3, 55.0, and 25.0 min, respectively. Data for the water bath are currently being collected.

Significance: The TDT sandwich already offers operational advantages such as dry heating and flexible heating rates. A comparison of the improved TDT sandwich method against traditional methods provides a basis for utilizing a more precise and efficient method for determining the thermal death kinetics of microorganisms.

Poster

P1-10 Whole Genome Sequencing Analysis for Top Seven Shiga Toxin-producing *Escherichia coli*

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Introduction: *Escherichia coli* O157:H7 and six other Shiga toxin-producing *E. coli* (STEC) serogroups O26, O45, O103, O111, O121, and O145 are often referred to as the top seven STEC. The use of whole genome sequencing (WGS) data to identify, subtype, and distinguish top seven STEC is rapidly growing. The performance of WGS must be examined to ensure appropriate implementation.

Purpose: The purpose of this study is to evaluate the performance of whole genome multi locus sequence typing (wgMLST) when applied to top seven STEC analysis.

Methods: A total of 20 top seven STEC strains and five O157 strains with other H antigens were selected for this study. All strains were cultured using non-selective agar, and DNA was extracted using the DNeasy UltraClean Microbial kit. WGS was performed using the Illumina MiSeq and raw sequence data were analyzed by wgMLST using BioNumerics software. Allele difference was determined based on the consensus results of assembly-based and -free calls. Dendrograms were generated using the unweighted pair group method with arithmetic mean (UPGMA) clustering method to show similarity coefficients and allele differences among samples.

Results: The results indicated that wgMLST analysis was able to distinguish *E. coli* strains with different serotypes. Within the top seven STEC groups, any two strains with different serotypes differed by 486 alleles or more. Serotypes O45:H2 and O103:H2 displayed the highest similarity (88.4%) to each other. O157:H7 strains were well distinguished from other non-O157 strains with only 9.6% allele similarity. Strains of the same serotype had less genetic differences; for example, six O157:H7 strains shared at least 98.2% allele similarity (81 allele differences).

Significance: This study indicates that wgMLST is an appropriate method for differentiating top seven STEC strains to the serotype level. The finding will support the application of WGS for foodborne pathogen analysis.

P1-11 WITHDRAWN

P1-12 The Relationship between Inactivation and Morphological Damage of *Aspergillus flavus* Treated by High Hydrostatic Pressure

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Introduction: Foodborne *Aspergillus flavus* contamination is a major concern for the food industry. Thus, there is an increased research interest in discovering effective treatment methods that preserve food quality and decontaminate food by inactivating *A.* flavus. High hydrostatic pressure processing (HHP) is considered one of the most promising food preservation techniques and is used for commercial pasteurization of an increasing number of food products.

Purpose: The purpose of this work was to investigate the mechanisms underlying the inactivation of *A. flavus* under HHP treatment.

Methods: *Aspergillus flavus* BCRC 30003 (1×10^5 conidia /ml) was subjected to pressure treatment at 100, 200, 300, 400, 500 or 600 MPa, with a holding time of five minutes at 25°C. After treatment with different levels of hydrostatic pressure, surviving cells were then analyzed by viable colony forming units, cell membrane damage by fluorescent dye propidium iodide (PI) uptake, morphological features by scanning electron microscopy (SEM), and membrane protein changes by SDS-PAGE.

Results: The results showed that a 600 MPa treatment for five min could considerably inactivate *A. flavus* counts, with increased uptake of propidium iodide (PI) and the number of viable organisms decreasing from 10^5 CFU/ml to no viable bacteria. Morphological damage to the cell wall, cell membrane, and cytoplasmic components by HHP treatments were observed on SEM images. The SDS-PAGE results showed that the protein bands differed between HHP-untreated and HHP-treated *A. flavus*, in that HHP decreased the protein content and caused partial protein degradation.

Significance: The findings of this study indicated that HHP inactivates *A. flavus* by causing morphological changes in the internal and external cellular structures, as well as through membrane damage, cell wall rupture, and membrane protein degradation.

P1-13 Influence of Asymptomatic *Escherichia coli* Inhabiting the Gut on Inflammation, Cell Proliferation, Oxidative Stress, and Angiogenesis in the Intestine

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❖ ***Developing Scientist Entrant***

Introduction: Even though some *Escherichia coli* strains live asymptomatically in the human gut, they may cause chronic problems from long-term exposure.

Purpose: This study investigated the influence of asymptomatic *E. coli* inhabiting the gut on chronic intestinal symptoms.

Methods: The concentrations of cytokines (IL-1β, IL-6, IL-10, IL-12p70, TNF-α, and IFN-γ) and prostaglandin E_2 (PGE_2) were measured in Raw 264.7 cells infected by *E. coli* strains (*E. coli* NCCP14037, NCCP14038, NCCP14039, and NCCP15661). *E. coli* was injected orally into eight-month old BALB/c mice, and the responses related to the inflammatory reaction, cell proliferation, cell apoptosis, oxidative stress and angiogenesis were investigated in the intestine by histopathological analysis, qRT-PCR, and western blot. A selected *E. coli* strain (*E. coli* NCCP14037), which has a greater influence in the intestine, was injected orally into eight-month old BALB/c mice, and the responses described above were investigated in the intestine. Also, the risk factors for *E. coli* were analyzed by RNA sequencing.

Results: *E. coli* had an effect on the production of pro-inflammatory cytokines (IL-6 and TNF-α) and PGE_2 in Raw 264.7 cell. Among four strains of *E. coli*, *E. coli* NCCP14037 was most likely to have a greater influence on the intestine by regulating the expression of genes (*SOD1* and *CAT*) and proteins (IL-6, IL-12, PCNA, COX-2, and iNOS) ($P<0.05$). In addition, the length of large intestine was shorten in *E. coli* NCCP14037-treated mice, and this result indicated that the inflammatory reaction occurred in the intestine. The result of RNA sequencing showed that curli, flagella, and fimbriae were highly expressed in *E. coli* NCCP14037.

Significance: Although some asymptomatic *E. coli* inhabits the intestine, its long-term exposure can cause inflammation, cell proliferation, oxidative stress, and angiogenesis in the intestine.

Poster

P1-14 Sporulation of Planktonic and Sessile *Clostridium perfringens* in Response to Chemical and Oxidative Stress

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Introduction: *Clostridium perfringens* is a major human pathogen causing gastroenteritis by enterotoxin production and has an ability to form spores and biofilms for environmental persistence and disease transmission. Biofilms and spores may restrict antimicrobial penetration and contribute to the recalcitrance of bacterial infections.

Purpose: This study aims to compare the chemical and environmental resistance properties of *C. perfringens* vegetative cells and spores in planktonic and sessile conditions.

Methods: Three strains of *C. perfringens* (TYJAM-D-66, CMM-C-80 and SDE-B-202) were isolated from meat supplied to a school cafeteria. Sporulation rate was determined after ero, one, two, five, and seven days at 37°C. Resistances of vegetative cells and spores against disinfectants (0.01% sodium hypochlorite solution, 5% hydrogen peroxide solution, and 80% alcohol) or aerobic conditions (18 h and 36 h) after biofilm formation was analyzed.

Results: Sporulation rate of sessile *C. perfringens* TYJAM-D-66 (*cpe*+) was about 19% at day five, while sporulation of planktonic cells was 14% at day two and decreased to 2% at day five. Sporulation rate of sessile CMM-C-80 (*cpe*-) and SDE-B-202 (*cpe*+) was only up to 0.26 and 0.67% respectively, at day seven. When exposed to aerobic conditions, TYJAM-D-66 vegetative cells were decreased by 1.35 to 1.70 log, CMM-C-80 by 5.13 to 5.36 log, and SDE-B-202 by 5.59 to 5.67 log. Spores decreased by 0.92 log. After the treatment of sodium hypochlorite, 9.23% of TYJAM-D-66 planktonic cells survived, while vegetative cells and spores on biofilm showed 53.62 and 82.33% survival, respectively. Comparative genomic analysis of the isolates was performed to understand the genomic characteristics including toxin profiles and genes involved in sporulation and biofilm formation.

Significance: Our results indicate resistance of vegetative cells and spores of *C. perfringens* to environmental stress after biofilm formation. This conclusion has a great significance for analyzing the relationship between the toxin gene, sporulation, and inhibition of *C. perfringens* biofilms.

P1-15 Use of Water Activity vs Moisture Content in Response Surface Models for Predicting Microbial Lethality during Extrusion of Low-moisture Foods

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❖ ***Developing Scientist Entrant***

Introduction: Low-moisture foods can no longer be considered microbiologically safe due to numerous food product recalls and foodborne illness outbreaks. Water activity (a_w) is one of the important factors that influence the ability of pathogens to survive in a desiccated environment. As moisture content is easy to measure, it is often used in models rather than water activity during the extrusion process.

Purpose: The purpose of this study is to determine if a_w is more useful than moisture content as an indicator of lethality of *Salmonella* spp. and *E. faecium* during extrusion of oat flour by evaluating the fit (R^2) of response surface models to observed data.

Methods: Oat flour samples inoculated with *Salmonella* spp. and *E. faecium* are formulated to different moisture levels (14 to 26% wet basis) and fat levels (five to 15% w/w) and are extruded in a single-screw extruder at different temperatures (65 to 95°C) and screw speeds (75 to 225 rpm). A custom high-temperature water activity test cell is being designed to determine the moisture sorption isotherms at temperatures greater than 60°C and at different fat contents. The a_w at different temperatures and fat contents will be used to replace moisture content in the response surface model.

Results: The goodness of fit of the response surface model was checked by the coefficient of determination. The model fits well, with R^2=0.83 and 0.84 for *Salmonella* and *E. faecium*, respectively. Moisture content showed a significant quadratic effect on the inactivation of both microorganisms during the extrusion process. Once a_w is measured, those values will be used to replace moisture content in the response surface model and goodness of fit will be evaluated.

Significance: The developed model would serve as an excellent tool in understanding the influence of a_w on thermal resistance of *Salmonella* spp. and *E. faecium* during extrusion of low-moisture foods.

P1-16 Thermal Inactivation of *Salmonella* Enteritidis PT 30 and *Enterococcus faecium* in Egg Powders at Different Water Activities

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Introduction: Numerous *Salmonella* outbreaks in recent years have been related to low-moisture food. This demands the scientific community develop effective thermal treatments for *Salmonella* in low moisture foods. However, the increased thermal resistance of *Salmonella* with low water activity (a_w) complicates the development of effective treatments.

Purpose: The aim of this study was to find the *D*-Value at 80°C of *Salmonella* Enteritidis PT 30 and *Enterococcus faecium* in egg powders.

Methods: *Salmonella enterica* serovar Enteritidis PT 30 and its surrogate *Enterococcus faecium* NRRL B-2354 were inoculated in 3 different egg powders (white, yolk, whole) at different a_w (0.3, 0.45, 0.6). The samples were treated at 80°C with a TDT (thermal death time) cell.

Results: The results showed that the inactivation of both microorganisms in the three egg powder was well modeled by first-order kinetics (R^2=0.94 to 0.99). Using this data the *D*-value was calculated for both microorganisms. The resultant D-Values varied from 5.27±0.12 min ($D_{80°C}$-Value for *S.* Enteritidis PT 30 at $a_{w,20°C}$ =0.60±.02) to 43.78±0.39 min ($D_{80°C}$-Value for *E. faecium* at $a_{w,20°C}$= 0.30+.02); it was observed that at lower a_w the D-Value increased for all the samples. The D-Value for *E. faecium* was higher for all the samples at the different a_w levels, compared to *S.* Enteritidis PT 30 D-Values.

Significance: This study furthers the scientific understanding of the impact of the a_w for the development of adequate thermal treatments in low moisture foods.

Poster

P1-17 Assessing Efficacy of Vacuum-assisted, Low-temperature Steam Decontamination of *Salmonella* spp., *Listeria monocytogenes*, Shiga Toxin-producing *Escherichia coli*, and a Surrogate (*Pediococcus acidilactici*) on Raisins

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❖ ***Developing Scientist Entrant***

Introduction: The awareness of safety concerns for low water activity foods and new FSMA regulations challenge processors and scientists to establish and document steps of preventive controls to maintain product safety.

Purpose: Determine efficacy of vacuum-assisted steam treatments for reduction of *Salmonella* Montevideo, Newport, and Tennessee; *Listeria monocytogenes* (1/2a, 1/2b, and Scott A); and *E. coli* O157:H7 and O121 on raisins, and evaluate the suitability of *Pediococcus acidilactici* as a potential surrogate organism.

Methods: Raisins were inoculated with a cocktail containing *Salmonella* spp., *L. monocytogenes*, *E. coli* and *P. acidilactici*. Raisin samples were then loaded into a chamber and a vacuum was used to create a negative pressure. Vacuum-assisted, low-temperature steam was applied at two different temperatures (62 and 72°C) for various times (n=9) and survivors were enumerated using non-selective media with a selective overlay maximize recovery. Experiments were replicated at least three times.

Results: At 62°C, a five-log reduction occurred after 14 min for *Salmonella* (5.0±0.8 log CFU/g), and after 20 min for *E. coli* (6.2±1.07 log CFU/g) and *L. monocytogenes* (5.7±0.74 log CFU/g). At 72°C, a five-log reduction occurred at 3.5 min for *E. coli* (5.0±1.32 log CFU/g), *L. monocytogenes* (5.0±0.74 log CFU/g) and *Salmonella* (5.0±1.2 log CFU/g). For the 62°C treatment, *P. acidilactici* was significantly more resistant than the pathogens for five, eight, 14, and 20-min treatments (P<0.05), but not consistently more resistant than another pathogen across 72°C treatments.

Significance: The findings suggest that vacuum-assisted steam may be an effective intervention for low water activity foods against common foodborne pathogens, providing a novel solution for contamination of dried fruits. These experiments also suggest *Pediococcus acidilactici* is significantly more resistant than the selected pathogens during certain vacuum-assisted, low-temperature steam treatments of low water activity foods, but also raise questions about its suitability as a surrogate at different treatment temperatures.

P1-18 Inactivation of *Salmonella enterica* and *Enterococcus faecium* in Cumin Seeds Using Gaseous Ethylene Oxide

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❖ ***Developing Scientist Entrant***

Introduction: Ethylene oxide (EtO) has historically been used to reduce bacterial populations in spices. Most previous studies were performed from the 1940s to 1980s with limited processing parameters (gas concentration, temperature and relative humidity (RH)) on inactivation action of gaseous EtO.

Purpose: The purpose of this study was to investigate the effects of processing parameters on the antimicrobial efficacy of gaseous EtO in cumin seeds.

Methods: Five grams of cumin seeds either inoculated with a five-strain *Salmonella enterica* cocktail or *Enterococcus faecium* were packed in a porous packet made of filter paper and placed on a petri dish in a Sterilizer-Vac 5XL Gas Sterilizer from 3M. Treatment conditions were 735.3 ppm for EtO concentration, 37°C, and 30% RH. The inoculated sample was treated for different exposure times until achieving a five-log reduction of *Salmonella* in cumin seeds. After treatment, cells were recovered on trypticase soy agar (TSA) supplemented with 0.05% (w/v) ammonium iron citrate and 0.03% (w/v) sodium thiosulfate (mTSA) for *Salmonella* and TSA supplemented with 0.05% (w/v) ammonium iron citrate, and 0.025% (w/v) esculin hydrate (eTSA) for *E. faecium* and incubated for 24±2 h at 37°C.

Results: For *Salmonella*, there was a log reduction of 2.75±0.02, 3.85±0.03, 3.95±0.02 and >5.15 after two, five, 10 and 15 min exposure time, respectively. While for *E. faecium,* there was a log reduction of 2.29±0.05, 2.69±0.00, 3.08±0.32 and 4.49±0.01 after the same exposure times. The results showed that *E. faecium* consistently had lower log reduction than *Salmonella* in cumin seeds under the same treatment conditions.

Significance: EtO holds potential as an effective intervention method for *Salmonella* in spices. *E. faecium* is a good surrogate for gaseous EtO sterilization in cumin seeds and can be used for further validation in spices industry.

P1-19 The Prevalence and Characteristics of Acid-resistant *E. coli* in Foodborne and Clinical Isolates in Korea

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Introduction: Pathogenic *E. coli* is known to be one of the most common pathogens causing food poisoning in Korea. Half of *E. coli* outbreaks have been linked to consumption of food of plant origin. The discovery of pathogenic *E. coli* having acid-resistance that helps survival has been reported.

Purpose: In this study, we investigated the acid-resistance and genetic characteristics of pathogenic *E. coli* obtained from surveillance and foodborne outbreaks.

Methods: The overnight cultured *E. coli* was exposed to acetic acid solution at pH 2.5 for 120 min. After 120 min of incubation, the survival rate was calculated by comparing the reduced bacterial concentration to the initial concentration. The closed whole genomes of selected strains with strong acid resistance were sequenced and analyzed to identify the gene functions as well as the genetic markers of acid resistance.

Results: Of total 414 pathogenic *E. coli,* 56 (23%) of 239 clinical isolates and 12 (seven percent) of 175 foodborne isolates showed strong acid resistance (less than two log CFU/g reduction) against pH 2.5 acetic acid solution. In terms of pathogenicity, four (12%) of 33 EAEC, six (8%) of 71 EHEC, 26 (14%) of 182 EPEC, and 32 (27%) of 121 ETEC showed strong acid resistance. The pan-genome analysis of selected strains showed that the genes related to the glutamate-dependent acid resistance system (AR2) were commonly found in all selected strains and the GadEWX regulons were highly conserved in all genomes of selected strains.

Significance: This can be used as scientific evidence of why the EPEC and ETEC are the major pathogens found in low pH fermented food in Korea.

P1-20 WITHDRAWN

P1-21 WITHDRAWN

P1-22 Ethylene Oxide Fumigation for Inactivation of *Salmonella* and *Enterococcus faecium* nrrl B-2354 in Black Pepper

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❖ ***Developing Scientist Entrant***

Introduction: Several multistate foodborne outbreaks have been linked to black pepper contaminated with *Salmonella*. Although ethylene oxide (EtO) fumigation has been used as a decontamination process for spices, a recent study reported highly variable microbial inactivation in EtO commercial facilities. There is thus a need to understand the effects of EtO processing parameters such as relsative humidity, temperature and concentration on the microbial inactivation kinetics.

Purpose: The purpose of this study was to investigate EtO fumigation for inactivating *Salmonella* in whole and ground black pepper and, evaluate *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella*.

Methods: Whole and ground black pepper samples were inoculated with *Salmonella* cocktail or *E. faecium.* Two concentrations of EtO (735.3 and 933.8 ppm), and three levels of temperatures (37, 46, 55°C) and relative humidity (30, 40, 50%) were applied to the inoculated black pepper samples. Different exposure times were conducted for each condition to achieve up to five-log reduction of *Salmonella*. For EtO treatment, inoculated samples were packed in air-permeable bags and then placed either on the petri dishes or at the bottom of retail bottles filled with uninoculated samples.

Results: Under the worst conditions (37°C, 30% RH, 735.3 ppm EtO) for samples in Petri dishes resulted in 4.58 and 3.79-log reductions of *Salmonella* in whole and ground black pepper, respectively, after an exposure time of 15 min. Corresponding inactivation values for *E. faecium* were 2.92 and 2.95-log reductions. For both whole and ground black pepper in the bottles, more than five-log reductions of *Salmonella* and *E. faecium* were achieved with two min of exposure time.

Significance: This study provides the basis for developing guidelines for EtO fumigation of spices to ensure food safety. *E. faecium* was found to be a conservative surrogate for *Salmonella* during EtO fumigation of black pepper.

P1-23 Behavior of Shiga Toxin-producing *Escherichia coli, Salmonella* spp., and *Listeria monocytogenes* on Dried Apricots Made with and without Sulfur Dioxide

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Introduction: Dry fruits are one of the most economically valuable specialty crop products in California. Unfortunately, limited literature information is available about the microbial safety of dried fruit products.

Purpose: The aim of this study was to evaluate the behavior of three common foodborne pathogens, Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella spp.*, and *Listeria monocytogenes*, on dried apricots made with and without sodium dioxide.

Methods: Dried apricots made with and without sodium dioxide were purchased from the local farmers' market in Davis CA. These apricots were inoculated with a five-strain STEC cocktail, a five-strain *Salmonella* cocktail, and a five-strain *L. monocytogenes*, cocktail separately. Apricots were inoculated and dried at ambient temperature for five days before they were stored in Ziploc bags at ambient temperature for six months. The behavior of inoculated pathogens was evaluated on days zero, two, five, 15, and 30 in the first month and every month for the rest of the storage period.

Results: pH values of the dried apricots made with sodium dioxide (apricot-SD) and without preservatives (apricot-NP) were 4.6 and 4.8 respectively. The water activities were 0.724 and 0.629 respectively. After inoculation, the pH and water activities of inoculated apricots increased significantly, with the pH of apricot-SD increased to 5.7 and that of apricot-NP increased to 5.1. The water activities of both types of products increased to ~0.85 after inoculation. Although the pH and water activities decreased significantly after five days of drying at ambient temperature, inoculated products still had slightly higher levels of pH and water activities compared to the uninoculated products. All inoculated pathogens survived the six months of storage. The five-day drying period led to an approximately one-log reduction on all pathogens.

Significance: Results of this study are important data for conducting a risk assessment of dried fruits.

P1-24 Inactivation of *Salmonella* Typhimurium during Red Chile Drying

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Introduction: Dried red chile powder has been associated with outbreaks of salmonellosis and there have been numerous recalls of product due to contamination with *Salmonella* spp.

Purpose: To determine the inactivation kinetics of *Salmonella* Typhimurium in artificially contaminated dried chile peppers. We evaluated heat treatments used during the manufacture of dried red chile to determine if they were sufficient to result in a five-log reduction in *Salmonella.*

Methods: Green chile peppers were dried in a lab dryer for one week at 90°F to about 50% moisture to simulate midseason chile peppers used in the drying industry. Red chile peppers were inoculated with *Salmonella* Typhimurium (ATCC 14028) for the lab scale inactivation studies at approximately 10^{7-8} CFU/g. We use a laboratory scale dryer for the heating trials and heated the peppers to 150, 160, 170 and 180°F for up to four h. The levels of *Salmonella* in the dried red chiles were enumerated by plating appropriate dilutions in triplicate onto XLD/TSAYE plates. After 24-h incubation at 35°C plates were enumerated as described in the BAM.

Results: The levels of *Salmonella* in the dried products were determined on XLD/TSAYE plates in triplicate at each time point. When chile peppers were dried at 150°F or 160°F less than three log of the inoculated organisms were inactivated. While higher temperatures resulted in significant thermal destruction of the pathogen (170°F for four h, greater than seven-log reduction; 180°F for two h, greater than five-log reduction).

Significance: Drying red chile at 170°F or greater for four hours resulted in acceptable inactivation of *Salmonella* spp. (greater than five-log reduction). Drying at 180°F required two hrs or less for five- log reductions of the pathogen on the chile. Lower drying temperatures were not sufficient to ensure a safe product.

Poster

P1-25 A Non-Ionizing Radiation Method (UV-C) to Control *Aspergillus flavus* and *Aspergillus parasticus* on Roasted Coffee Beans

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Introduction: *Aspergillus* spp. are a predominant mold of coffee bean products, and *A. flavus* and *A. parasticus* are the main pathogenic molds that can produce aflatoxins. Since coffee is consumed worldwide, the accumulation of aflatoxins due to steady ingestion leads to carcinogenesis. Non-ionizing radiation (UV-C) can be applied to the final disinfection methods of foods as a surface sterilization technology.

Purpose: This study investigated the effects of UV-C on the reduction of *A. flavus* and *A. parasticus* on a roasted coffee bean. Also, physiochemical properties and sensory evaluation were measured.

Methods: Coffee beans were divided into a round and a cracked surface, and six log of fungi spores were inoculated. Each surface was treated by UV-C for maximum of two hours. To examine the effect of UV-C on spores, the spores formed in the coffee bean were observed by FE-SEM and irradiated samples were measured for physiochemical (pH, moisture, and Hunter color) changes and sensory evaluation (seven-point hedonic scale).

Results: The populations of *A. flavus* and *A. parasticus* irradiated by UV-C on both surfaces were significantly decreased ($P<0.05$) as irradiation time increased. When UV-C was irradiated on a round surface for two hours, *A. flavus* was decreased by 2.16 log, and *A. parasticus* by 1.03 log. On a cracked surface, *A. flavus* was reduced by 0.71 log, and *A. parasticus* by 0.37 log. There was no big difference in physiochemical properties after UV-C irradiation, but 'a', and 'b' of Hunter color and pH was significantly decreased ($P<0.05$) after two hours. Also in sensory evaluation, there was no difference in color, appearance, texture, and overall acceptability, but the flavor in sensory evaluation showed significant differences ($P<0.05$) after two hours.

Significance: This study suggests that non-ionizing radiation (UV-C) on roasted coffee beans may be effective in reducing *A. flavus* and *A. parasticus* with fewer physiochemical changes and sensory changes.

P1-26 Inactivation of *Salmonella* and Surrogate Bacteria on Brazil Nuts and Pine Nuts Exposed to Commercial Propylene Oxide Processing Conditions

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Introduction: Fumigation by gaseous propylene oxide (PPO) has been demonstrated to reduce *Salmonella enterica* on some tree nuts. A surrogate whose inactivation pattern is similar to *Salmonella* is needed to avoid direct handling of *Salmonella* in process validation within the processing facility.

Purpose: To determine the suitability of *Enterococcus faecium* and *Pediococcus acidilactici* as potential surrogates for indicating *Salmonella* inactivation on whole Brazil and pine nuts subjected to PPO processing in accordance with the USEPA label instructions.

Methods: Brazil nuts or pine nuts were co-inoculated with a cocktail of tryptic soy agar-grown *Salmonella enterica* strains and one of two potential surrogates: *Enterococcus faecium* NRRL B2354*, or Pediococcus acidilactici* ATCC 8042. Samples were dried, packaged and placed in polywoven polypropylene bags within non-inoculated nuts. Commercial PPO treatments (PPO<2.5 kg/m^3) were performed by Cosmed in dedicated chambers, using a proprietary process. Samples were returned for enumeration by plating onto TSA overlaid with appropriate selective media. Processing was completed three times, with six samples per process. Mean reductions in log CFU/g were compared using an ANOVA with Tukey's posthoc test and a matched pair *t*-test. $P<0.05$ was considered significant.

Results: Inoculation levels on pine nuts were one log higher than on Brazil nuts, indicating nut size and surface area were factors that influence bacterial numbers. Log reductions of *E. faecium* (6.52±0.96 for Brazil, 6.46±0.82 for pine) and *P. acidilactici* (5.99±0.89 for Brazil, 5.79±0.79 for pine) were significantly lower than *Salmonella* (6.82±0.62 for Brazil, 7.56±0.69 for pine) on both nuts ($P<0.05$); however, there were instances when *Salmonella* had a lower reduction on individual Brazil nut samples.

Significance: *Enterococcus faecium* NRRL B2354 and *Pediococcus acidilacti* ATCC 8042 may be considered as surrogates for *Salmonella* when inoculated onto Brazil and pine nuts processed using PPO fumigation with these conditions. *Pediococcus acidilactici* may be a more conservative choice as a surrogate for *Salmonella* inactivation. Surrogates should be used to validate different product configurations and processing parameters.

P1-27 Study of *Listeria monocytogenes* in Turkey Meat Samples from Independent, Urban Delis Provides a Critical Triangulation Point for a Multistate Outbreak Investigation

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Introduction: Research suggests that small and independent delis are less likely to follow proper sanitation procedures, including slicer inspection, which could lead to a higher likelihood of these delis being a reservoir for *L. monocytogenes* growth and cross-contamination.

Purpose: To determine the incidence of *L. monocytogenes* in counter-sliced turkey deli meat obtained from independent delis in an urban city.

Methods: Turkey deli meat, counter-sliced on site, was collected from 133 delis in an urban area, mostly from independent establishments. The samples were analyzed for *L. monocytogenes* using USDA MLG methodology for isolation and confirmation. The selection criteria for delis included using the city's restaurant inspection and grading system. The majority of delis had an 'A' or top grade but also had food safety violations in their online record.

Results: Two samples, from separate delis, were confirmed positive for *L. monocytogenes* (1.5%). Analysis of the genomic sequences of one of the samples revealed a close match to a cluster of six clinical listeriosis cases, which are part of an ongoing multi-state outbreak spanning four different states. The second sample matched a clinical case in a neighboring state. Both samples were obtained from delis that did not have the top inspection grade.

Significance: Although a snapshot of one urban area, this study is the first report of the current incidence of *L. monocytogenes* on counter-sliced deli meat from independent deli establishments. This study suggests that these delis can potentially serve as sources of *L. monocytogenes* contamination or contribute to downstream foodborne listeriosis. Information provided by city inspection and grading systems, in addition to the letter grade, may serve as a tool to identify delis with potential *L. monocytogenes* contamination issues and serve as a basis for product and environmental sampling by public health authorities.

P1-28 Comparison of Food Establishment Characteristics between Viral and Bacterial-caused Foodborne Outbreaks Reported to the National Environmental Assessment Reporting System

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Introduction: Food establishment characteristics are voluntarily reported to the CDC's National Environmental Assessment Reporting System (NEARS) by state and local health departments who investigate foodborne outbreaks. Understanding the differences between establishments that have bacterial or viral outbreaks, such as those in food safety policies, practices, and other characteristics, can enable development of interventions to prevent foodborne illness.

Purpose: The purpose of this study was to evaluate characteristics between establishments that had bacterial versus viral outbreaks.

Methods: We used a case-case methodology to compare characteristics between food establishments that had either a confirmed or suspected outbreak due to a bacterial or viral agent. Data was extracted from NEARS for outbreaks that occurred during 2014-2017. Variables were analyzed using chi-square and t-tests.

Results: The dataset contained data on 349 food establishments. We found that establishments that had bacterial outbreaks differed significantly ($p<0.05$) from establishments that had viral outbreaks on several characteristics. For instance, establishments with bacterial outbreaks, compared to viral outbreaks, had

- 7 higher odds of observed potential cross-contamination from raw to ready-to-eat food
- 12% fewer cleaning policies
- 58 lower odds of having wiping cloths stored in sanitizer solution
- 51 lower odds of using a mechanical warewasher
- 48 lower odds of having a written policy requiring food temperatures to be checked
- 59 lower odds of having food temperature records
- 39 lower odds of using a commonly validated procedure to rapidly cool food
- 25% fewer refrigeration units.

Significance: These data suggest that there are systematic differences in food safety policies and practices between restaurants with bacterial and viral outbreaks. For example, these data suggest that restaurants with bacterial outbreaks have reduced refrigeration capacity to maintain food at recommended temperatures, a factor often associated with growth of bacteria. Information like this is valuable in developing intervention efforts to prevent foodborne illness.

P1-29 Determining the Perceived Cost of Implementing a Vomit Clean-up Plan

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Introduction: Evidence suggests an organization's willingness and commitment to implementing food safety practices are affected by perceptions regarding the challenges and costs of doing so. In 2009, the United States Food and Drug Administration added a regulatory provision to the Food Code requiring food service establishments to have bodily fluid clean-up procedures in place.

Purpose: To determine the perceived cost of implementing a vomit clean-up plan in a foodservice operation.

Methods: Foodservice workers from retail, commercial, and institutional settings who attended one of 146 educational sessions offered by 34 educators were surveyed before attending food safety training.

Results: A total of 388 participants completed the survey. The incidence of vomit events reported in the past three months varied considerably by setting (1.45% for retail to 21.4% for institutional foodservice operations). Among the 54 respondents (13.9%) who reported their establishment had an event, most indicated either they did not know the amount of time or expenditure dedicated to addressing these events (69.4%), or they reported there was no cost to doing so (15.7%). Estimates of time and other expense of addressing each vomit event varied widely among the 14.8% of respondents who provided estimates of this information. Overall, it was felt that 10 to 30 minutes would be required for cleaning and that, on average, less than $20 to $300 would be needed for cleaning supplies, training and clean-up time, and cost of replacing any food. These results varied significantly from the actual time and cost estimates of implementing currently recommended vomit clean-up procedures. Those working in commercial environments had a much better understanding of the time and other expenses associated with addressing a vomiting event than did workers from other settings.

Significance: The perceived cost of implementing a vomit clean-up plan should be integrated into existing food safety training to increase adoption.

P1-30 Perceived Benefits and Barriers to Implementation of a Traceability System in School Foodservice Establishments in North Carolina, South Carolina and Georgia

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Introduction: Significant logistical, contractual, and food safety challenges exist when sourcing food for school foodservice operations (SFOs) from small- to medium-sized (SMS) farms. Each step from ordering to packaging to service can present significant barriers for traceability of the product from farm to table.

Purpose: The aim of this exploratory study was to determine the perceived benefits and barriers of SFOs in implementing a traceability system for produce purchased by SFOs from SMS farms.

Methods: Qualitative research results from an earlier study and results from other previously published studies that explored institutional foodservice procurement were used to design a web-based survey. Items measured current buying activity; buying directly from SMS farms; benefits and challenges of buying from SMS farms; traceability system; traceability implementation and costs; and organizational characteristics. The response format included three- and five-point Likert scales, closed-choice items, and yes/no scales. The instrument was piloted with five SFO buyers before dissemination. The survey was administered to 411 SFO buyers from school districts throughout North Carolina, South Carolina, and Georgia.

Results: The response rate was 29.9% (n=123 SFOs). Approximately 74% of respondents reported having some type of traceability system in place for food products. Preparing for a food recall was most often cited as a benefit of 91.26% of buyers. Although 74.19% reported they would need to purchase software specific to a traceability system, 57.73% reported they would be limited by the technology expenses associated with implementation. Nearly one-third (32.02%) reported that they would not be able to spend anything on technology for a traceability system and 30.93% would not be able to spend anything on maintaining a traceability system.

Significance: Although a seemingly simple concept to implement, significant challenges, such as cost and technology, need to be addressed before traceability systems are implemented in SFOs.

P1-31 Characterization of *Escherichia coli* O157:H7 Stationary Phase Acid Resistance and Survival in a Model Vegetable Fermentation System

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Introduction: Existing data show that *Escherichia coli* O157: H7 (STEC) and related serotypes vary in acid resistance phenotype. Some strains may survive for three or more weeks in fermented vegetables, depending on acid conditions and environmental variables.

Poster

Purpose: To determine if STEC acid resistance correlates with growth and death of STEC in vegetable fermentations in a model laboratory vegetable fermentation system.

Methods: We examined seven selected STEC stains to determine growth rates in laboratory media at various pH values (pH 4.2 to 6.8) and measured survival in simulated stomach acid (SA) challenges. Two strains, B201 (acid-sensitive) and B241 (resistant) were selected for further analysis of acid resistance in lactic acid solutions under aerobic and anaerobic conditions at pH 3.3 in cucumber juice (CJ) and fermented cucumber juice (FCJ). We then compared the growth and death of B201 and B241 in a model fermentation system to determine survival time during competitive growth with *Lactobacillus plantarum* strain MOP3. Experiments were done in triplicate.

Results: STEC strains fell into statistically distinct groups based on acid sensitivity, although there was variation based on the type of acid challenge (stomach acid vs lactic acid or acetic acid). B201 and B241 were statistically different ($P<0.05$) when challenged with SA, lactic acid in CJ, or aqueous lactic acid solution. However, there was no statistical difference ($P>0.05$) between these strains in the FCJ. Interestingly, there were also no statistical differences ($P>0.05$) in the survival (CFU/ml) of B201 or B241 in competition with MOP3 with regard to survival time, pH, or total and protonated lactic acid concentration.

Significance: The results indicate that the acid resistant phenotype with stationary phase cells may not accurately predict STEC survival in a vegetable fermentation system. Further research may be needed to determine factors influencing STEC die-off in vegetable fermentations.

P1-32 A Buffer Capacity Model for Predicting pH Changes Due to Addition of Low Acid Ingredients in Acid Foods

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Introduction: The pH of most acid food products depends on undefined and complex buffering of ingredients and is critically important for regulatory purposes (21 CFR part 114) and food safety.

Purpose: Our objective was to develop and validate a method for predicting pH changes with the addition of a variety of low acid ingredients into acetic acid formulations typical of salad dressing products

Methods: A variety ($n>20$) of acetic acid formulations with low acid ingredients (garlic powder, onion powder, mustard flour, spices, etc.) were titrated individually and in combination in water at concentrations typical of dressing products. Titration curves (pH 2 to 12) were generated with NaOH and HCl as titrants. Titration curves were used to generate buffer capacity (BC=dN/dpH) curves and fit the BC model. A matrix of pKa and concentration values for undefined buffers in ingredients was estimated by curve fitting (Matlab fmincon algorithm). For each ingredient the matrix was then used to predict pH using a custom Matlab algorithm. All pKa values were adjusted for two percent NaCl.

Results: The pH prediction model was validated using 39 mixtures of acetic or citric acid and ammonia (observed vs. predicted pH, R^2=0.987, RMSE=0.22 pH units). Titration of most low acid dressing ingredients showed little buffering (BC<0.005) compared to acetic acid (BC=0.24) for the pH range (pH 2 to 12), resulting in small (<0.1) pH changes in mixtures with acetic acid at 2.5%. Unexpectedly, corn syrup was found to have BC=0.017 at pH values greater than 11, possibly due to processing aids.

Significance: The BC models were used to estimate buffering and therefore the concentrations of low acid ingredients that prevent a pH rise above 4.6. Using the model to quantify the effect of ingredients on pH may benefit regulatory agencies and manufacturers in assessing product safety.

P1-33 The Ability of Collection Solutions to Maintain the Viability of *Listeria monocytogenes* after Sampling Inoculated Stainless Steel Surfaces

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Introduction: Routine environmental monitoring is important for managing pathogens in food production facilities. Environmental samples are often held at refrigerated temperatures prior to processing in the laboratory. Oftentimes, samples are sent overnight to offsite laboratories and typical holding times are 24 to 48 hours. If sample delivery is delayed, this may extend to 72 hours, often leading to re-sampling.

Purpose: To compare three commonly used collection solutions for their ability to maintain the viability of *Listeria monocytogenes* with 72 hours of holding at refrigerated temperatures following sampling of inoculated stainless-steel surfaces.

Methods: Twenty-five microliter aliquots with between 50,000 and 120,000 *L. monocytogenes* cells were inoculated onto one ft^2 stainless-steel coupons and dried under ambient conditions for 72 hours. Samples were collected from the inoculated surfaces with polyurethane foam sponges hydrated with HiCap Neutralizing Broth, D/E Neutralizing Broth, or Neutralizing Buffer (NB). UVM enrichments were started immediately after sampling or after 72 hours of refrigerated storage. Positive samples were identified using isolation on chromogenic media and blackening of Demi-Fraser Broth.

Results: In one pairwise comparison, *L. monocytogenes* was recovered from 10 out of 10 coupons when HiCap sponges were enriched immediately and nine out of 10 sponges when the sponges were held for 72 hours. *L. monocytogenes* was only recovered from one out of 10 NB sponges that were enriched immediately and zero out of 10 sponges that were held for 72 hours. In a study comparing the recovering of *L. monocytogenes* from sponges with HiCap or D/E, comparable recovery was seen when enriched immediately. When the D/E sponges held for 72 hours, there was a 50% reduction in *L. monocytogenes* recovery. No reduction was observed with the HiCap sponges.

Significance: The broth used to hydrate a sponge is important to the recovery of injured *L. monocytogenes* from surfaces, especially when sponges are held for extended periods before processing.

P1-34 Survival and Growth of *Arcobacter* spp. in Human Consumption Water at Different Temperatures

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Introduction: *Arcobacter* has been classified as an emerging zoonotic pathogen, associated with the consumption of different products including water. This bacteria has the ability to colonize the biofilms present in distribution pipes, acquiring protection from disinfection processes. This is one reason why water has been considered as one of the main transmission routes of this bacterium.

Purpose: The aim of this work was to evaluate the survival and growth of *Arcobacter* spp. inoculated into chlorinated and non-chlorinated water at different temperatures.

Methods: Two different concentrations of *Arcobacter* (10^3 and 10^5 CFU/mL) were inoculated into three different water matrices (chlorinated at concentration of 0.5 mg/L, non-chlorinated, and water with 11% organic matter added) and incubated at zero, five, 12 and 25°C for 15 days in order to evaluate its growth and survival. Counts were performed at one, three, five, seven, nine, 11, 13 and 15 days of incubation in duplicate. Bacterial growth was evaluated using blood agar plates incubated at room temperature for 72 h.

Results: *Arcobacter* was able to grow in the three different matrices evaluated; the chlorinated matrix showed less growth. Also, the survival of this bacteria is greater when organic matter is present in water as well as when incubation temperature increases. The number of bacteria decreases with time, but bacteria did not disappear completely.

Significance: Results obtained In Vitro show that water used for human consumption may represent a risk for health, but the survival of the bacteria in the natural environment might be different. Further research is recommended.

P1-35 Evaluating the Impact of Cooling Techniques on *Escherichia coli* Populations in Taco Meat

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Introduction: Storing large quantities of leftover food products may result in improper (slow) cooling, which has been identified by the United States Food and Drug Administration as a factor that contributes to outbreaks of foodborne illness. A variety of techniques are used in school foodservice settings to cool leftover food products that are intended for future meal preparation. Research that investigates the effect of various cooling methods on foodborne pathogen populations in food products is necessary to prevent foodborne illness.

Purpose: This research was conducted to characterize changes in *Escherichia coli* populations in taco meat subjected to a variety of cooling methods commonly employed in school foodservice operations.

Methods: Precooked taco meat was heated to 165°F, portioned into commercial serving pans at two and three-inch depths, cooled to 135 to -140°F, and then inoculated with *E. coli* (target concentration of 10^4 CFU/g) as a Shiga toxin-producing *E. coli* surrogate. All pans were uncovered or covered, with or without an air gap, and then stored in a commercial walk-in freezer (-20°C), or placed in ice water baths in a commercial walk-in refrigerator (4°C). *E. coli* populations were enumerated by spread plating onto MacConkey agar at zero, four, eight, 12 and 24 hours.

Results: Time was the only significant (P=0.0022) variable (n=12 samples per time point), with populations declining over time. The largest and most significant population change occurred between zero and four hours, when a decrease of 0.31 log CFU/g was observed. When comparing zero and 24 hours, *E. coli* populations declined by 0.20 log CFU/g.

Significance: *E. coli* populations did not increase during cooling. Combined with the lack of statistical significance for other variables, this illustrates that *E. coli* populations were effectively controlled by the cooling methods investigated.

P1-36 Strengthening Food Safety Provisions on Cruise Ships: The Vessel Sanitation Program Cooperative Revision Model

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Introduction: The Centers for Disease Control and Prevention (CDC) Vessel Sanitation Program (VSP) bases operational and construction inspections on two foundational VSP guidance documents with important food safety provisions: the Operations Manual and the Construction Guidelines. Worldwide, these documents are considered gold standards in cruise ship sanitation and construction.

Purpose: VSP used a cooperative model to revise these important documents with cruise industry stakeholders; this method can serve as a model framework for other collaborative efforts between government, industry, and other stakeholders.

Methods: VSP developed a new change request form (CRF) and instructions. As part of the revision process, stakeholders, including cruise lines, shipyards, vendors, outfitters, consultants, manufacturers, and international partner-nation agencies, submitted suggestions using the CRF. VSP and stakeholders held 14 days of in-person meetings and two web-based meetings between April 2015 and October 2016 to review the submitted CRFs. During these meetings, submitters presented their changes and discussed the reasons and evidence for requesting the changes. During the group discussion, VSP took one of three actions: accepted each CRF as submitted, accepted it as amended, or did not take action on it.

Results: Three hundred seven CRFs were submitted during the review process. Of these, 112 were accepted as submitted or as amended and 34 were withdrawn. VSP released the final Operations Manual and Construction Guidelines in April 2018 with stronger food safety provisions aligned with the current U.S. Food and Drug Administration Food Code.

Significance: The VSP mission is to prevent the introduction of acute gastroenteritis (AGE) into the United States from cruise ships sailing from foreign to domestic ports. While ships carry approximately 12 million passengers per year in and out of U.S. ports, AGE cases on cruise ships are relatively infrequent.

P1-37 Predictive Modeling of the Effect of ε-Polylysine Hydrochloride on Growth and Thermal Inactivation of *Listeria monocytogenes* in Fish Balls

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Introduction: Limited information is currently available concerning the effect and potential application of ε- polylysine hydrochloride (ε-PLH) on growth and thermal inactivation of *Listeria monocytogenes* in fish balls.

Purpose: The purpose of this study was to investigate the effectiveness of ε-PLH for inhibiting the growth and enhancing thermal inactivation of *L. monocytogenes*.

Methods: Fish balls, supplemented with ε-PLH, were inoculated with *L. monocytogenes* and incubated at 3.4, 8, 12, or 16°C for growth studies, or heated at 60, 62.5, 65, or 67.5°C for thermal inactivation tests. The growth curves were fitted to the Huang primary model, and the Huang and Ratkowsky square-root models (SRM) were used as the secondary models. The survival during heating was analyzed with a log-linear model.

Results: The results showed that, while the lag time of *L. monocytogenes* was affected by both ε-PLH concentration and temperature, the specific growth rate was unaffected by ε-PLH. Under the same temperature, a tenfold increase of the lag time would be expected for every 565 ppm in the increase of concentration. The estimated minimum growth temperature was –2.04 and 0.29°C when estimated with the Ratkowsky and the Huang SRM, respectively. Validation at 10°C showed that the Huang primary model, in combination with either the Huang or Ratkowsky SRM, could accurately predict the growth of *L. monocytogenes*. The thermal resistance of the pathogen was significantly reduced by an increase in temperature or ε-PLH. The thermal z value of *L. monocytogenes* was 5.78°C, and the ε-PLH z value was 1642 ppm.

Significance: ε-PLH can be used to enhance thermal inactivation and control the growth of *L. monocytogenes* during storage. The models can be used to design more effective thermal processes and assess the inhibition of *L. monocytogenes,* thus improving food safety.

Poster

P1-38 The Evaluation of Facilities and Hygiene Prerequisites within the National School Nutrition Programme in South African Schools

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Introduction: The incidence of poverty has increased over the years, especially in rural areas of developing countries, thus affecting learners in poverty. The National School Nutrition Programme (NSNP), part of an Integrated Nutrition Programme (INP), was introduced to reduce the incidence of food insecurity as well as the child mortality rate in South Africa. Even though the programme had good intentions it also poses a number of challenges which still need to be resolved.

Purpose: Previous studies highlighted that among the challenges facing the NSNP is infrastructure; food preparation facilities and storage rooms are lacking. Governments' attention to food safety has increased due to the potential health and economic impact of foodborne outbreaks. This paper gives an overview of the extent to which the South African government improved the prerequisite requirements since the inception of the NSNP within central South African schools.

Methods: The facilities and hygiene operating prerequisites of 98 randomly selected schools were evaluated using an inspection checklist.

Results: The results show that although the focus was given to basic infrastructure such as location (>50%) and structures (>60%); other requirements such as maintenance of such structures (<50%), food preparation equipment (50%) and hygienic operating prerequisites still needed attention (<40%).

Significance: The lack of these requirements might increase the possibility of food contamination and reduction of the shelf life of the food served to learners. With the number of different microorganisms associated with foodborne disease status, programs within the NSNP may pose latent risks for children.

P1-39 Implementation of Novel Technology and Its Implications for a Food Safety Culture in University Dining Halls

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Introduction: A positive food safety culture is important in food establishments, especially when thousands are served daily. Buffet facilities have been implicated as a common locations for foodborne illness outbreaks due to their association with improper food storage; therefore, it is imperative staff have the tools, value correctly monitoring temperatures, and can make process changes if necessary.

Purpose: The purpose of our work was to assess the overall food safety behaviors and culture of surrounding the implementation of temperature monitoring technology as it relates to active managerial control (AMC).

Methods: Daily paper logs for recording holding temperature of foods that need time and temperature control for safety (TCS) foods were replaced with a digital food safety management system. Handheld digital thermometers were used to obtain and upload temperatures in real time. Anytime a temperature read below a threshold of 135°F, the serving line team member was alerted and guided through predefined corrective action steps to resolve the deviation. The behaviors of serving line team members (*n*=66) were observed. Decision making by line (*n*=5) and executive management (*n*=2) on food safety approaches was also observed.

Results: Overall, 90% of serving line team members reported the equipment was easy to use when completing daily food safety checklists. The threshold supported more consistent AMC and awareness from the entire staff. Improvements in collecting daily information for line and executive management included being able to easily find data, ensuring proper training for serving line team members, and no longer storing paper records. Barriers identified were weak communication between all employment levels, lack of engagement, and technology aversion.

Significance: Using this equipment allows for AMC since the system records, analyzes, and reports data. The shared data on the impact of electronic temperature monitoring equipment on decision-making overall answered questions on efficiency and food safety.

P1-40 Characterization of *Salmonella enterica* Isolates from Selected United States Swine Feed Mills by Whole-genome Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Recent multistate foodborne outbreaks have highlighted the importance of using rapid methods to trace contaminations in the food chain and the need of implementing these tools in accordance to new regulatory framework. Genomic techniques, such as whole genome sequencing (WGS), allow for rapid identification of pathogens and have led to the development of databases (GenomeTrakr) with epidemiological information able to assist microbial monitoring and surveillance across the food industry.

Purpose: We used WGS to characterize *Salmonella* enterica isolates from selected United States swine feed mills in relation to season and feed production stages.

Methods: *Salmonella* isolates (*n*=57) were collected from 11 facilities located in Kansas, North Carolina, Iowa, Indiana and Oklahoma, during a previous study. Samples were analyzed following USDA guidelines and confirmed by PCR. WGS was carried out on either an Illumina MiSeq or NextSeq sequencer. *De novo* genome assemblies were obtained with the Shovill pipeline version 0.9. NCBI Pathogen Detection was used to determine antibiotic resistance genes and SNP (single nucleotide polymorphism) clusters.

Results: Isolates belonged to 16 different serotypes, with eight detected in multiple isolates: *Salmonella* Agona, Mbandaka, Senftenberg, Schwarzengrund, Rissen, Hartford, Kimbu and Typhimurium. The remaining eight serotypes, including I 4,[5], 12:i-, were observed in one isolate each. *Salmonella* Agona and Mbandaka were detected across all three seasons. A limited serotypes diversity was observed in Kansas as compared to North Carolina. Isolates were divided across 18 SNP clusters. No matches with clinical samples were found, while strains were matched with other environmental isolates from the NCBI database. AMR genes showed that 40% of the strains carried at least one antimicrobial resistance gene including those encoding for tetracycline, phenicol, aminoglycoside and β-lactam resistance.

Significance: Our analysis shows the presence of pathogenic *Salmonella enterica* in feed mills and underscores the potential role of these environment as pathogen entry route into the human food chain.

Poster

P1-41 Prevalence of *Salmonella* and *Escherichia coli* in Selected United States Swine Feed Mills and Assessment of Potential Contamination Risk Factors

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* has been detected in animal feeds and pork products, raising questions about the role of feed and feed mill environments in introducing contamination into the feed-to-fork supply chain. Research elucidating pathogens occurrence patterns and related risk factors might help design microbial risk assessment and control strategies in these environments.

Purpose: The purpose of this study was to determine the prevalence of *Salmonella* and *E. coli* at different sites within mills and identify potential risk factors associated with their prevalence in feed processing facilities.

Methods: A total of 135 samples from selected sites including floors, equipment, shoe surfaces, and feed, were collected during fall 2018 in six feed mills in the Midwestern United States. Each sample site was analyzed for the presence of both *Salmonella* and *E. coli* with culture methods, followed by PCR confirmation. A survey regarding production volumes and other parameters, hygiene practices, and microbial testing capabilities was conducted in each facility.

Results: From the total of 135 samples obtained, eleven (8.2%) contained *Salmonella* spp. and 31 (23.0%) contained *E. coli*. All sampled mills had at least one site confirmed to contain *Salmonella* spp. or *E. coli*, with six sites confirmed for both. Floors had the highest number of confirmed samples across sampling sites, suggesting that employee foot traffic may be a biosecurity risk. Survey responses support that mills with higher bacteria loads are older, suggesting that age of the mill may be a risk factor for enteric pathogen contamination. Other risk factors evaluated did not appear to relate to *Salmonella* or *E. coli* prevalence. In addition, it was noted that not all mills have microbiological testing on site.

Significance: The data documents a relatively high prevalence of *E. coli* and *Salmonella* in United States swine feed mills. This information could be used to evaluate risks and design mitigation strategies.

P1-42 Knowledge Discovery from Epidemiological Data for Assisting Foodborne Outbreak Investigation

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Introduction: Consumption of foods contaminated by human pathogens is responsible for a large portion of human illnesses. Understanding the interplay between pathogens and foods is critical for outbreak prevention.

Purpose: The purpose of this study was to discover food-pathogen relationships from historical outbreak data.

Methods: Epidemiological data on foodborne disease outbreaks reported to the CDC from 1998 through 2017 was analyzed with network analysis (NA) and machine learning. In the NA, degree of centrality was used to identify the important foods in foodborne outbreaks. K-nearest neighbor (KNN), naïve bayes (NB), and support-vector machines (SVMs) were used to predict the top *n* possible pathogens for food sources. Average accuracy (percentage of predicting correctly) was used to select the optimal model.

Results: Salads, chicken, and beef were found to be the top three foods responsible overall for foodborne outbreaks. NA found that chicken was the most central food in the outbreaks related to *Salmonella* with a degree centrality of 0.65; beef was the most important food in the outbreaks related to *Escherichia* with a degree centrality of 0.57; and rice was the main contributor to outbreaks induced by *Bacillus cereus* with a degree centrality of 0.41. In addition, specific patterns of foods were found in different outbreaks. Given a set of food sources, the accuracy of predicting the one most-possible pathogen was 0.56±0.04 for KNN, 0.62±0.05 for SVMs, and 0.59±0.03 for NB. The average accuracies for predicting the top three most possible pathogens were higher than 85% for all models, with SVMs having the highest accuracy. The metrics for the F-score, precision, and recall also indicated that SVMs was the optimal model for predicting food-pathogen relations.

Significance: Findings in food-pathogen relations provided "first-step" insights in source attribution that can be used for assisting future outbreak investigations.

P1-43 Scoping Review of Chronic Sequelae Associated with Common Foodborne Illnesses

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Introduction: In order to strengthen the burden estimates for chronic sequelae of foodborne illness, a scoping review of the current literature for common foodborne pathogens and their associated sequelae was conducted.

Purpose: To describe the results of a scoping review of chronic sequelae associated with common foodborne illnesses.

Methods: A comprehensive search was conducted in PubMed, EMBASE, and Web of Science for peer-reviewed articles published between January 1, 2000 and April 1, 2018. Papers available in English, of any epidemiological study design, for 10 common foodborne pathogens (*Campylobacter, Salmonella, E. coli, Listeria, Shigella, Cryptosporidium, Cyclospora, Giardia, Yersinia,* and norovirus) and their associated gastrointestinal- and joint-related sequelae were included.

Results: Of the 6,348 titles screened for inclusion, 380 articles underwent full-text review, of which 130 were included for data extraction. Of the bacterial pathogens included in the search terms, the most commonly reported were *Salmonella* (*n*=95) and *Campylobacter* (*n*=90); *E. coli* (*n*=43), *Shigella* (*n*=41), *Yersinia* (*n*=35), and *Listeria* (*n*=8) all had fewer results. Norovirus was the only virus included in our search with 23 papers that reported mostly gastrointestinal-related sequelae, with reactive arthritis reported once. For parasitic diseases, *Giardia* (*n*=22) and *Cryptosporidium* (*n*=18) had the most papers and no results were found for *Cyclospora*. The most commonly reported gastrointestinal outcomes were IBS (*n*=119) and IBD (*n*=29) and reactive arthritis (*n*=100) or 'joint pain' (*n*=18) for joint-related sequelae. *Salmonella* and *Campylobacter* were most often associated with a variety of outcomes with reactive arthritis (*n*=30 and *n*=23) and IBS (*n*=18 and *n*=21) being reported most often.

Significance: This review showed there are still a relatively small number of studies being conducted to understand specific pathogen/outcome relationships. This scoping review shows where important gaps in the impact of chronic sequelae from common foodborne illnesses still exist and where more focused research would best be implemented.

Poster

P1-44 *Salmonella* Food Poisoning Outbreaks and Climate Factors in South Korea

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Introduction: The outbreak of *Salmonella* food poisoning in South Korea has a long history. The numbers of outbreaks have been variable in recent years, however, its rate is still higher among bacterial food poisoning.

Purpose: The purpose of this study was to evaluate the relationship between the variation of climate factors and the outbreaks of *Salmonella* food poisoning of South Korea.

Methods: We used time series data on food poisoning statistics from the Ministry of Food and Drug Safety of Korea and from Korea Statistics. The climate data measured by the Automatic Weather System from the Korea Meteorological Administration were used. Regression analysis was adopted for trends of *Salmonella* food poisoning outbreaks over the past years (2002 to 2015), and Pearson's correlation analysis was employed to establish the relationship between the climate factors and the outbreaks of *Salmonella* food poisoning in these years.

Results: The numbers of annual outbreaks of *Salmonella* food poisoning have been decreasing with no significance, and *Salmonella* spp. was the second most causative pathogenic bacteria. *Salmonella* food poisoning occurred most frequently in summer, followed by spring, autumn, and even in winter. The annual outbreak of *Salmonella* food poisoning was found to be positively correlated with an average annual temperature, the highest annual temperature, the lowest annual temperature, precipitation, the number of days with rainfall, and humidity (P<0.05).

Significance: The results indicate that there is a strong association between climate factors and *Salmonella* food poisoning outbreaks in South Korea. It should be noted that differently from earlier years, *Salmonella* food poisoning occurred even in winter in the 2000s. Climate change, especially warming climate, is supposed to have affected the pattern of the food poisoning outbreaks.

P1-45 A Large Outbreak of *Salmonella* Food Poisoning Due to Egg White and Possible Preventive Measures

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Introduction: Outbreaks of *Salmonella* food poisoning are problems in many countries. Recently a large outbreak of *Salmonella* food poisoning occurred at food-service establishments including schools in South Korea.

Purpose: This study attempts to summarize information relating to the incident, together with advice on how the outbreak may be reduced or prevented.

Methods: In September 2018, over two thousand students and staff were hit with gastrointestinal symptoms, including hospital admissions after school lunch. An epidemiological investigation by the Korean government was set up to identify the source of infection.

Results: A total of 2,112 students and staff from more than 55 schools across the nation reported symptoms of food poisoning. It was found that the symptoms were significantly associated with chocolate cakes in the school meals. The cakes were manufactured by a small cake maker and distributed by a major food supplier. The cakes were distributed to 175 schools, two kindergartens, 12 restaurants and one children's center. An epidemiologic inspection revealed that *Salmonella* Thompson was isolated from fecal samples and implicated food, in particular egg white, one ingredient of the cake.

Significance: The probable source of the microorganism can be suspected as a group of eggs. In the Korean egg industry, a portion of eggs with cracked shells are used for manufacturing baked goods. Since it is difficult to be free of *Salmonella*, it is recommended that all cracked eggs and their products using for manufacturing should be pasteurized. Egg suppliers/sellers in Korea are not required to have any certification related to handling eggs. Periodic education of the egg suppliers/sellers for egg safety is necessary, such as hygiene of farms and workers in the egg industry, including distributors and retailers. For food safety, relevant standards of hygiene are also necessary in production, processing, handling and transportation. This should be controlled with thorough and regular inspection of facilities and equipment.

P1-46 Occurrence of *Cyclospora cayetanensis* in Florida, 2014–2018

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❖ ***Developing Scientist Entrant***

Introduction: Infections with *Cyclospora cayetanensis* are often linked to consumption of contaminated fresh produce. Infections may be acquired locally or while traveling. The annual number of cyclosporiasis in Florida not linked to foodborne outbreaks is a cause for concern.

Purpose: This study evaluated the role of different types of travel among *Cyclospora* cases in Florida.

Methods: *Cyclospora* outbreak data for 2014-2018 was obtained using the Cyclospora National Hypothesis Generating Questionnaire form. Travel was classified as domestic in- or out-of-state, international, and non-travelers. Infection rates were calculated using population information provided by the US Census Bureau for 2014 to 2017.

Results: There were 277 cases epidemically or clinically linked to *Cyclospora*, and, 44.8% of the cases between 2014 and 2018 were domestically acquired. Among travelers, 12.6% and 26.4% reported domestic or international travel, respectively. Annual travel distributions were similar in most years, except for 2016. For the five years, there were more cases of *Cyclospora* in those who had traveled domestically than internationally. The year 2017 had the highest number of cases with 109, from which 5.5%, 21.1%, and 65.1% traveled domestically, internationally, and not at all, respectively. The counties with the highest rates for 2014, 2015, 2016, 2017, and 2018 were Putnam, Indian River, Flagler, Lee, and DeSoto respectively, with infection rates of 1.4, 0.68, 1.8, 3.1, and 2.7 per 100,000 inhabitants, respectively. The annual infection rates of *Cyclospora* in Florida varied from 0.2 per 100,000 in 2014 to 0.5 per 100,000 in 2018, although the highest rate was in 2017 at 0.6 per 100,000.

Significance: These results revealed that in 2014 to 2018, most cases of *Cyclospora* in Florida occurred among people not reporting international travel.

P1-47 A Systematic Review of Older Consumers' Food Safety Knowledge and Practices at Home

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Introduction: Older adults are a high-risk population for foodborne illness because of weakened immune systems, chronic diseases, and a resulting increased likelihood of complications. In 2017, seniors were estimated to have outnumbered children in Canada, and in the United States, up to 20% of the population will be of retirement age by 2030. Thus, there is a growing concern about food safety as the proportion of elderly increases.

Purpose: A systematic review was undertaken to identify, characterize, and synthesize the published research on the knowledge, attitudes, and practices of older adults (60+) toward food handling in the domestic setting.

Methods: The review consisted of a comprehensive search strategy, relevance screening, and article characterization, risk-of-bias assessment, data extraction, and meta-analysis to synthesize all available studies in this area. The study set out to determine the prevalence of safe food handling knowledge, behaviours, and high-risk food consumption practices among the elderly living at home.

Results: A total of 57 relevant studies published between 1996-2018 were identified. Most studies used a cross-sectional design (86%), were conducted in the United States (58%), and contained self-reported food safety practices (77%). Results from the meta-analysis showed most outcomes had large heterogeneity across studies. Knowledge gaps included older adults' awareness of *Listeria*, and knowledge on the safe operating temperatures of refrigerators. Older consumers had high reporting of refrigerating leftovers within two hours of cooking (85% to 90%, n=6 studies), not using a food thermometer to check whether the food was cooked (9% to 55%, n=6 studies), and consuming undercooked eggs (8% to 50%, n=8 studies).

Significance: Gaps in knowledge and practices were identified which could be used to inform future education interventions. Research gaps were also identified, including investigation of consumption of certain high-risk foods (e.g., soft cheeses, raw fish), and storage practices.

P1-48 Longitudinal Survey on the Prevalence of *Escherichia coli* O157:H7 in Bovine Feces and Slaughtered Carcasses from Selected Abattoirs in Southern Nigeria

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Introduction: Although cattle are considered as the primary reservoir for *Escherichia coli* O157:H7, a foodborne pathogen causing life-threatening diseases and foodborne outbreaks worldwide, there is a paucity of information regarding the epidemiology of *E. coli* O157:H7 in cattle at slaughter.

Purpose: This study investigated the prevalence of *E. coli* O157:H7 in bovine faeces and slaughtered carcasses from selected abattoirs in Cross River State, southern Nigeria.

Methods: A total of 360 fresh samples each of bovine faeces and carcasses were collected from major abattoirs and examined for the presence *E. coli* O157:H7 using standard microbiological methods.

Results: The overall prevalence of *E. coli* O157:H7 in bovine faeces and slaughtered carcasses were 71 of 360 (19.72%) and 107 of 360 (29.72%). With regards to sampling areas, the northern, central and southern senatorial districts had the prevalence of 13.33%, 25.80% and 20.0% from bovine faeces while 22.50%, 38.33% and 28.33% were recorded from bovine slaughtered carcasses respectively. The longitudinal prevalence of *E. coli* O157:H7 from bovine faeces was high in January (29.73%), December (25.00%) and October (23.26%) as 43.24%, 37.21% and 35.00% was recorded in January, October and December from slaughtered bovine carcasses. The ratio of faeces/carcass contamination was highest in September (1:2.3), June and August (1:1.6). There was a significant difference (P<0.05) in the prevalence of *E. coli* O157:H7 from bovine faeces and slaughtered carcass while the faecal/carcass contamination ratio did not differ significantly (P>0.05) among the various sampling areas.

Significance: The presence of *E. coli* O157:H7 from bovine faeces and slaughtered carcass highlight the potential threat to public health. Continuous surveillance of this pathogen and the incorporation of hazard analysis and critical control points (HACCP) in the abattoirs are of immense public health importance.

P1-49 1+1=3: Whole Genome MlST and Whole Genome SNP, a Powerful Combination for Typing and Outbreak Surveillance of *Cronobacter* spp.

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Introduction: *Cronobacter* spp. is linked with serious infections such as meningitis, septicaemia and necrotizing enterocolitis. Although infections are rare, they are often very serious for young infants, leading to death. In 2004, the World Health Organization requested the establishment of a molecular typing method to enable international control of the organism. Nowadays, costs for whole genome sequencing (WGS) are dropping and hundreds of genomes became publically available on NCBI/SRA. Key challenge is to rapidly compute and interpret the relevant information from this growing amount of data.

Purpose: In this study, we compare two subsequent pipelines for high resolution WGS-based molecular typing.

Methods: First, whole genome multilocus sequence typing (wgMLST) is applied to WGS data from all 320+ isolates available from SRA, with the purpose to detect clusters of highly related strains. A cluster defined by wgMLST can then be further characterized by whole genome single-nucleotide polymorphism analysis (wgSNP). SNP variants are detected by mapping the WGS reads to a reference chosen from within the cluster to maximize the resolution. Both analysis pipelines were run on the BioNumerics Calculation Engine, which is fully integrated with the BioNumerics 7.6 software.

Results: Data was analyzed in less than eight hours. Five potentially interesting clusters containing isolates from both clinical and food or environmental sources were identified using wgMLST. The added resolution of wgSNP against an internal reference sequence increased the confidence in the detected clusters, supporting epidemiologists in their source tracking efforts and detection of potential outbreaks. This may open many perspectives for cost efficient food safety and public health monitoring programs.

Significance: The combination of both wgMLST, which allows fast and scalable analysis, and wgSNP, which provides a higher resolution, offers a powerful tool to rapidly provide a robust, portable and high resolution picture of molecular typing data.

P1-50 Whole Genome MlST as a Tool to Screen for Potential Outbreaks Quickly and Easily, Applied to a *Listeria monocytogenes* Outbreak in South Africa

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen, especially in vulnerable patient groups. Complete *L. monocytogenes* genome sequencing has become more routine and increased the number of publicly available genomes on the Sequence Read Archive (SRA) of NCBI. Rapid and automated processing of this data is necessary to ensure a reliable and easy to follow workflow in routine surveillance, reducing the time needed to detect and contain an outbreak. Whole genome or core genome MLST (wgMLST and cgMLST) are particularly useful for this application, as the results are stable and comparable within a species. They are therefore suitable for compiling a database for outbreak screening.

Purpose: This study demonstrates the use of wgMLST for quick detection of outbreaks without prior knowledge of possible epidemiogical links.

Methods: A database containing all isolates submitted to SRA (appr. 25,000) before June 2018 had been previously constructed, containing de novo assemblies and cg/wgMLST allele IDs. Strains marked as originating from South Africa (63 in total) were screened against the complete database.

Results: The isolates were screened against the database in less than three minutes. In the results, we observed five clusters with a clonal relationship and two sporadic strains. A cluster of ST6 isolates containing the majority of South-African isolates was identified. An ST6 isolate had been identified as the

Poster

cause of an outbreak in South Africa linked to deli meats, so this cluster was investigated in more detail. The South-African strains formed a tight subcluster within this group. No evidence of a recent spread of this outbreak isolate from or to other countries was seen.

Significance: We demonstrate that wgMLST is suitable for the quick detection and investigation of outbreak isolates and related strains. This makes it an appropriate technique for global outbreak surveillance.

P1-51 Biosecurity Evaluation and Compliance in Broiler Breeder Farm Units in Southwest Nigeria: Implications for Poultry Farm Workers' Health and Chicken Meat Consumers

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❖ ***Developing Scientist Entrant***

Introduction: Breeder broiler farms (BBFs) occupy a strategic position in the chicken meat production process. There are threats from pathogens but biosecurity measures remain a vital tool to curb these threats.

Purpose: Evaluation of biosecurity compliance levels in BBFs in Nigeria as a measure of risk of infection in the poultry industry, occupational risk to farmworkers and foodborne risk to broiler meat consumers.

Methods: Seventy-two BBFs were selected by the snowballing method, comprising large BBFs (>2500 birds; *n*=47) and small to medium BBFs (≤2500 birds; *n*=25). Using 133 variables in a questionnaire/checklist through observational methods, records, procedures and activities of the farms were used to score the checklist. Scores were entered and filtered in Microsoft Excel, and transferred into SPSS version 15 and Stata software for analysis. Setting a good compliance level at 75% and $P \leq 0.05$, a comparative analysis of compliance was done with large and small to medium farms using univariate logistic regression models and multivariable analyses. Pairwise comparison of interactions between related biosecurity variables was calculated with Pearson's correlation coefficient and *P*-value between pairs of variables within each group.

Results: There is no single farm with 100% compliance, and certain items have zero compliance scores, including: feed delivery site location, dead haul truck access, unscheduled tools and equipment maintenance, irregular farm-level surveillance, lack of sequence in bird inspection or vaccination, and flock movement among others. Overall mean compliance was 22.28±1.40% (CI 95%: 19.52 to 25.04) and 77.72±1.40% (CI 95%: 74.96 to 80.48) failed the compliance test for biosecurity.

Significance: The outcomes have implications for poultry and human health in terms of high risks of infection, occupational hazards, and foodborne infections in consumers of broiler meat. Regulators will have to consider implementation of auditable and monitored biosecurity systems in BBFs to reduce zoonoses from broilers.

P1-52 Prevalence and Serotyping of *Salmonella* spp. in Broiler Production Value Chains and the Environment in Nigeria: Implications for Public Health

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❖ ***Developing Scientist Entrant***

Introduction: The poultry industry is constantly challenged with *Salmonella* contamination and outbreaks. However, there is still a dearth of information on the status of *Salmonella* in broiler chickens and their shared environments with humans in Africa due to lack of intercontinental surveillance.

Purpose: This study surveyed *Salmonella* in the Nigerian broiler production value-chain (NBPVC) to provide baseline information on *Salmonella* surveillance in Nigeria.

Methods: A cross-sectional study was carried out using relevant sample matrices covering six stages of production in 60 sites based on a farm-to-fork approach. Sampling sites were strategically distributed to cover different sectors of the production lines. A total of 1,135 samples originating from 60 sampling sites (breeder-farms, hatcheries, grow-out farms, abattoirs, live-bird markets and retail outlets. Classical bacteriological (culture and biochemical tests using modified ISO 6597) and molecular (*invA* gene detection by PCR) protocols for *Salmonella* isolation were carried out. Identified isolates were serotyped using the Kauffman-White-Le Minor scheme. Obtained data were summarized and subjected to descriptive and Kappa analyses. Prevalence of *Salmonella* isolated per stage and location were expressed as percentages.

Results: The study observed a *Salmonella* prevalence of 55% and 23% based on location and overall samples respectively. *Salmonella enterica* subsp. *enterica* serovar Kingston (27%) and Kentucky (23%) had the highest occurrences among the representative strains (*n*=50). Prevalence of *Salmonella* in live bird markets (100%); hatcheries (57%), breeder farms (56%), grow-out farms (55%), abattoirs (33%), and retail outlets (25%) was observed with no significant differences based on the two confirmatory methods (agreement score = 95.28%; $\kappa<0.0001$).

Significance: High *Salmonella* prevalence and identified serotypes in this study require urgent attention due to food safety, economic, and public health concerns. Regulators will have to consider active surveillance with implementation of an auditable and monitored biosecurity system in NBPVC to target and reduce elimination of *Salmonella* from broilers in Nigeria.

P1-53 Prevalence, Molecular Characteristics and Whole Genome Sequence Analysis of CTX-M Type ESBL-producing *Escherichia coli* Isolated from Raw Milk Cheese in Egypt

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Introduction: CTX-M type extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* have been recovered from different types of food. Here we report the prevalence and the genome makeup of strains isolated from raw milk cheese.

Purpose: To elucidate the prevalence and molecular characteristics of CTX-M type ESBL-producing *E. coli* in Egyptian raw milk cheese (karish cheese), and to determine the resistome and virulome of nine strains by whole genome sequencing and typing.

Methods: A total of 200 samples of karish cheese (25 g each) were screened for cefotaxime resistant *E. coli* using eosin methylene blue agar supplemented with cefotaxime (two mg/liter). Isolated strains were further characterized by biochemical tests, susceptibility to 15 antimicrobials and PCR-based interrogation of a large pool of antibiotic resistance markers. Genomic DNA was extracted from nine select *E. coli* strains using the Qiagen DNeasy kit. Sequencing was carried out on the Illumina MiSeq platform and whole genome sequences were analyzed *in silico* for multilocus sequence type (MLST), antibiotic drug resistance and virulence gene complement.

Results: Thirty-seven CTX-M type ESBL-producing *E. coli* strains were detected from 200 samples (37 (18.5%) of 200). Different types of β-lactamases were identified including, TEM, SHV, OXA and KPC at 75.6, 21.6, 16.2 and 2.7%, respectively. Additionally resistance to tetracycline (*tet A* and *B)* and quinolones (*qnr*B, *qnr*S) were detected in the majority of isolates. MLST typing classified the nine strains subjected to sequencing as follows: ST10 (#3), ST 38 (#2) and one strain each for ST1421, ST 515, and ST361. One strain features a novel ST type. The identified virulence gene inventory encodes different functions (e.g. *eilA*, *lpfA*, *astA*, *gad*, *iss*, *pssA*, and *air*).

Significance: Considering that, raw milk is consumed without further treatment; our results highlight that karish cheese is likely an important vehicle for dissemination of CTX-M type ESBL-producing *E. coli* in Egypt.

P1-54 *Megasphaera elsdenii* and Ruminally-protected Lysine Impact on *Escherichia coli* O157:H7 Prevalence in Finishing Cattle

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❖ ***Developing Scientist Entrant***

Introduction: Feedlot cattle commonly shed the foodborne pathogen *Escherichia coli* O157:H7. *Megasphaera elsdenii* , a lactic acid utilizing bacterium, is administered to cattle to avoid rumen lactate accumulation during forage- to concentrate-based diet transition. The impact of administering *M. elsdenii* on foodborne pathogen shedding has not been explored.

Purpose: The purpose of this study was to quantify *E. coli* O157:H7 prevalence in finishing cattle administered *M. elsdenii.*

Methods: Cattle (*n*=448) were assigned to one of four treatments in a randomized complete design with a two by two factorial arrangement of treatments containing: ruminally-protected lysine (LYS; Kemin Industries, Des Moines, IA) fed at zero or 0.45% of diet dry matter; with or without *M. elsdenii*. Lyophilized *M. elsdenii* was rehydrated in 20 ml anaerobic diluent and administered as an oral gavage providing 1×10^{10} CFU along with a daily feed top-dressing providing 1×10^{7} CFU per animal per day. Rectoanal mucosal swabs were obtained from animals twice before harvest, yielding 896. Swabs were incubated in gram-negative broth at 37°C for six hours, analyzed by immunomagnetic separation (IMS) with anti-O157 beads, spread-plated onto Chromagar O157, and incubated for 18 to 24 hours at 37°C. Latex agglutination was used to presumptively identify suspect colonies as *E. coli* O157 and polymerase chain reaction was used for *E. coli* O157:H7 confirmation.

Results: Accounting for feedlot pen clustering effects ($P<0.01$), there were no effects of lysine, *M. elsdenii*, or the lysine and *M. elsdenii* interaction ($P>0.30$) on *E. coli* O157:H7 prevalence: control, 8.8%; lysine only, 8.2%; *M. elsdenii* only, 10.3%; and both, 10.3%.

Significance: Administering *M. elsdenii* and supplementing cattle diets with lysine did not alter fecal shedding of *E. coli* O157:H7 in feedlot cattle.

P1-55 Molecular Screening for ESBL Genes in *Escherichia coli* Strains Isolated from Livestock and Bivalve Molluscs in Sicily, Italy

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Introduction: ***Escherichia coli*** is mainly a commensal enteric bacterium, and its presence in food or water is an indicator of hygiene and faecal contamination. However, pathogenic *E. coli* strains can be responsible for severe outbreaks, and their antimicrobial resistance is an important issue for public health.

Purpose: A molecular screening for the presence of extended spectrum beta-lactamase (ESBL) genes was performed in *E. coli* strains isolated from Sicilian farms of livestock and bivalve molluscs (mussels and clams) to evaluate the circulation of antimicrobial resistant strains in animals bred for food consumption.

Methods: *E. coli* isolates from livestock (n=225) and molluscs (n=216) were analyzed for the presence of beta-lactamase genes (Tem, Shv, Ctx-MI Oxa; Ctx-M I, Ctx-M II, Cmy II and DHA) through two specific multiplex PCRs. Phylogenetic analysis was performed on ESBL-positive strains.

Results: Of 225 isolates of *E.coli* from livestock farms, 72 (32%) were mainly Tem gene positive with a few isolates carrying one more ESBL genes simultaneously. Among livestock species, a higher prevalence was observed in intensive farm animals. In contrast, the isolates from molluscs showed a prevalence of 68% (147/216 positive) with 18 isolates showing the simultaneous presence of one or more ESBL genes. Phylogenetic analysis showed the prevalence of phylogenetic group A and B1 in livestock isolates, while phylogenetic groups D and B1 and others were detected in the isolates of molluscs.

Significance: Molecular screening of *E. coli* in Sicily showed a lower prevalence of ESBL genes in isolates from free-ranging animals compared to intensive farms. The higher ESBL prevalence in isolates from molluscs bred in small lakes in areas with high human density indicates the big impact of human activity in the spreading of antimicrobial resistance.

P1-56 Public Communication as a Control Measure in Produce-related Multi-Jurisdictional Enteric Illness Outbreaks in Canada

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Introduction: Source identification is challenging in produce-related outbreak investigations due to low product specificity and traceability gaps. The opportunities to implement timely product control measures are limited. Therefore public communications play a significant role as a health protection strategy.

Purpose: Describe the experience of applying public communications in Canadian multi-jurisdictional produce-related enteric illness outbreaks between 2017 and 2018.

Methods: Epidemiologic data were analyzed during multi-jurisdictional outbreak investigations to determine the likely vehicle of infection. Public health communications were disseminated through the web, social media and traditional media. The content of Public Health messaging was modified over the course of each investigation.

Results: Between December 2017 and December 2018 the Public Health Agency of Canada investigated four multi-jurisdictional produce-related enteric illness outbreaks: 3 *E. coli* outbreaks associated with romaine lettuce exposure and one *Salmonella* Infantis outbreak associated with long English cucumber exposure. There were 21 public health notices, 28 tweets, and 14 Facebook posts issued as part of the public communications approach in response to these illness outbreaks. High weekly web (165,000 visits) and social media (1,140,000 Facebook views) traffic was observed during the outbreak periods. Information about safe food handling practices was always provided in the initial notices. Advisories to avoid consumption of the produce item identified as the source of the outbreak were issued in two outbreaks. Public communications were updated regularly as new evidence accrued. A final public health notice notifying the conclusion of the outbreak was posted in all investigations.

Significance: In multi-jurisdictional produce-related enteric illness outbreaks, public communication is often challenged by its content and timing. It provides a key mechanism to allow Canadians to make informed decisions regarding their health. The process of communication has been refined through these experiences and will continue to be considered as a control measure.

P1-57 Chronic Sequelae of Foodborne Parasitic Gastroenteritis: A Systematic Review

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Introduction: *Giardia*, *Cryptosporidium*, and *Cyclospora* are important foodborne parasites causing parasitic gastroenteritis whose disease burden has historically been calculated from acute infections only, as their chronic sequelae have been poorly understood.

Purpose: Evaluate the prevalence and burden of long-term sequelae of select foodborne parasitic infections based on a systematic literature review, and provide estimates of prevalence, severity, and duration of chronic sequelae of parasitic gastroenteritis where data allows.

Methods: A systematic review was conducted of literature identified in PubMed, Embase and Web of Science using either one of 26 terms related to long-term sequelae or 20 terms related to disease burden, and the search terms *Giardia*, *Cryptosporidium*, or *Cyclospora*. Literature from 1/1/2000 to 4/1/2018 was considered for inclusion. Study designs encompassed in the analysis included prospective and retrospective cohort, case-control, cross-sectional, randomized control trial, review, systematic review, and disease burden studies.

Results: The literature search for *Giardia* found 18 articles: six prospective cohort studies, three retrospective cohort studies, one review, one systematic review, six disease burden studies, and one randomized control trial. For *Cryptosporidium*, the search found 13 articles: one prospective cohort study, two retrospective cohort studies, one case-control study, one cross-sectional study, one review, one systematic review, and six disease burden studies. For *Cyclospora*, the two articles found were both disease burden studies. Preliminary results show *Giardia* was associated with irritable bowel syndrome, chronic fatigue syndrome, functional dyspepsia, and reactive arthritis in the included studies, as well as microscopic duodenal inflammation, which may provide a possible mechanism of action. *Cryptosporidium* was associated with ulcerative colitis, persistent gastrointestinal symptoms, and joint pain in the included studies.

Significance: Understanding the incidence, severity, and duration of chronic sequelae of parasitic gastroenteritis will help provide more accurate public health impact of disease burden and economic estimates.

P1-58 Correlating *Salmonella* Isolates: Multi-drug Resistance and Serotype Concordance between CDC PulseNet Illness Clusters and FSIS-regulated Establishment Samples

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Introduction: The United States Department of Agriculture, Food Safety and Inspection Service (FSIS) samples meat, poultry, and processed egg products for *Salmonella* to verify process control in regulated establishments. FSIS evaluates the sampling results to determine if human salmonellosis clusters are associated with FSIS-regulated products.

Purpose: *Salmonella* isolates recovered from samples collected from FSIS-regulated establishments during 2013 through 2017 were analyzed to identify strains that were included in human salmonellosis clusters and multiple-drug resistance (MDR) was assessed as a variable of particular interest to the agency.

Methods: PulseNet provided a list of *Salmonella* isolates recovered from samples collected at FSIS-regulated establishments and included in Centers for Disease Control and Prevention (CDC) PulseNet clusters. The isolates were analyzed by serotype, PFGE pattern, and antimicrobial susceptibility. FSIS isolates were further analyzed by the type of products that were derived from (chicken, turkey, pork, or beef) to evaluate differences of *Salmonella* strains among the products.

Results: During 2013–2017, 19,031 *Salmonella* isolates were recovered from samples collected at FSIS-regulated establishments including the National Antimicrobial Resistance Monitoring System (NARMS) samples, 945 of which were included in 142 PulseNet clusters, representing 28 distinct serotypes. The most common serotypes were *Salmonella* Infantis (18%), Typhimurium (16%), and Heidelberg (7%). Of the 142 clusters, 85 (60%) included isolates from chicken, 42 (30%) from beef, 23 (16%) from turkey, and 23 (16%) from pork. The most common serotypes differed by commodity. Of the 945 isolates, 395 (42%) were MDR and included in 34 clusters. FSIS monitored 109 (77%) of the 142 clusters, 27 of 109 (25%) were investigated, nine of 27 (33%) included MDR strains, and seven of 27 (26%) resulted in FSIS regulatory actions.

Significance: Monitoring *Salmonella* isolates from FSIS establishment samples and focusing on certain strains that are most likely associated with illnesses are critical for early detection of outbreaks possibly associated with FSIS-regulated products, especially for strains with those that are multidrug resistant (MDR).

P1-59 Kitchen Kaizen: Preliminary Findings of a Hands-on Consumer Food Safety Workshop

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Introduction: About one in six Americans will experience foodborne illness. Foodborne illness is highly preventable, yet consumers still engage in risky food handling behaviors. Empowering consumers with the knowledge and resources to engage in safe food handling at home can translate to saving $133 to $391 on average per resident each year.

Purpose: Kitchen Kaizen is a hands-on approach to teach consumers important but less obvious behaviors focused on clean (versus sanitize), separate (not washing raw poultry), cook (calibrating a food thermometer), and chill (refrigerator temperatures) while reviewing core behaviors. Those who attend the workshop will gain a greater knowledge (how's and why's) and confidence to safely handle food at home.

Methods: An educator facilitates a 75-minute workshop that incorporates behavior theory and dialogue learning principles with adults to cover: i) food safety trends, ii) hand-washing, iii) food/appliance thermometers, iv) washing/not-washing, v) cleaning/sanitizing, and vi) leftovers. Each topic is five to 10 minutes in length, where participants have a discussion on a given prompt, receive evidence-based handouts, and have a hands-on activity that they can take and apply safe food handling at home. A pre and post-survey was administered, and a paired-sample *t*-test used SPSS 25.0.

Results: Since 2017, 272 adults participated, 48.53% of the participants completed both surveys (n=132). The majority of respondents identified as female (88.63%), obtained a college degree or higher (57.48%), and most respondents were in a household making $75,000+ (33.3%). Respondents self-efficacy, attitude, and behavioral intention significantly (P<0.05) increased to calibrate a food thermometer, check the refrigerator temperature, make sanitizer, and prepare food safely for themselves and others.

Significance: Results from Kitchen Kaizen show trends that the workshop is creating knowledge and behavior improvements. Participants were provided with materials to help improve home food safety. Wider application to this teaching model in food safety may improve consumer behavior to consistently engage in safe food handling.

P1-60 Third Party Accreditation Final Rule: VQIP and Regulatory Audits, One Year after the ANSI Accreditation

Claudio Gallottini

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Introduction: After the first year that the Accredited Third-Party Certification Rule (ATPC) became effective, we analyzed the results of international market comprehension of the Voluntary Qualified Importer Program (VQIP), Regulatory Audits, and Consultative Audits under the Food Safety Modernization Act (FSMA) .

Purpose: Understanding the ATPC trends, opportunities, and potential for national import chains.

Methods: When ATPC become effective FDA opened the possibility for importers to voluntarily participate in the VQIP program. The results of the first year have been null. The FDA also opened the possibility of Regulatory Audits: to date in the first part of 2019 we made many different Consultative Audits with only one request for VQIP from a European Union importer chain. Therefore we studied the commercial proposals of the first six top ranked Certification Bodies (CBs) in the food world, looking at their web pages.

Results: In all the CBs' web pages, FSMA ATPC appears to be not ready or available to the international food industry sector. The common wording is "*...In preparation for the much-anticipated FSMA certification*" or "*...our solution is 100% FSMA ready*". Another area of confusion is the auditors' qualifications. Ninety-nine percent of all the auditors or trainers used by these CBs are trained only about the Preventive Control Qualified Individual-Human Food Rule, without experience or knowledge about the whole of United States federal food safety laws and regulations (including FSMA and the other six rules).

Significance: Regulatory audits and the whole ATPC could be a strong tool for United States Importers to qualify their foreign supply chain. The lack of awareness in national and international CBs represent a strong obstacle for the success of this FDA ATPC Rule and also for public health purposes.

P1-61 What Do International Governments Tell Consumers about Domestic Food Safety?

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❖ ***Developing Scientist Entrant***

Introduction: Domestic food preparation has been associated with a sporadic incidence of foodborne illness throughout the world. Consequently, internationally, governments have developed sources of domestic food safety advice targeting consumers. To date, there has not been a review of international government based consumer food safety advice, therefore, very little is known about how these sources relate and compare to each other.

Purpose: To identify commonalities and differences between food safety advice from international government agencies, and assess their suitability and adequacy of advice.

Methods: An inclusion criterion was utilized to identify online domestic food safety consumer advice sources from international government agencies (n=14), a content analysis-approach was utilized to assess and compare sources

Results: A total of 494 food safety practices were noted from the reviewed sources (n=14), in which five key food safety themes (cook, chill, clean, cross-contamination, check) were identified utilizing the content analysis approach. These highlighted 47 food safety practices which were present in three or more Government sources. The most frequent food safety practice (n=33) was to ensure perishable/cooked foods are kept refrigerated.

Significance: All government agency websites had many commonalities regarding food safety advice, however, the detail provided to inform consumers how these actions are adequately carried out, or why they need to engage with the practice differed greatly. The inclusion of consumer guidance detailing cooking was lacking.

P1-62 Three Years of the Food Safety Modernization Act: Compliance Data from Europe with a Focus on the Italian Food Industry's Response

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Introduction: We assessed the Food Safety Modernization Act (FSMA) response in European Union countries after three years of requirements being in effect for FSMA rule compliance.

Purpose: Understand the delay of some European Union countries in complying with new FSMA prevention and control requirements.

Methods: Official FDA data published in February 2016 on the FDA web portal showed that at least 30,000 European Union food industries were registered with the FDA. To date, looking at one rule, the Preventive Controls for Human Food, we could count around 3,000 Preventive Controls Qualified Individuals for Human Food (PCQI-HF) trained peoples in all the European Union 28 (official data published by the Food Safety Preventive Controls Alliance). We investigate the reason behind this situation with a direct mailing to 10,000 Europen Union food industries in seven countries (IT, FR, GR, SP, UK, DE, NL).

Results: After three years of FSMA the European Union food industry's responses have taken place at different speeds. From the Food Safety Preventive Controls Alliance official metrics, which are continuously updated, no data are available from France, Germany, Netherlands and the UK. The lack of training in these European Union countries is linked to incorrect understanding of the main changes introduced by FSMA (for example, the Food Safety Culture concept). These changes have been accepted by Spain and Italy to improve the level of food safety standards and use it to push United States export in 2018 at +10% in comparison to 2017.

Significance: FSMA not only has legislative weight but could be an opportunity if well incorporated abroad by foreign food industries. The benefit of this understanding could be achievement of public health goals stated by the FDA in FSMA and its rules.

Poster

Poster

P1-63 Food Safety Careers: Development of an Optimized Recruitment Strategy Using the Social Cognitive Career Theory

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Introduction: The food industry in the United States plays an important role with significant economic contributions as an employer, with an estimated 27,000 establishments employing 1.46 million workers according to the Committee for Economic Development. There is the need for empirical research on best practices for recruitment purposes of prospective food safety students.

Purpose: To determine the most effective method for recruitment through the i) development of three outreach materials using social cognitive career theory (SCCT) as a framework, and ii) comparison of recruitment effectiveness of the materials.

Methods: A southeastern, 18 to 20-year-old target audience participated in this study. The developed outreach materials promoted the Food Science Program at NCSU and included: a recruitment website, a video, and an in-person presentation. A survey research instrument of a five-point Likert scale and adapted STEM-semantics-survey questions was administered before and after each recruitment method. Interest and attitudes towards food safety careers were measured. Three treatment groups were used, with *n*=66 participants each, *n*=198 total. Different combinations of the three aspects of the SCCT (self-efficacy, outcome expectations, how to apply personal goals) were also compared to detect if a component was more influential.

Results: One-Way ANOVA was used to test for significant differences in the change of response at a confidence level of 90%. The standard video (0.97±1.44) was significantly more effective than the website (0.36±0.97) at increasing understanding of what food safety careers are like (*P*=0.017) on a five-point Likert scale. The in-person presentation was found to have the greatest impact on attitudes towards food safety careers.

Significance: Each recruitment presentation improved feelings of self-efficacy towards food safety careers, increased knowledge about food safety careers, and improved overall attitude towards food safety careers: the SCCT is an effective method to use in recruitment presentations.

P1-64 Food Safety Knowledge and Safe Food Handling Confidence among Pregnant Women in Louisiana

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Introduction: Compared with the general population, pregnant women are more susceptible to foodborne illnesses because they undergo significant physiological changes to accommodate their developing fetuses including immunological as well as hormonal changes. The down-regulation of the immune system prevents rejection of the fetus which also increases pregnant women's susceptibility to foodborne pathogens and risk of developing more severe outcomes from those pathogens.

Purpose: i) To assess pregnant women's confidence to handle food safely, and ii) to assess their perception of food safety knowledge.

Methods: A questionnaire was designed to assess the pregnant women's confidence to handle food safely and their perception of food safety knowledge in the state of Louisiana. Data were analyzed using descriptive statistics.

Results: Two hundred twenty-two questionnaires were collected. The majority of participants were white (75.0%) between the ages of 26 and 30 (45.8%), with a four-year college degree (42.5%). The dependent variable, Confidence to Keep Foods Safe for Consumption, was measured on a six point scale. The overall mean value for the group was 5.19 (SD = 0.81). The independent variables had overall mean values ranging from 5.32 (Separating and Cleaning Knowledge; SD = 0.71) to 4.52 (Using a Thermometer Knowledge; SD = 1.43. General Food Safety Knowledge (M = 4.79; SD = 1.11) and Food Storage Knowledge (M = 4.64; SD = 0.98) had relatively similar means.

Significance: To protect pregnant women and their fetuses from the serious consequences of foodborne illness, food safety education is critical. Studies to assess the confidence, knowledge and behavior of the pregnant women will be beneficial for the food safety education.

P1-65 Food Handling and Causes of Food Waste in Urban Mexican Households

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Introduction: Food quality and safety throughout the supply chain depends on adequate cold chain management. Deficiencies in food handling causes biological deterioration and waste of foodstuffs.

Purpose: This study is focused on highlighting the weaknesses of household food handling that lead to unnecessary food waste or discarding of food.

Methods: The target was an urban population of Guadalajara, the second largest city in Mexico. Fieldwork consisted of gathering information from an in-person survey in public places. A total of 50 interviews were conducted. Each member of the sample was randomly selected; 66% were women, 68.8% had a college degree and over 50% were within the highest income level. The data set was analyzed using the frequency procedure for categorical data and the options of the answers were compared using chi-square at a *P*-value of 0.05.

Results: More than 72% eliminated food from their refrigerators for cleaning *(P*<0.0001*)*, and 68% believed that food waste did not increase environmental pollution (*P*<0.0001). Around half of the people interviewed indicated that the main reason for food waste was food decomposition after being cooked in any way (*P*=0.0003).

Significance: The results indicate that consumers in urban Mexican households should be trained by private, academic and government sectors to reduce unnecessary food waste and to be aware of the negative environmental impact of wasting food.

P1-66 Consumer Attitudes Toward Food Safety Risks in Lebanon

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Introduction: Internationally, the home is associated with the incidence of food poisoning; food safety education is required to improve consumer food-handling behaviors. Understanding cognitive behavioral influences, such as attitudes, is important for the development of educational initiatives; to date, little is known about Lebanese consumers' attitudes towards food safety.

Purpose: This study aimed to explore Lebanese consumers' attitudes towards food safety risks and perceptions associated with acquiring food poisoning in Lebanon

Methods: A quantitative, self-complete food-safety questionnaire was distributed to a convenience sample (*n*=97) of consumers in Beirut, Lebanon. Attitudinal responses (*n*=21) were given upon a five-point Likert-type rating scale; the perceived likelihood of getting food poisoning was assessed using a variation of a visual-analog-scale.

Results: Overall, 60 to 68% of consumers believed they knew all of the food preparation/storage practices required to ensure food safety, however, a similar proportion (62%) believed their current food-safety behaviors needed improvement. Despite this, over half (51%) were confident that the way they prepared food at home would not cause food poisoning. Lebanese consumers perceived getting food poisoning from food prepared in the home was less likely (32%) than from food-service establishments/outlets such as sit-in restaurants, takeaways and market stalls (61 to 68%) demonstrating a perception associated with optimistic bias. Data showed that 51% thought food safety is not a priority for consumers in Lebanon with 63% believing that electricity interruptions do have an impact upon food safety and 58% suggesting that food safety in the home is affected. Nearly half of respondents (45%) were concerned about the safety of home drinking water and 50% believed this has impacts upon food safety.

Significance: Consumer attitudes towards food safety risks in Lebanon were variable; data indicated awareness of increased food safety risks due to the countries' unique public health infrastructure. Failure to associate the home with food poisoning incidence may hinder educational efforts; data from this study can inform the development of educational initiatives designed to raise awareness of food safety issues, bring about behavioral improvement and reduce the risk of food poisoning in Lebanon.

P1-67 Leaving Established Pedagogy: Understanding the Educational Needs of Generation Z to Better Craft Food Safety Education

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❖ ***Developing Scientist Entrant***

Introduction: Substantial literature has been focused on how Generation Z engages differently in the classroom and workforce than preceding generations. The body of literature on the subject of what motivates this population and how it learns is growing exponentially; however, to date, no research has been done on Food Science and related disciplines.

Purpose: It is imperative to understand the next generation of food scientists and food production workers. Given the changing regulations and increased demand for food safety, it behooves instructors and instructional designers to seek to understand and potentially change their pedagogy to maximize the efficacy of their teaching on Generation Z.

Methods: To better understand this group, interviews were conducted with 16 undergraduates randomly selected from across the country. They were asked to share their educational preferences- how they best learn and what motivates them to incorporate food safety culture into their practices.

Results: Analysis of themes showed a common desire for community, perceived importance, and participation. Technology, while mentioned, was most often an assumption rather than a stated desire.

Significance: Acknowledging these needs as new curriculums and training are developed is important if the target population is Generation Z. Working and presenting in mediums familiar to Generation Z will better engage them in learning environments.

P1-68 Evaluation of Food Safety Recommendations in Egg Noodle Online Video Streaming and Blog Recipes

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❖ ***Developing Scientist Entrant***

Introduction: The Web is frequently used to share recipes and food preparation techniques. Free video streaming, food blogs, and free online forums enable consumers to easily post and share recipes. Without screening, videos and blogs could promote food handling behaviors which may lead to a higher risk for foodborne illness.

Purpose: Evaluate the food safety content and potential risk of popular online video streaming and blog recipes by using 'making egg noodles' as an example.

Methods: Keywords "egg noodles," "recipes," and "homemade" were searched on YouTube, Google, and Pinterest to collect online shared video and blog recipes. Criteria of selection were developed to measure material popularity and relevance to the project. Videos were sorted by view count, and only a view count of over 200 was selected. No view minimum was established for blogs; this information is not available on every blog. The search yielded 49 YouTube videos and 59 blogs on this topic. Videos and blogs were reviewed and coded for 35 items including ingredients, processing procedure, food safety information, and environmental cleanliness. Food handling practices and safety violations per USDA guidelines were also recorded.

Results: Food safety recommendations were seldom provided in either video (6%) or blogs (10%); while blogs contained fewer unsafe food handling practices than videos. Most kitchens, 82%, were visibly clean in videos. Many videos (41%) contained at least one cross-contamination event; cross-contamination events were not specifically described in blogs. Approximately half videos and 3% blogs mentioned eggs from backyard chickens, which were promoted by individuals as "extra fancy" or "healthy." Pets were observed in the background of several videos, in the kitchens.

Significance: The mishandling and lack of food safety recommendations on popular recipes show that efforts are needed to develop strategies for online shared-recipe use and to promote safe food handling to consumers.

P1-69 Evaluation of Food Safety Curricula Effects on Students' Food Handling Behaviors: An Observation Study

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❖ ***Developing Scientist Entrant***

Introduction: More youth are becoming involved in food preparation at home; however, previous studies showed that youth lack knowledge of and fail to practice safe food handling. Food safety curricula were developed and evaluated based on self-reported knowledge and actual behavior change. Observation can be used to address the discrepancies between self-reported and actual behaviors. Stationary cameras are commonly used for observation, but wearable cameras are an emerging alternative.

Purpose: i) Compare the effectiveness of an informal and an academic-standard aligned curriculum in improving students' food safety knowledge and food handling practices; ii) explore the use of GoPro wearable cameras as an alternative to stationary cameras for data collection.

Methods: One-hundred two high school students were divided into two groups; each group was taught using either informal or academic-standard aligned curriculum. Pre- and post-surveys measured knowledge and self-efficacy changes. Stationary and GoPro cameras collected pre- and post-behavioral data as students cooked a meal. SPSS was used to analyze survey data by Student's *t*-test, significance level 0.05. Videos were reviewed for 13 behaviors

Poster

including hand washing, glove use, and cross-contamination. Camera advantages and limitations were assessed by evaluating environment capturing capabilities and audio quality.

Results: Student knowledge changed significantly for informal (P=0.015) and aligned (P=0.006) curriculum, but the change between curricula was not significantly different (P=0.742). Students self-reported being confident they can prepare food safely, but when observed, students still cross-contaminated and failed to wash hands. GoPro cameras were more effective at monitoring hand washing and activities away from the cooking stations. Stationary cameras captured more cooking station activities, including GoPro-wearers' body touches.

Significance: This study highlights the need for behavior evaluation beyond self-reported knowledge assessment and demonstrates the synergistic effect of utilizing stationary and GoPro cameras for observation data collection.

P1-70 Food Safety in the Classroom: Evaluation of Curriculum Alignment to State Standards Using the Delphi Method

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❖ ***Developing Scientist Entrant***

Introduction: Classroom food safety education in the United States public schools is declining with nationwide elimination of many family and consumer science courses. Adaptation to changing academic standards necessitates incorporating food safety topics into science and agriculture disciplines. However, many food safety programs require adaptation to academic standards prior to classroom implementation, presenting a challenge to educators with limited food safety knowledge.

Purpose: Use the Delphi method to evaluate a food safety curriculum developed by authors and aligned to "*Indiana State Academic Standards for Agriculture Life Science: Food*" for high school students.

Methods: The food safety curriculum allows students to assume quality assurance team member roles in a fictitious food company and provide science-based solutions to food safety problems. The Delphi method, which uses expert panel evaluation to determine consensus, was employed. A panel of 20 education experts evaluated the curriculum via an anonymous online survey. The survey included curriculum alignment to academic standards, predicted student engagement, and ease of implementation. Experts chose the degree to which they agreed with each survey statement using a five-point Linkert scale, and overall consensus of expert opinions was evaluated.

Results: Experts (73%) agreed that food safety education is important and the curriculum evaluated aligns with academic standards. They concurred that the food safety curriculum needs to be relevant, rigorous, and promote critical thinking and problem-solving. One expert stated, "...bringing in more science-based curriculum helps make this [food safety] content more relevant to students...the level of rigor and detail in this curriculum helps make it more interesting and applicable to students..." Another stated, "The [HACCP] activity sheet is intense and really involves the student to use critical thinking and problem-solving skills."

Significance: This study showcases how the alignment of food safety curriculum can be accomplished to demonstrate food safety relevance in students' lives and career development.

P1-71 Evaluation of Story of Your Dinner Education Campaign Video and Blog-style Recipes

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Introduction: Proper home food preparation is one of the steps to protect consumers from foodborne illness. Story of Your Dinner, a consumer food safety education campaign, was developed and initiated by the Partnership for Food Safety Education (PFSE).

Purpose: To evaluate the effectiveness of the campaign video and the blog-style recipes containing food safety recommendations.

Methods: Part I: Evaluation of the campaign video. Food safety educators were recruited to identify consumers nationwide to view the campaign video and respond to pre- and post-surveys, which consist of self-risk assessment, perceived behavior control, and food safety knowledge questions. Part 2: Evaluation of the blog-style recipes. Consumers were recruited at Purdue University in Indiana. They received either a chicken recipe on a blog-style recipe card or video the first week and the other format the second. They were required to prepare the dish as instructed in the recipes and complete online questionnaires.

Results: Part I, 81 consumers (60% male, 40% 35 to 54 years old) participated. They demonstrated significant knowledge and practice increase in: "not washing raw meat under running water" (33% to 86%), "the maximum time food should be allowed to sit unrefrigerated" (56% to 73%), and "the recommended temperature range for the refrigerator" (49% to 64%).

Part II, 23 consumers participated. Most (86%) found both recipe formats were easy to follow and the amount of food safety recommendations was considered just about right (64%). The majority of consumers noticed the difference in food safety recommendations between the video and card, like "washing hands" with 20 seconds (video) or without (card). More consumers were aware of the message of "using thermometers" from recipe card than video.

Significance: The campaign video increased consumers' food safety knowledge and self-report practice compliance. Recipes with food safety recommendations were effective tools to emphasize recommended practices.

P1-72 Evaluation of Food Safety Education among Indiana Veteran Farmers

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Introduction: Farming is a potential career for military veterans. Government resources are available to help veterans' transition to farming. However, relatively little research has been conducted to understand the challenges and opportunities faced by veteran farmers. Veteran farmers, particularly those operating small acreage farms, may struggle to balance profits and the increasing food safety regulatory requirements.

Purpose: Evaluate the attitude, practice, and knowledge among veteran farmers towards food safety, and identify major barriers to food safety education.

Methods: This study used a web-based survey (Qualtrics, Provo, UT) to evaluate farm food safety knowledge, attitude, and practice of veteran farmers and focus groups to identify major barriers to food safety education. The survey was composed of six blocks, including demographic characteristics, military background, farming practices, food safety knowledge and attitude, and barriers to food safety education. It was distributed to an e-mail listserv from the Farmer Veteran Coalition Chapter of Indiana. Data were analyzed using SPSS.

Results: Thirty-four Indiana veteran farmers completed this online-survey, and 62% of the veteran farmers considered themselves extremely comfortable or somewhat comfortable with their food safety knowledge. However, among those currently farming (n=22), only six farmers had food safety training. Of the thirteen farmers who grew produce, most of them (77%) did not have a food safety plan for their farm. Less than eight percent of them collected water

and soil samples for safety testing, and no action was taken when a field was contaminated with flood water. The major barriers to food safety education identified are lack of learning time, an overwhelming amount of information, and lack of educational materials.

Significance: The findings shed light on the veteran farmers' barriers to food safety practices and education programs. It will guide extension educators and policymakers to develop audience-targeted food safety programs for veteran and other socially disadvantaged farmers.

P1-73 What is It like to Have a Shared-use Kitchen: A Pilot Study with Young Adults

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Introduction: A recent national survey reported that nearly one-third of United States adults lived in a shared household. Food safety situations and perception of roommates' food handling in shared-use residential kitchens have not been adequately explored.

Purpose: This pilot study uses a young adult population to examine the effect of living with or without roommates on the perception of kitchen cleanliness, food safety knowledge, and practices.

Methods: An online survey (Qualtrics, Provo, UT) was developed containing questions about demographics, the perception of kitchen cleanliness, food safety knowledge, and practice. Upon approval of Purdue University IRB, the survey link was sent to a listserv of all registered undergraduate and graduate students at Purdue University, West Lafayette.

Results: A total of 2,214 students (60% female, 79% 18 to 24 years old) completed the survey. Students believed that their safe food handling practices were better than their roommates'. Most students (66%) were confident about their own practice to keep the kitchen clean but fewer (25%) were confident about their roommates'. The past experience affected students' perception of shared-use kitchens and roommates' practices. Many believed that living with roommates meant the kitchens were less clean. Over half (56%) of students who lived with roommates believed that kitchen cleanliness would be better without roommates. However, fewer students who live alone (35%) reported that the condition would be worse if they shared kitchens with roommates. Over 90% of students responded that they were willing to improve their safe food handling practices and communicate with their roommates if materials were available.

Significance: This study sheds light on shared-use kitchen situations including subsidized housing. The material on home kitchen food safety and communication skills with roommates should be developed to improve the condition of the shared-use kitchens and enhance the efficacy of consumer food safety education.

P1-74 Development and Formative Evaluation of a Social Media Intervention Design

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Introduction: With an increase in the use of social media platforms to obtain information, Safe Plates Food Safety Information Center was created to provide evidence-based food safety information to engage consumer audiences and provide an open-learning environment.

Purpose: The purpose of this exploratory research study was to evaluate social engagement, interaction and reach among consumers and food safety professionals during two coordinated social media outreach campaigns.

Methods: The Transtheoretical Model and the Integrated Behavioral Model served as theoretical frameworks for program planning and implementation for two campaigns. The first focused on hurricane disaster preparedness and recovery, and the second focused on holiday food safety. Sixteen information sheets, 63 informational images, nine memes and three blog posts were shared on Facebook, Twitter and Instagram**.** Hootsuite was utilized to track social media presence, reach and engagement. A formative evaluation was also conducted with food safety extension professionals who served as sharing nodes.

Results: Over the course of the two campaigns, 220,520 individuals were reached including 1,402 shares or retweets of disseminated food safety information. Over 370 posts on Facebook, Twitter and Instagram reached 138,410 people during the localized disaster preparedness campaign. During the holiday campaign, 184 posts reached 82,110 individuals. Qualitative data gleaned from food safety professionals suggested that the social media approach filled a resource gap for their clientele. Food safety extension professionals were more likely to share posts and interact on Facebook (compared to Twitter and Instagram). They also reported that having hardcopy versions of information sheets during power outages was important.

Significance: Insight was gained on social media intervention during two coordinated campaigns. Increased engagement and spread of evidence-based resources were achieved. Findings emphasize a need to continue development and testing of social media strategies for consumer-focused food safety messaging and a niche for training other food safety extension professionals.

P1-75 Interactive Food Safety Investigation Challenge Engages Post-Secondary Students in One Health Concepts

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Introduction: Prior research studies demonstrated the utility of foodborne disease investigations and the interactive challenge format for engaging students and exercising critical thinking and communication skills.

Purpose: Student engagement in One Health concepts was evaluated after completion of a novel interactive mock foodborne disease investigation in which data clues were presented as manipulatives.

Methods: Post-secondary pre-veterinary medicine and animal biosciences (92%) and food science (seven percent) students (n=137) in an introductory animal and food sciences laboratory course utilized the investigation with IRB approval. Students received a brief written introduction to foodborne illness, an outbreak scenario, and tasks to complete in groups for various phases of the investigation including epidemiology, laboratory, environment, traceback, recall, and prevention. Successful organization, compilation, and interpretation of data clues yielded a numerical code to unlock a combination and yield a small reward. Groups presented findings and answers to critical thought questions on their investigative role. Visuals supported students' presentations and connected concepts during debriefing. Assessments included a subjective student survey on engagement and knowledge gains and an objective measure of content understanding through a final exam.

Results: Students rated familiarity with the subject prior to the exercise as "some" (44%) and "a little" (48%). Students agreed or strongly agreed that the exercise increased their understanding of outbreak investigations (92.7%), safe food production (90.5%), and environmental water as a transmission vehicle (86.9%). Volunteered learned concepts indicated enhanced appreciation for the complexity of food safety. Students agreed or strongly agreed that the exercise format was enjoyable (77.4%) and cited the clues and group interaction among the most enjoyable features. Correct response rates for final exam questions were 82% or greater for 72% of the questions and indicated understanding of overarching concepts.

Significance: These data demonstrate the value of a foodborne disease investigation presented in a challenge game format for engaging students in one-health concepts.

Poster

Poster

P1-76 How Does the Food Safety Knowledge of Student Dietitians Compare at a University in Wales, Lebanon and Ohio?

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Introduction: Registered dietitians (RDs) are the only healthcare professionals regulated by law to assess, diagnose and treat dietary/nutritional problems. Given that food needs to be safe and nutritious to maximize food-related health and wellbeing; food-safety is part of the dietetic-curriculum designed for the training of RDs. Despite this, gaps in RDs food-safety knowledge have been identified; furthermore, data detailing student-dietitians food safety knowledge is limited.

Purpose: Assess and compare food safety knowledge of student-dietitians in three accredited dietetics programs in utilizing different food safety teaching strategies.

Methods: Food-safety questionnaires were completed by student-dietitians in accredited university programs in Beirut, Lebanon (*n*=30), Cardiff, UK (*n*=78) and Columbus, Ohio (*n*=104).

Results: From the 212 student-dietitians that participated, 79% recalled having received food-safety training/education, recall differed significantly ($P<0.05$) between institutions (Cardiff 100%; Beirut 97%;USA 58%). Student-dietitians in Cardiff participated in a one-day food safety training award, and students in Beirut attended food-service practicums and food-microbiology lectures, whereas in Ohio, students completed microbiology and foodservice sanitation courses, and 43% completed ServSafe certification. Foodborne pathogen awareness was different in all three regions ($P<0.05$). The awareness among students in Beirut was the lowest for each pathogen, students in Columbus had the highest awareness of *Campylobacter* and *Clostridium* while Cardiff students had the highest awareness of *Listeria*, *E. coli* and *Staphylococcus.* Although the majority indicated awareness of food-safety practices, significant differences were determined ($p<0.05$) by the institution. The majority of students (Beirut 100%; Ohio 99%; Cardiff 89%) were aware of the need to use a meat thermometer to check core temperature. Knowledge of the 'use-by' date indicating food safety was significantly greater among student-dietitians in Cardiff (81%) than student-dietitians in Beirut (30%) and Ohio (74%). Ohio students had the lowest awareness of cross-contamination practices such as washing raw poultry (39%), compared to Cardiff (75%) and Beirut (47%).

Significance: Differences between institutions may suggest teaching-approach affects knowledge retention. There is a need to determine the best practices in teaching student-dietitians food safety and explore the interpretation of dietetic curriculum requirements in institutions that deliver accredited training.

P1-77 Sport and Exercise Nutritionists' Perceptions of Food Safety Risks among Athletes

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Introduction: Food safety is essential for athletes as they are reported to be susceptible to infection after acute exercise. The incidence of foodborne illness among athletes participating at events has frequently made media headlines in recent years. Sport and exercise nutritionists (SENs) currently provide food-related advice/information to enable optimum nutrition for performance. However, the role SENs play in identifying and mitigating the risk of foodborne infection for athletes requires investigation.

Purpose: To identify SEN's perceptions of athletes' food-preparation practices and food-consumption habits that may increase risks associated with foodborne illness.

Methods: SEN working with elite athletes (*n*=23), participated in a series of focus groups (*n*=3). Each group discussion followed a structured route and included perceptions of food preparation/consumption associated with key scenarios (home/training/traveling and competing away from home).

Results: SEN reported food-preparation/consumption habits unique to athletes that may increase the risk of foodborne illness. Advanced preparation, cooking and prolonged storage were unsafe behaviors frequently indicated. SEN attributed responsibility for food safety to individual athletes and food providers (athlete villages/hotels) during competitions; however, they also perceived their contribution for the prevention of food-poisoning to be "*a very high priority*" and athlete illness from food-poisoning was reported as "*a big concern*". Concern was particularly high when athletes competed away from home when *"at the highest risk"* due to limited access to appropriate refrigeration facilities, food sources, food-preparation/storage practices were *"out of [SENs] control*". Consequences of foodborne illness were identified as days of lost training, it was discussed that one of the main objectives of SEN *"is to minimize days lost at training through injury and illness"*.

Significance: SEN play an important role in advising food-preparation/consumption practices to athletes and in reducing the risk of foodborne illnesses. This study identified SEN's awareness/concern for athlete food safety and implementation of unsafe behaviors. Further research is required to determine SEN food safety training to facilitate the delivery of sufficient food-safety information to athletes.

P1-78 Utilizing Remote Covert Observation in Food Manufacturing and Processing Environments to Assess Hand Hygiene Compliance

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Introduction: Ensuring hand hygiene (HH) compliance in food manufacturing/processing environments is of utmost importance for food safety. However, assessment methods can influence the validity and reliability of information that can be captured. Although informative, food safety cognition (e.g., knowledge/attitudes/self-reported practices/intentions) are not indicative of actual behavior and may be subject to biases. Similarly, researcher presence in overt observation of behavior can cause reactivity bias, whereas remote covert observation (using CCTV cameras) can provide repeated and comprehensive analysis over a sustained period where familiarity with existing workplace CCTV cameras may reduce reactivity bias.

Purpose: This mixed-methods research approach explores the use of covert observation in food manufacturing/processing environments.

Methods: The study combines a desk-based review of professional food handler food safety studies (*n*=20), interviews with managing directors and technical managers/supervisors from food-manufacturing/processing business (*n*=11), an evaluation audit of CCTV cameras (*n*=122) in food manufacturing/processing businesses (*n*=3) and structured remote covert observation of HH practices in food businesses (*n*=2).

Results: Food safety cognition from survey-based methods were most frequently included in food-handler research studies (80%); observational data, particularly from manufacturing/processing environments, were lacking. Interviews indicated positive attitudes toward utilizing covert observation to assess HH compliance. Although food businesses had CCTV cameras in operation, however, these were predominantly used for security or monitoring productivity; none had resources or time to conduct frequent/structured observations. The audit identified that 66% of HH areas (*n*=24) were featured in CCTV, however, not all provided viewpoints to facilitate meaningful observation of HH compliance. The covert observational analysis in a bakery sector manufacturers determined that only 2% of HH attempts (entering production) were compliant with company protocol. Another investigation in a ready-to-eat manufacturer identified significant differences ($P<0.05$) between compliance with HH behaviors in high-care and high-risk areas.

Significance: Remote covert observation is a useful yet underutilized resource that provides valuable insight into actual food safety practices and HH compliance in food manufacturing/processing environments. The study has identified current HH practices and characterized site-specific issues that may result from organisational subcultures.

P1-79 Aligning Food Safety Culture Assessment Tools with the Global Food Safety Initiative's Position: A Comparative Analysis

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Introduction: Reliance on complex supply-chains to consistently deliver safe-food in dynamic political climates suggests the omnipresent food safety culture focus has never been more important. Definitive guidance demonstrating 'good' culture in practice is rare, thus, the recent Global Food Safety Initiative's (GFSI) position-paper, offering a compelling framework indicative of food safety culture excellence, is a welcome addition to this subject-field.

Purpose: This study conducted a comparative analysis of the tools available to assess food safety/organisational culture conducive to food-manufacturing environments in parallel with attributes included in the GFSI framework.

Methods: Electronic searches utilising academic databases facilitated collection of studies incorporating tools developed to assess and evaluate food safety culture. Methods were scrutinised prior to analysing content which facilitated comparison of applicable tools to the key dimensions (and sub-components) identified in GFSI's 'A Culture of Food Safety' position paper (vision/mission, people, consistency, adaptability, hazard/risk awareness).

Results: Six tools were identified as meeting predefined inclusion criteria. Mixed methods (in various combinations) included self-report surveys, interviews, observations, questionnaires, focus-groups, environmental and performance metrics; all (*n*= 6) triangulated data gathering/analysis to control for confounding factors (e.g., social-desirability or systematic bias). Two studies were predominantly developed for commercial purposes and thus precise details were unavailable. Three studies reported findings following application in food-manufacturing/processing at single locations (Canada, Belgium, Zimbabwe). While each study demonstrated high research standards none of the tools address every attribute contained within the GFSI framework.

Significance: While this novel review identified adequate tools to support the application of GFSI's guiding framework to a food-manufacturing environment, none address every dimension. Further validation in additional geographical locations (recognising national culture and organisational demography influences) alongside increased scrutiny of policy/document quality (against which participatory mechanisms are indirectly examined) may add to the valuable work already undertaken.

P1-80 Seeing is Believing: CCTV Perspectives in Food Manufacturing

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Introduction: Closed circuit television (CCTV) in slaughterhouses in England has recently (November 2018) become a mandatory requirement for monitoring regulatory compliance and animal welfare. Such a requirement is not mandatory in food and drink manufacturing and processing businesses; however, surveillance systems are sometimes installed for monitoring safe food practices, security or simply to encourage workforce productivity.

Purpose: This study aimed to evaluate CCTV camera positions in a food-manufacturing business across three subsidiary sites (including raw meat, cooked meats and ready-to-eat food sectors) to assess the field-of-views and potential surveillance system refinement.

Methods: A total of 122 onsite cameras were assessed by considering each camera viewpoint individually via company surveillance software. Recorded determinants included food hygiene behaviour, security, production-workflow and vantage points.

Results: For the food manufacturing business under study, CCTV was utilised for security, food safety (e.g., hand-hygiene behaviour) and workforce monitoring. From a security perspective, 25% of cameras focused on vulnerable access/egress points (e.g., main entry doors/corridors) and personal security (e.g., staff lockers) while 35% of cameras were positioned in stores (ambient/refrigerated) and dispatch/warehouses; areas populated the least by food operatives. Hand sinks (at point of entry/during production) appeared in eight percent of camera field-of-views, but only five percent offered monitoring perspectives on precise food handler behaviour. With minor camera-angle adjustment, a further seven percent could provide increased hand hygiene observation opportunities during production (providing coverage of 16 of 24 hand sinks present).

Significance: While not a substitute for management presence within production, CCTV if strategically positioned can offer substantial insight into workflow processes, inform training and proactively indicate health, safety and food hygiene malpractices. A well-designed system, modified and adapted as production layouts change, offer food operators evidence of due diligence and compliance in the event of an investigation. Conducting regular and structured CCTV assessments are practical and may identify field-of-views that are ineffective, duplicated or defunct, releasing cameras for valuable use elsewhere in the business.

P1-81 Overcoming Biological Adsorption Limitations of Bacteriophages through Use of Short Tail Fibers Capable of Targeting Highly Conserved Core Regions within Bacterial Lipopolysaccharides

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❖ ***Developing Scientist Entrant***

Introduction: Irrespective of current available technologies, there remains a steadily increasing global threat from bacterial contaminants in food and water, and an overall financial burden from spoilage-derived food waste. Compared to current methods, bacteriophage-based separation and detection assays have a multitude of superior attributes that have yet to be fully harnessed due to biological hurdles (e.g., host range specificity). While model organism T4's long tail fibers (LTF) narrowly target *E. coli*'s OmpC its short tail fibers (STFs) adsorb to the host's conserved lipopolysaccharide (LPS) inner core.

Purpose: The objective of this study was to demonstrate a new approach to broadening bacteriophage host adsorption capabilities by harnessing the endogenous ability of phage STFs to bind to highly-conserved inner-core regions within host LPS, while also preventing host resistance mediated by non-essential surface mutations.

Methods: T4's STF gene or LTF genes were expressed as a fusion protein with an upstream green fluorescent protein (GFP). Both were cloned using standard techniques. GFP-STF and GFP-LTF protein production was achieved using an IPTG-inducible element. Subsequent isolation of these protein fusions was then done using metal chromatography via N-terminal his-tags. Using fluorescent microscopy, adsorption capabilities were compared to one another using adsorption assays across the ECOR reference library, a collection of 72 *E. coli* strains.

Poster

Poster

Results: We expressed the GFP-LTF and GFP-STF at 35 mg/l and 160 mg/l, respectively. Both tail fibers bound ECOR strains outside T4's host range, suggesting the barrier to T4 infection in many of the *E. coli* strains lies with host defenses and not adsorption alone.

Significance: Bacteriophage-based technologies can be harnessed to provide cost-effective, accurate, rapid, and practical prevention or diagnostic tools within food safety, yet are hindered by host-range limitations.We present findings suggesting that the barrier to infection for many bacterial targets may lie with host defenses and not adsorption alone.

P1-82 Engaging Undergraduate Students in the Importance of Food Microbiology and Safety

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❖ ***Developing Scientist Entrant***

Introduction: Despite the importance of STEM disciplines, the interests and scores of United States students in this field seems to be lagging every year. Food science is the perfect tool to enhance STEM education, because of the universality, cultural importance and scientific diversity of food itself. Strategies to capture the attention and creativity of students to solve critical supply and food safety challenges need to be used in classroom settings.

Purpose: Our study focused on the assessment of different teaching tools to engage undergraduate students into the importance of food safety and microbiology.

Methods: During the Food Microbiology laboratory class (60 undergraduate students), three engagement strategies were used along the semester: outbreak case studies, agar art and a group research project. Case studies were presented through short stories to teach microbiological and epidemiological principles and practices. An agar art contest was conducted as a way to learn and highlight bacterial morphological diversity. Finally, students were challenged with a research group project using two ready-to-use plating alternatives to validate food preservation strategies.

Results: Quizzes, laboratory notebook and exams were used to evaluate learning outcomes. By the end of the semester, 89% undergraduate students learned about foodborne pathogen characteristics, how to differentiate symptoms, onset times and identify possible contamination vehicles through case studies in-class discussion. The agar art contest was accomplished while studying differential media and isolation technique of morphological diverse colonies. During the final research project presentations, students demonstrated a statistical correlation between the two enumeration techniques applied in the validation studies and no statistical difference between the methods ($P<0.05$). They also offered several creative solutions to food safety challenges, such as the use of hot sauce for microwaved chicken wings stored overtime.

Significance: The data collected in this study demonstrates that creative engagement strategies are beneficial for supporting students' learning in food microbiology and safety.

P1-83 Development and Evaluation of a Food Safety Training for Exempt Home Food Operations and Home Bakeries in Iowa

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Introduction: In the state of Iowa, home-based food operations make and sell products out of their homes for purchase by consumers. However, these producers are largely unregulated, and many are not licensed or inspected.

Purpose: The objective of this study was to develop a pilot food safety training for home-based food operations in Iowa and assess participants' attitude and behaviors towards three food safety practices using seven constructs from the theory of planned behavior.

Methods: A pilot training was developed and addressed topics including Iowa laws, food safety basics and application to the home kitchen, production, and at the point-of-sale. The training was disseminated at five locations around the state and participants were asked to complete a pretest, post-test, and six to eight-week follow-up evaluation, using a Likert scale. The assessment included an evaluation of areas of attitude, behavior, intention, perceived behavioral control, and willingness to conform to social pressures related to food safety.

Results: Results from the evaluation show that participants (n=51) had positive feelings (responses of 5.00 or higher) for six constructs, except for attitude, which was slightly lower (4.00 or higher) for all testing times. Participants responses slightly, but not significantly, increased between pretest and post-test times, but then returned to levels close to the original responses, six to eight weeks after the training. Significant differences were observed between pretest and post-test times within behavior towards preparation environment and perceived behavioral control constructs for all participants, but then returned to scores like the pretest scores during the six to eight-week follow-up evaluation.

Significance: Overall feelings toward food safety were positive from participants and the evaluation indicates a need to affect long-term participant attitude and behavioral changes within all constructs. The results from this study will provide researchers and educators with the guidance of areas within a food safety training for home food producers.

P1-84 Characterization of the Environment and Risk Management Practices for Strawberry Farms in the Southeastern United States

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Introduction: Strawberries are among the top five produce commodities in the United States, accounting for over 53,000 acres of land. In the southeastern United States, there are many small-scale growers, some harvesting on less than one acre. As implementation of risk management practices can be affected by scale; small-scale growers may face unique barriers.

Purpose: To characterize the current risk management practices of strawberry growers in the southeastern United States.

Methods: A 43-question survey was developed using Qualtrics software to collect information regarding grower's location, business characteristics, farm characteristics, and risk management practices. Questions were designed based on the Produce Safety Rule (PSR) as well as feedback from expert reviews. The survey was distributed to individual growers and professional organizations.

Results: A total of n=17 participants completed the survey thus far. Farm size ranged from 10 to 1200 acres with 0.3 to 600 acres dedicated to strawberry production. Nearly half of respondents indicated their annual farm income was between $25,000 to 250,000 with one grower reporting >$500,000. Strawberries were not the only commodity for 94% of growers with 62.5% and 37.5% producing crops or crops/livestock, respectively. Of those growing more than one crop, 56.25% were covered by the PSR, however, 37.5% were unsure. A majority of growers reported using risk management practices but were less likely to document them. For example, 82% of growers reported their employees had attended food safety training, meanwhile, only 41% had documented training procedures. Growers who kept documentation were most likely to have written procedures, followed by corrective actions, and training records ($p<0.01$).

Significance: Our results indicate that strawberry growers within the southeastern United States have significant differences in their documentation of risk management practices. As this is an integral part of the PSR, strawberry growers may benefit from additional education regarding documentation.

P1-85 Food Safety Education and Outreach for Florida Farmers

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Introduction: In Florida, the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) and the Florida Department of Agriculture and Consumer Services (FDACS) collaborated to provide education and outreach through Produce Safety Alliance (PSA) Grower Training Courses and On-Farm Readiness Reviews (OFRR) to assist farmers in meeting the requirements of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR).

Purpose: To determine if PSA training was successful in improving the level of knowledge of the PSR and foundational food safety principals that Florida farmers have and to determine the level of farm preparedness for FSMA PSR compliance.

Methods: A directional dependent samples t-test was used to determine if there was a significant increase in knowledge after completion of the PSA training (n=754). For the OFRRs (n=9), qualitative data was submitted anonymously and compiled for percentages. This data was submitted to an online survey developed by the OFRR team through the National Association of State Departments of Agriculture.

Results: For PSA training, results showed post-test scores were statistically and significantly higher than pre-test scores (t=33.25, $P< 0.001$), indicating a significant increase in knowledge after participation in the training. Out of 25 points, participants scored an average of 16.45 on the pretest and 20.66 on the post-test. After collecting anonymous data, the three areas of farms required the most improvement were health and hygiene, preharvest worker training, and preharvest water. Of the farms that were assessed, 44.44% met the FSMA PSR requirements, 33.33% needed minor improvements, and 22.22% needed significant improvements to meet the PSR requirements.

Significance: The results of the PSA Grower Training and the OFRR program demonstrated improvement of the knowledge and compliance level of Florida farms regarding the FSMA PSR.

P1-86 Food Handler Awareness of Allergen Management Systems in Welsh Food Manufacturing Businesses

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Introduction: Food and drink manufacturing businesses are legally required to train food handlers and manage allergens onsite. Similarly, the BRC Global Food Safety Standard requires businesses to ensure all staff are trained effectively and requires management of allergens too. Although research regarding allergen awareness of food handlers in restaurants is available, data detailing food handler awareness of allergen management systems in food-manufacturing environments is lacking.

Purpose: To understand food handler awareness of the management of allergens.

Methods: Food-handlers (n=51) from food-manufacturing businesses in Wales (n=10) participated a short course covering the five themes of the BRC guidelines for allergen management in food manufacturing: significance of process, suppliers, separation, scheduling and sanitation. Food handler knowledge was measured pre- and post-intervention, and technical managers (n=4) were interviewed regarding intervention impact and effectiveness (four to eight weeks post-intervention).

Results: Food handlers reported an increase in familiarity with allergen management procedures following the training intervention, the increase was statistically significant ($P<0.05$) from 49% to 82%. Confidence in awareness of allergen management paperwork increased from 25% to 75%, ($P<0.001$) and using allergen management paperwork significantly increased from 37% to 78%, ($P<0.001$).

Confidence in the ability to list allergen documentation increased from 25% to 75% ($P<0.001$) as did trainees confidence in knowing allergen management responsibilities, which significantly increased from 51% to 90%, ($P<0.001$).

Technical managers reported improvements in paperwork following intervention for 33% of companies. Furthermore, 33% are more aware of non-conformances. For most (67%) overall allergen management has improved. Records have improved since the training for 100% of companies.

Significance: This study has designed, delivered and evaluated training required by food-manufacturing businesses which increased food handler awareness of allergen management. Food handlers tended to have some knowledge of allergens to carry out their role but evidence shows that a short two-hour session on allergen management could improve knowledge and confidence significantly.

P1-87 A Support Package to Support Small Food Manufacturing Businesses in Wales in Overcoming Barriers to Obtain Food Safety Certification: A Pilot Study

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Introduction: The Welsh government has identified, that to enable the growth of the food industry in Wales, there is a need to support small food-manufacturing businesses to obtain food-safety certification. SALSA (Safe and Local Supplier Audit) is a robust and effective food-safety certification scheme appropriate for smaller food-producers/processors/manufacturers, which is seen as a precursor to obtaining more complex, international certification such as BRC (British Retail Consortium). Data suggest 38% of food-manufacturing businesses in Wales don't have food-safety certification. Previous research has identified the barriers for Welsh businesses to obtain food-safety certification as 'knowledge and skills', 'time, cost and resources' and 'access to information'. Consequently, the Welsh government wants to determine the most appropriate way to support food manufacturers/processors in Wales to overcome barriers and obtain food-safety certification.

Purpose: To develop and pilot a bespoke support package for small food-manufacturing/processing businesses in Wales to obtain food-safety certification.

Methods: A support package was designed to overcome identified barriers by addressing three areas; 'knowledge and skills development', 'accessing financial support' and 'improving information and communication'. The package consisted of six support-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 food-manufacturers (n=62) expressed an interest in joining the support-programme, eligible businesses (n=9) joined the programme.

Results: Participation in the support-programme took businesses four to 10 months to complete. Two-thirds of the businesses completed the programme (n=6). Knowledge of the SALSA scheme and attitudes towards food-safety certification increased significantly ($P<0.05$) following each support mechanism. Seven companies underwent the official SALSA audit, however, only those that had complete the support-package programme (n=6) obtained SALSA certification.

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Significance: This pilot study has successfully designed, developed, delivered and evaluated a support-package that has resulted in 100% of small food-manufacturing businesses that completed the support-package obtaining food-safety certification. The launch of the support-package will assist to accelerate food-industry sector growth in-line with Welsh Government aspirations.

P1-88 Use of a Multidisciplinary Program Approach to Assist Food Entrepreneurs in Mitigating Business, Financial and Food Safety Risks

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Introduction: The locally grown produce and food products market continues to grow, and producers are seeking opportunities to meet this demand. This multidisciplinary project addressed food safety and business development for Mississippi stakeholders including limited resource producers, beginning farmers, and small farms/ranches.

Purpose: The project aim was to increase participants awareness and skills related to food safety and regulations, business/marketing strategies, and financial recordkeeping for agribusinesses to mitigate financial, marketing, and legal risks.

Methods: The "Food as a Business for Producers" workshops incorporated information, presentations, and access to experts with topics on proper business set-up, costing and pricing, online marketing, labor regulations, agricultural state programs, food processing regulations, and food safety. Five workshops (*n*=72 participants total) were held in regional locations in Mississippi. Participants evaluated their change in knowledge using a Likert scale pre- and post-workshop (1=Very Little…5=Very much). A six-month follow-up online survey was emailed to participants to assess practice implementation.

Results: On average across topics, participants had "Little" knowledge before the workshop and "Much" knowledge after the workshop (*n*=59; 82% response rate). Using a Wilcoxon signed-rank test to evaluate change in knowledge perception, participants had knowledge gains ($P<0.01$) indicating workshop effectiveness. Financially, 46% of the attendees indicated that they expect the practices they were going to adopt as a result of the workshops to save them money. Fifty-nine percent also indicated that practice adoption would result in higher sales, approximately 38% higher on average. The six-month survey respondents (*n*=22; 29% response rate) revealed that 77 to 91% have adopted (to different degrees) practices. Also, of the six-month survey respondents, fourteen participants indicated that implementing practices has resulted in cost reductions and/or an increase in sales.

Significance: The multidisciplinary workshops were successful at increasing knowledge and practice implementation related to business management, marketing, financial recordkeeping, and food safety.

P1-89 Development of a Hands-on and Demonstration-based Produce Food Safety Training Curriculum

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Introduction: A "Build Your Own Farm Food Safety Manual" workshop has been offered in Florida since 2010 to assist in the development of food safety plans, improvement of food safety practices, and expansion of market access. Additional produce-oriented food safety training increased substantially after the release of standardized FSMA-related curricula. Previous produce-targeted workshop participants have indicated, through focus groups and workshop evaluations, a need for more hands-on scenarios and examples.

Purpose: The objectives of this project are to develop training demonstration sites; hands-on curriculum modules; deliver the training to target audiences, and assess short- and medium-term learning and impact.

Methods: Results from previously conducted focus groups and workshop evaluations were used to guide the development of a hands-on and demonstration-based curriculum. The hands-on curriculum included seven modules: regulations and recordkeeping; agricultural water, well inspections, and water testing; employee hygiene, handwashing stations, and toilets; preharvest field assessments; packinghouse hazard assessment; cleaning and sanitizing harvest containers and food contact surfaces; and verification of sanitation. Two demonstration sites in Florida were developed at working research farms to conduct the 6.5 hour hands-on and demonstration-based workshops. A total of 34 individuals participated in one of two pilot workshops in 2018.

Results: Participants benefitted from a practical application of farm food safety evaluation conducted in an informal setting that encouraged independent thinking and group problem-solving. A 16 question pre- and post-test evaluation was developed and used to measure participant knowledge gain. Responses were tabulated and analyzed with a paired sample *t*-rest. Matching pre- and post-test results were obtained from 15 participants. All participants significantly ($P=0.000$) increased knowledge; average post-test scores (82%) were higher than pretest scores (36%).

Significance: Improved understanding of foundational food safety principles and practices that support the production of safe produce, as well as FSMA compliance, through the visualization of key food safety issues can be developed through hands-on, problem-based exercises at demonstration farms.

P1-90 Health Professionals as a Trusted Source for Food Safety Education: A Pilot Study in China and Peru

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✦ ***Undergraduate Student Award Entrant***

Introduction: Consumers trust health professionals (doctors, nurses, and dietitians) when sourcing food safety information. However, from previous studies in the United States, only half of the health professionals were confident in their food safety knowledge, and less than half of the health professionals provided food safety education to their patients. Little information has been reported outside the United States.

Purpose: Evaluate food safety education attitudes and practices of health professionals in China and Peru, and identify the major barriers of food safety education from a global perspective.

Methods: Face-to-face semi-structured interviews were used to collect data among health professionals (*n*=41). They were recruited from local hospitals in China and Peru. All interviews were conducted in their native language (Chinese or Spanish). Interviews were recorded, transcribed, translated into English, and then coded and analyzed.

Results: The most frequently mentioned food safety concern in China was food adulteration, while, in Peru, it was contaminated water. Most (59%) health professionals said they were confident about their microbial food safety knowledge. However, only 20% of them had food safety training. All of the health professionals recognized the need to educate patients in food safety, but only 39% said they delivered food safety education to patients. They were more likely to talk about diet and nutrition instead of food safety. Significantly more health professionals delivered food safety education when the hospitals required it ($P<0.05$). The top three major barriers in food safety education were "patients' interest," "time restriction" and "limited materials." The top four most desirable education topics, were "hand washing," "choosing safe food," "handling raw meat and poultry," and "avoiding cross-contamination."

Significance: The findings can guide the decision making of policymakers, educators and health professionals and support the development of next-generation food safety education strategies for public health.

P1-91 Effective Delivery of an Online Good Manufacturing Practices Course to Teach Regulatory Requirements and Food Safety Practices

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Introduction: An on-demand, online Good Manufacturing Practices (GMPs) course was developed to support regulatory personnel and food industry members in understanding new requirements outlined in 21 CFR 117.

Purpose: Review of a voluntary post-course evaluation as well as analysis of user activity data collected in the learning management system provides an opportunity to improve the effectiveness of course delivery and target new audiences.

Methods: Post-course evaluation data from 617 respondents that completed the course (out of 747 course participants) found that the largest clusters of participants worked in food processing facilities (235 (38.09%) of 617) and food wholesaler/distributor (71 (11.51%) of 617). Additionally, most respondents worked in firms with 10 to 25 employees (120 (19.45%) of 617) or fewer than 10 employees (114 (18.48%) of 617). A majority of the respondents 532 (86.22%) of 617) would recommend the training to others. Analysis of user activity was completed including the time of day, the day of the week, and date the course was accessed by a subset of 239 individuals that enrolled and completed the course between August 1, 2018, and December 31, 2018. Consultants (71.2 d) and federal government/regulatory personnel (57.33 d) took the longest to complete the course.

Results: Participants feel the online platform is an effective way to convey information and the course is predominantly being used by smaller firms with 25 or fewer employees. The 12 weeks allotted to complete the course is sufficient for all identified groups. There is great potential to expand both the number of participants because of the multitude of small processors providing food to United States consumers and the types of participants due to changes in the Food Safety Modernization Act that now include fruit and vegetable packers.

Significance: Increasing the understanding of regulatory expectations and supporting proper implementation of food safety practices through effective educational opportunities will reduce food safety risks and keep processors active in the marketplace.

P1-92 Investigating the Effect of Washing Raw Chicken on Cross-Contamination to Kitchen Surfaces and Ready-to-Eat Food Products

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❖ ***Developing Scientist Entrant***

Introduction: Cross-contamination of surfaces and RTE foods while preparing raw poultry is well documented, but the effect of chicken washing on cross-contamination is unknown. The impact of current messaging about poultry washing on cross-contamination is also unknown.

Purpose: This study was conducted to determine how washing raw poultry affects the frequency and degree of cross-contamination to kitchen surfaces and a vegetable salad during meal preparation and evaluate the efficacy of a text-based food safety message on washing chicken.

Methods: A meal of chicken thighs inoculated with 10^8-10^{10} CFU/g of a generic *E. coli* DH5-α strain tagged with a green fluorescent protein and a vegetable salad was prepared by participants (*n*=281) in a test kitchen. Half of the participants received an email cautioning against chicken washing (intervention group) and half did not (control group). Food preparation surfaces and prepared salad were sampled after meal preparation and *E. coli* were cultured on selective media for enumeration. Statistical analysis was completed using R.

Results: *E. coli* DH5-α contamination occurred more frequently when consumers washed chicken thighs (frequency 17.9% for wash vs. 9.9% for no-wash), especially for sinks, but salad contamination was not significantly impacted (25.8% vs. 19.6% for wash vs. no-wash, respectively). Sinks before cleaning had the highest prevalence of cross-contamination, despite wash status (60.3% for wash and 35.6% for no-wash, $P=0.0011$). Cleaning of sinks and countertops reduced the contamination prevalence significantly ($P<0.0001$), 60.3% to 14.3% (sinks) and 22.6% to 2.2% (countertops) for washers; and 35.6% to 5.1% (sinks) and 9.2% to 1.6% (countertops) for non-washers. Overall, food safety messaging was effective at changing consumer behavior ($P<0.0500$).

Significance: This study provided important information on how poultry washing impacts cross-contamination and suggests that subtle food safety messaging can be effective.

P1-93 Understanding Poultry Washing and Post-washing Cleaning and Sanitizing Behaviors of Consumers

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Introduction: The United States Centers for Disease Control and Prevention (CDC) has identified contaminated equipment/prevention of contamination as a contributing factor to foodborne illness. Risks associated with the washing of poultry during meal preparation have been described in the literature but little is known about how consumers actually carry out this task.

Purpose: This study was conducted to investigate the variability of how individuals who self-report washing chicken in their homes prior to preparation carry out cleaning and sanitizing practices.

Methods: A sample of 300 participants who self-reported washing poultry was recruited from various North Carolina communities, and asked to prepare raw chicken pieces in a simulated home kitchen while being observed by researchers. One-half of the participants were provided with text-based and image enhanced messages frequently used by the Food Safety and Inspection Service on social media in emails prior to the preparation event (intervention group); the other half served as a control group. Videos of participants' preparation events were recorded and coded following completion of the preparation steps. Cleaning and sanitizing actions were coded and adherence to public health messages was measured.

Results: Cleaning and sanitizing of the sink area was not typically carried out as a two-step process as defined by public health messages. While a rinse or clean (with soap) step occurred in 65% of observations, a two-step clean and sanitize process occurred in only four percent of observations when the chicken was washed. Consumers paid most attention to cleaning and sanitizing utensils and cutting boards that would be further used with ready-to-eat foods.

Significance: Data collected through direct observation more accurately reflects consumer food handling behaviors than data collected through self-reported surveys and allows for more targeted message development. Messages targeting consumers should be updated to include the focus on sink cleaning and sanitizing during poultry preparation.

P1-94 Creation and Implementation of a Social Marketing Campaign for Beef Food Safety

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Introduction: Food safety-related messages are most effective when they are meaningful to the intended audience, contain accurate information, are delivered repeatedly, and are distributed at appropriate times. A social marketing approach to food safety messages has been suggested by public health agencies to reach consumers and impact risk-reduction behaviors.

Purpose: This exploratory study was conducted to create a roadmap of the design and execution of a social marketing media campaign intervention.

Methods: A multidisciplinary team of food safety professionals developed the framework for a social marketing campaign by applying the theory of planned behavior. Within the constraints of a prescribed budget, the team developed a concept focusing on safely cooking ground beef hamburgers and hired a marketing firm to assist with content creation, themes, visuals and place media. Campaign components included a slogan, responsive website, display and video advertisements, radio PSAs, press releases, broadcast interviews, movie theater previews, and a social media quiz.

Results: The resulting output was a 16-week food safety campaign, "160 is Good", promoted in the summer of 2017. The campaign targeted the residents of Fayetteville, NC (market population ~ 200,000). Campaign media placement resulted in an estimated 11,489,658 total impressions. An impression is defined as a message heard, seen or interacted with. Impacts included 8,328,300 local radio station impressions (from 2,292 radio commercials); 562,313 impressions from online videos and movie theater previews; 1,667,578 website banner advertisements impressions; and, 944,527 online audio service impressions. Digital click-throughs and video watch rates surpassed other similar advertising activities. This highly successful campaign reached an estimated 73.2% of the market population at least once and many individuals multiple times.

Significance: This study provides important information on costs, lessons learned and potential reach of a food safety media campaign that can be used by food safety message developers in education, government and industry sectors.

P1-95 Needs Assessment Survey of Processors of Human Food in Tennessee for Meeting the Requirements of the Food Safety Modernization Act

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Introduction: the Food Safety Modernization Act (FSMA) is the most extensive legislation related to the microbial safety of food manufacturing and agricultural production in the country since the 1930s. The legislation aims to ensure the safety of imported as well as domestically-grown foods by shifting the focus from the response to contamination to preventive measures.

Purpose: The purpose of the current study was to assess the needs of the producers of human food in middle Tennessee for meeting the requirements of the legislation.

Methods: A survey (n=17) with a five-point hedonic scale and a demographics section was administered to processors of human foods in middle Tennessee to assess their existing knowledge and training needs for meeting the regulatory requirements of the Human Food Rule of FSMA. The content was analyzed using OpenEpi software at type one error level of five percent.

Results: Number of employees in the processing plants were ranging from one to eight (average two employees) and the processors were in business for an average of eight years (ranging from one to 30 years). 58% of respondents indicated they do not know if they are exempt from the Preventive Control for Human Food Rule. Thirty-six percent of processors indicated they possess "poor knowledge" of "allergen concerns of food products," while 28% of processors indicated they possess "good knowledge" of "hazards during transportation of food products." Thirty-three percent of processors indicated they possess "average knowledge" of "agencies that regulate food processing facilities and food products."

Significance: Assimilating the needs of the manufacturers is of importance for emerging entrepreneurs, enhancing the prospect of expanding their operations which otherwise would have to remain low in profit in order to stay within the FSMA exemptions.

P1-96 Sensitivity of *Bacillus amyloliquefaciens*, *Geobacillus stearothermophilus*, and *Bacillus atrophaeus* to Elevated Hydrostatic Pressure in the Presence of Mild Heat, Nisin and Lysozyme

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❖ ***Developing Scientist Entrant***

Introduction: Commercial adoption of high-pressure processing is gaining momentum and industrial importance with recent advances in the engineering of pressure-based pasteurization units. The main limit of the technology is limited efficacy for inactivation of microbial spores.

Purpose: The current study investigated the synergism of elevated hydrostatic pressure, mild heat, and two antimicrobials (nisin and lysozyme) for inactivation of three spore suspensions.

Methods: Various times (zero, three, five, and 10 minutes) at a pressure intensity level of 650 MPa (94K PSI) of elevated hydrostatic pressure (Hub880 Explorer, Pressure BioScience Inc), were investigated at 50°C for inactivation of *Bacillus amyloliquefaciens*, *Geobacillus stearothermophilus* (ATCC 7953), and *Bacillus atrophaeus* (ATCC 9372). The selected strains are currently considered as the most pressure-resistant natural isolates, the biological indicator for heat-based sterilization, and indicator for heat- and chemical-based decontamination interventions, respectively. The spore suspensions were exposed to treatments at the above-mentioned intensity with and without the presence of lysozyme (22.4 ml/l) and nisin (5000 IU/ml) in HEPES buffer. ANOVA was conducted followed by LSD-based mean separation by OpenEpi software.

Results: Counts of *Bacillus amyloliquefaciens* were 6.75±0.1 log CFU/ml prior to the treatment and were reduced to 4.32±0.1 log CFU/ml after the treatment for 10 minutes at 650 MPa and 50°C. These reductions were augmented in the presence of nisin where the spore suspension was reduced by 2.21 log CFU/ml. Nisin was similarly efficacious (P<0.05) for reducing *Bacillus atrophaeus* by 3.31 log CFU/ml after the above-mentioned treatment for 10 minutes while it was not capable of significant reductions (P≥0.05) of *Geobacillus stearothermophilus.* Lysozyme, similarly, lead to 2.62 and 2.65-log CFU/ml reductions of *Bacillus amyloliquefaciens* and *Bacillus atrophaeus,* respectively.

Significance: Results of the current study indicate an optimized pressure-based intervention in the presence of mild heat and antimicrobial agents could be efficacious for inactivation of >99% of microbial spores.

P1-97 Updates on a Planning Activity Project for Development and Implementation of an Intercollegiate MPH Degree Tracked in Food Safety and Foodborne Diseases Epidemiology

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Introduction: Food and water safety and the history of epidemiology are interconnected since the historic 1854 outbreak in water in Soho, London. Since then, various advancements and epidemiological studies have assured the safety of the public against food and waterborne infectious diseases. With existing and expanding infrastructures such as FoodNet and PulseNet as well as ongoing surveillance systems such as NARMS and PR/HACCP verification testing program, there is an increasing need for graduates with food safety, public health, and applied epidemiology backgrounds.

Purpose: This study discusses progress made on a recently funded planning activity project of USDA National Institute of Food and Agriculture (Project number TENX- 2017-06088) for development of a Master of Public Health (MPH) program tracked in Food Safety and Foodborne Diseases Epidemiology.

Methods: The current presentation articulates i) development of a foodborne diseases epidemiology and food safety advisory panel, ii) planned faculty enhancement in food safety epidemiology and risk assessment, iii) development of food safety and foodborne diseases epidemiology curricula, and iv) planning annual meetings of the advisory panel. An evidence-based formative and summative evaluation plan, recruitment plan, and plan for track's CEPH certification will additionally be presented.

Results: The main products of this planning activity project are a validated curriculum with an experiential learning practicum section, a formative and summative evaluation plan, and an advisory panel to oversee continuation and implementation of the endeavor. Matriculated students could benefit from the training to join various research programs in academe, surveillance programs in state or federal government, or careers in the private food industry.

Significance: This presentation could foster collaboration and exchange of ideas for the current project during this planning, development, and implementation phase.

P1-98 Advances in Validation Studies for Pressure-based Pasteurization of Microbial Pathogens, Pressure-Adapted Microorganisms, and Bacterial Spores

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Introduction: According to a report from the Centers for Disease Control and Prevention, advances made by food scientists for development of safe and nutritious food products were one of the top 10 public health achievements of the 20th century. Enhanced global commerce, increased in proportion of at-risk populations, and consumers' demand for non-traditional commodities and minimally processed products provide breeding ground for emerging, novel, and reemerging foodborne infectious diseases. This indicates the need for innovative solutions for assuring the safety of the food supplies using novel and emerging technologies.

Purpose: Current study is summary of recent validation studies conducted in the Public Health Microbiology laboratory of Tennessee State University for enhancing the industrial adoption of pressure-based pasteurization of various food commodities.

Methods: Special emphasis will be placed on presentation of hurdle validation studies, investigating decontamination of wild-type, rifampicin-resistant, and pressure-adopted serovars of non-typhoidal *Salmonella*, various serogroups of Shiga toxin-producing *Escherichia coli*, public health significant serotypes of *Listeria monocytogenes*, and *Cronobacter sakazakii*. Inactivation of *Bacillus amyloliquefaciens*, *Geobacillus stearothermophilus*, and *Bacillus atrophaeus* will additionally be discussed. The selected spore-forming strains are 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, respectively. Validation studies were conducted in PULSE tube using barocycler Hub440 and Hubb880 instruments at various levels of hydrostatic pressure (100 to 650 MPa).

Results: Although utilization of elevated hydrostatic pressure at 87K PSI (600 MPa) for three min is a common practice in private food industry, results of our validation studies indicate duration and intensity of the operation could be modified based on synergism of pressure-based interventions with mild heat and natural antimicrobials. Reductions (P<0.05) in excess of five log CFU/ml were observed for an array of product-pathogen combinations at hydrostatic pressure levels less than the current common industry practice.

Significance: Validation studies considering various intrinsic and extrinsic factors of the product could lead to optimized and economically feasible utilization of high pressure processing in new array of products.

P1-99 Pressure-based Pasteurization of Wild-type and Acid-Adapted *Escherichia coli* O157 and Non-typhoidal *Salmonella* Serovars in Orange Juice

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❖ ***Developing Scientist Entrant***

Introduction: Recent epidemiological studies indicate non-typhoidal *Salmonella* serovars and *Escherichia coli* O157 are among the leading causes of foodborne illness, hospitalization, and death episodes, and disability adjusted life year loss in the United States.

Purpose: The current study investigated efficacy of elevated hydrostatic pressure for inactivation of wild-type and acid-adapted *Escherichia coli* O157 and non-typhoidal *Salmonella* serovars in orange juice.

Methods: Time intervals of zero (untreated control), two, four, six, and eight min and intensity levels of 150 and 400 MPa of elevated hydrostatic pressure (Pressure BioScience Inc.) were investigated in two independent experiments for decontamination of acid-adapted (AA) and wild-type (WT) non-typhoidal *Salmonella* serovars (ATCC 13076, 8387, 6962, 9270, 14028) and *Escherichia coli* O157:H7 mixtures (ATCC BAA460, 43888, 43894, 35150, 43889, 43890) inoculated in sterilized orange juice. Experiment temperature was maintained at 4°C using a circulating water bath attached to a stainless steel jacket surrounding the treatment chamber. Results were analyzed by GLM procedure of SAS using Tukey- and Dunnett-adjusted ANOVA.

Results: At 150 MPa, for treatments of two to eight minutes, D-values of 8.00 and 8.92 min were observed for WT and AA *Escherichia coli* O157 at 4°C, respectively. Before treatment, *Escherichia coli* counts were 6.45±0.2 and 5.17±0.6 log CFU/ml for AA and WT phenotypes, respectively, and were reduced (P<0.05) to 1.02±0.4 and 2.15±0.4, respectively, after an eight-min treatment at 400 MPa. The AA and WT *Salmonella* counts were 4.11±0.8 and 4.32±0.8 log CFU/ml, respectively, and were both reduced (P<0.05) to the detection limit after eight-min treatment at 400 MPa.

Significance: Results obtained from this experiment could be incorporated as part of hazard analysis in FSMA-based management systems and risk assessment analyses for mitigating the public health burden of non-typhoidal *Salmonella* serovars and Shiga toxin-producing *Escherichia coli*.

Poster

Poster

P1-100 Photodynamic Treatment of *Bacillus cereus* Strains: Estimating the Inactivation Kinetic Parameters of Four Strains from Different Sources

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❖ ***Developing Scientist Entrant***

Introduction: The contamination of foods with pathogens is responsible for mortality and morbidity that impacts lives and countries' economies and social development. *Bacillus cereus* is a spore-forming bacteria commonly found in soil and associated witho foodborne diseases and to food spoilage. Therefore, *B. cereus* is an interesting microorganism to be employed as a model for photodynamic treatment (PDT).

Purpose: The main aim of this work was to evaluate and promote technological advances in the use of PDT for the inactivation of *B. cereus* strains.

Methods: A total of 12 strains of *B. cereus* isolated from different types of foods and outbreaks in Brazil were used. The resistance of the isolates to PDT was measured at different concentrations (1; 2.5; 5, 10; 12.5; 25; 50; 75; 100; 200 µM) of New Methylene Blue (NMB) for 60 min using a light source of 96 LEDs (RED; 650 nm). Then, the *B. cereus* strains were selected according to their PDT resistance and a further experiment was conducted with five, 50 and 100 µM of NMB for 120 min in order to obtain the inactivation kinetic parameters. The variability in the inactivation kinetic parameters was determined.

Results: From the 12 strains pretested, four strains (B63, 436, B3 and 14579) were selected based on cluster analysis according to their PDT resistance. The less resistant strains to PDT were 436 and 14579 with 7.5 and 7.5-log reduction, respectively, using 50 µM of NMB. The strains B3 and B63 with 2.8 and 2.9-log reductions were considered the most resistant to PDT. The discrimination of kinetic parameters of inactivation and variability will be presented.

Significance: This work will allow gaining insights into the feasibility of using PDT for inactivation of *B. cereus*.

P1-101 Processing of Dried Beef (Biltong) without a Heat Lethality Step to Achieve USDA-FSIS Validation (Five-Log Reduction) of *Salmonella*

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❖ ***Developing Scientist Entrant***

Introduction: In some parts of the world drying of meat products is a means of preservation whereby products lose moisture but retain protein and provide nutrition. In the United States, dried beef products (beef jerky) are a popular snack product but their manufacture often requires the use of a heat lethality step to provide adequate reduction of pathogens of concern (five-log reduction of *Salmonella* as per USDA-FSIS requirements). However, other types of 'dried beef' that are produced worldwide do not use heat, but rather, use salt, spices, and drying for microbial control.

Purpose: Our objective was to examine a process for the manufacture of dried beef (biltong) to try to achieve a five-log reduction of *Salmonella* without a heat kill step.

Methods: Beef obtained locally was sliced (one by two by three in) and inoculated with a five-serovar mixture of *Salmonella*. The beef was processed by dipping (water control vs antimicrobial), vacuum tumbling (spice and vinegar marinade), and dried in a temperature- and humidity-controlled oven (77°F/25°C; 55% RH) for four to six days.

Results: All three replicate trials using antimicrobial dip, spice/vinegar marinade, and drying at the specified temperature/humidity provided greater than five-log reduction (6.16-, 5.79-, 6.09-log reduction) with water activity levels of 0.84 or below within two to four days drying. Controls without antimicrobial dip treatment provided 4.16-, 4.3-, and 5.15-log reductions. All trials were performed in triplicate replication and repeated measures one-way ANOVA was carried out to see significant differences (P<0.05) in the response to different treatments from controls.

Significance: Prior references to dried beef products suggest that heat/steam is required to reach USDA-required lethality levels. Our data suggests that five-log reduction of *Salmonella* can be achieved without using established lethality temperatures while retaining flavor and palatability, yet still provides a microbially-safe product.

P1-102 Inactivation of Shiga Toxin-producing *Escherichia coli, Salmonella enterica* and Natural Microflora on Artificially Inoculated Wheat Grains by Atmospheric Cold Plasma

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❖ ***Developing Scientist Entrant***

Introduction: Enteric pathogens and high microbial counts on wheat grains can reduce the microbial safety and shelf life of refrigerated raw wheat-based foods. Air activated by an electric field forms atmospheric cold plasma (ACP) which can rapidly kill microorganisms.

Purpose: The effectiveness of ACP was investigated for killing Shiga toxin-producing *Escherichia coli* and *Salmonella enterica* as well as natural microflora on wheat grains.

Methods: Wheat grain samples (10 g) were inoculated with a five-strain mixture of *E. coli* or *S. enterica* to obtain an initial count of ~seven log CFU/g. Moistened grains (inoculated and non-inoculated) were sealed in plastic bags with atmospheric air and exposed to ACP (44 kV) for zero (control), five, 10, 15 and 20 min. Pathogen survivors were evaluated from colony counts on thin agar layer (TAL) media and selective (SEL) agar. Dichloran rose bengal chloramphenicol agar was used to enumerate yeast and molds (25°C, five d), whereas plate count agar was used for mesophiles (35°C, 48 h) and psychrotrophs (7°C, seven d). Pour plating using tryptic soy agar with 6% (w/v) yeast extract with an overlay of violet red bile agar was used to enumerate *Enterobactericeae* (35°C, 24 h).

Results: ACP treatment (20 min) reduced viable counts (log CFU/g) of *E. coli* on TAL medium and SEL agar by 3.09 and 4.84, respectively. *S. enterica* counts decreased by 4.40 (TAL) and 4.32 (SEL agar). Log CFU/g reductions of mesophiles, psychrotrophs, and *Enterobactericeae* were 0.96, 2.14 and 1.38, respectively. In contrast, yeast and molds were completely inactivated (3.29 log CFU/g reduction) after 10 min of ACP treatment (P<0.05).

Significance: ACP has good potential for destroying pathogens and spoilage microorganisms on wheat grains. Flour made from ACP-treated wheat can be of high microbial quality for enhanced safety and shelf-life of refrigerated raw wheat-based products.

Poster

P1-103 Survival and Inactivation of Wild-type and Rifampicin-resistant *Cronobacter sakazakii* and Background Microflora of Infant Formula Using Mild Hydrostatic Pressure

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Introduction: *Cronobacter sakazakii* could survive in dry, low moisture environments such as infant formula. Infections caused by the bacterium are often fatal in infants born premature and those younger than two months.

Purpose: Purpose of this study is to investigate effects of mild hydrostatic pressure for decontamination of infant formula from wild-type and rifampicin resistant *Cronobacter sakazakii.*

Methods: Up to nine cycles of mild elevated hydrostatic pressure (350 MPa, 30 s) were investigated for inactivation of a four-strain mixture of wild-type and rifampicin-resistant *Cronobacter sakazakii,* at four and 55°C, respectively. In a companion experiment, survival of the four4-strain mixture of *Cronobacter sakazakii* in infant formula was investigated during aerobic storage at 10 and 25°C. The experiments were conducted in two biologically independent repetitions, as blocking factors of a randomized complete block design, containing three repetitions per cycle/phenotype within each block. Study was analyzed by LSD-based ANOVA using OpenEpi software.

Results: Counts of *Cronobacter sakazakii* were reduced (P<0.05) from 5.50±0.5 to 4.74±0.2 log CFU/g (approximately 90% reduction) during 14-day aerobic storage at 25°C. Counts of background microflora were similarly reduced (P<0.05) from 7.19±0.4 to 5.03±0.2 log CFU/g (approximately 99% reduction), during 14-day aerobic storage at 25°C. At 55°C, >2.04 up to 6.56-log reductios (P<0.05) of wild-type *Cronobacter sakazakii* were observed as result of application of elevated hydrostatic pressure at 350 MPa (51K PSI). At 4°C, >1.06 and >5.23 reductions (P<0.05) of wild-type *Cronobacter sakazakii* were observed as result of application of elevated hydrostatic pressure at 350 MPa (51K PSI).

Significance: Overall the survival of the pathogen and spoilage organism were similar (P>0.05) during storage at 10°C and 25°C. Reductions of rifampicin-resistant phenotype at both temperatures were similar (P≥0.05) to wild-type pathogen, thus indicating these phenotypes could be used interchangeably in microbiological challenge studies.

P1-104 Effects of Come-up and Come-down Times on Efficacy of Pressure-based Pasteurization of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and Non-Typhoidal *Salmonella* Serovars

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Introduction: Industrial adoption of microbiological challenge studies are often curtailed due to differences in come-up and come-down times of research and commercial units. Limited information is currently available to quantify the effects of these parameters on efficacy of pressure-based pasteurization.

Purpose: Current study investigated effects of come-up and come-down times on performance of pressure-based pasteurization for inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and non-typhoidal *Salmonella* serovars.

Methods: Hydrostatic pressure (350 MPa, 4°C) and thermal-assisted elevated pressure (350 MPa, 55°C) were applied at various time intervals (zero to five minutes) and come-up and come-down times of three seconds (control) to five minutes for inactivation of strain mixtures of wild-type of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and non-typhoidal *Salmonella* serovars, inoculated in phosphate buffered saline at target level of 7.5 log CFU/ml. Experiments were conducted in two biologically independent repetitions, as blocking factors of a randomized complete block design and were conducted in Barocycler Reaction PULSE tubes. Results were statistically analyzed using LSD-based ANOVA by OpenEpi.

Results: For high-pressure treatments at 4°C, come-up times of 30 s, one min, two min, and three min were responsible for modest reductions (P<0.05) of 0.36, 0.42, 0.55, and 0.66 log CFU/ml of *Escherichia coli* O157:H7, respectively, at 350 MPa compared to control (standard three-second come-up time). Similarly, come-down times of one, three, and five min resulted in modest reduction of *Listeria monocytogenes* for 0.62, 1.11, 0.93 log CFU/ml, respectively, at 350 MPa and at 4°C. Predictably, these reductions were augmented (P<0.05) at higher temperatures.

Significance: Results of this study indicates that come-up and come-down times of a commercial unit could pose a negligible or significant effect on decontamination efficacy of a pressure-based intervention depending on duration and temperature of the treatment as well as the microbial pathogens involved.

P1-105 How Virginia Extension Agents Engage with the Public about Food Processing Perceptions

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Introduction: Virginia Cooperative Extension (VCE) agents serve as a resource for public dissemination of food information; for agents to dispel myths to consumers surrounding food processing technologies (FPT), they must understand the science behind them.

Purpose: The purpose of this study was to assess VCE agents' FPT knowledge, perceptions, and purchasing intentions, as well as to conduct a needs assessment for how they can best interact with clientele on this topic.

Methods: A Qualtrics survey was distributed electronically to extension agents across Virginia to assess their understanding and perceptions of both conventional (pasteurization, microwave technology, high-pressure processing, etc.) and emerging FPTs (photo-inactivating light, cold plasma, gaseous antimicrobials, etc.). Survey questions determined whether agents possessed adequate resources for each FPT. Only fully completed responses of currently employed VCE agents were recorded.

Results: Forty-nine Extension agents completed the survey: 19 family and consumer science agents, 24 agriculture and natural resources agents, six 4-H Youth agents, and three "other" agents (check all that apply). The majority of agents (67%) expressed concerns about 'processed' foods in general. Some agents had concerns about FPTs that are commonly utilized by the food industry, such as pasteurization (16%) and microwave technology (53%). Agents were more likely to be supportive of light-exposed foods to enhance safety and quality in comparison to gas-exposed foods. Agents reported that clientele asked about specific FPTs, however, they did not feel they have adequate resources to provide education about them (pasteurization=23%, microwave technology=10%, HPP=20%, irradiation=17%, forms of light=13%, forms of gas=10%, cold plasma=7%).

Significance: Because there is consumer demand for raw and/or minimally processed foods, FPTs are needed to control foodborne pathogens. Extension agents are viewed as relatable, trustworthy sources of information who can influence consumer acceptance through educational interventions (trainings, infosheets, etc.); but, they may not possess adequate resources to support this initiative.

Poster

P1-106 Exploring Engineered Water Nanostructures as an Antimicrobial Platform for Fresh Produce Decontamination

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce is susceptible to microbial contamination. However, washing fresh produce with chemical disinfectants has chemical risks from residues and toxic byproducts, and more importantly, is not suitable for organic produce and delicate fruits. A novel nanotechnology-based antimicrobial technology was developed with engineered water nanostructures (EWNS) synthesized using electrospray and ionization of aqueous suspension of antimicrobials. Such "waterless" nanocarrier platform can achieve food disinfection by delivering minuscule quantities of antimicrobials in an aerosol form and in a targeted manner.

Purpose: Assess the potential of EWNS platform to deliver different active ingredients in a targeted manner for inactivating foodborne pathogens, such as *E. coli* and *Listeria innocua*.

Methods: *E. coli* ATCC 25922 and *L. innocua* ATCC 33090 were spot-inoculated onto stainless steel coupons and exposed to different EWNS based nanoparticles that incorporate active ingredients of interest. The physical and chemical properties of iEWNS nanoparticles (i denotes the active ingredient) were measured. The delivered dose of active ingredient was also estimated as a function of exposure time. Bacteria were recovered on tryptic soy agar and reductions were calculated.

Results: iEWNS nanoparticles could significantly reduce (P<0.05) *E. coli* and *L. innocua* on coupons (~10^6 CFU/coupon) by greater than five log in a matter of minutes of exposure. For instance, one percent hydrogen peroxide based EWNS nanoparticles could inactivate greater than five log of *E. coli* and *L. innocua* on coupons in five and 15 min, respectively. When using three percent hydrogen peroxide based EWNS nanoparticles, *E. coli* was reduced by 2.2 log in 30 seconds. It is worth noting that to achieve a three-log reduction of *E. coli*, only 0.078 to 114.53 ng/cm^2 of iEWNS nanoparticles were delivered.

Significance: These data suggested that the iEWNS platform is effective against *E. coli* and *L. innocua* by only delivering nanogram levels of active ingredients.

P1-107 Evaluation of Initial and Post-High Pressure Pasteurization Treatment Storage Temperatures as Critical Process Factors

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Introduction: Sensitivity of pathogenic microorganisms to temperature during HPP and the impact of temperature as a critical factor during refrigerated HPP processing of foods is not clearly understood.

Purpose: To obtain greater understanding of the impact of initial product temperature and post-HPP storage temperature on the destruction of selected pathogens in apple and orange juices (pH=4.0).

Methods: Separate five-strain cocktails of *Escherichia coli* O157:H7, *Salmonella*, or *Listeria monocytogenes* were acid-adapted, inoculated into apple and orange juices, and HPP-treated at 85,000 psi for three minutes. Products and pressurization fluid were adjusted to initial temperatures of one, four, or 10°C prior to HPP, and stored at one, four, or 10°C post-HPP. Survivors were enumerated using MPN methodology immediately after HPP, and after one and three days of storage post-HPP (n=3). Results were analyzed using ANOVA.

Results: After one and three days of storage at 4°C post-HPP, *E. coli* was reduced by 3.04±0.19 and 3.67±0.53 log in apple juice and 5.74±0.51 and 7.29±0.23 log in orange juice, respectively. *Salmonella* and *L. monocytogenes* were not recovered from either product after one day post-HPP, demonstrating log reductions of both organisms >6.98 and >7.52 log. Results of studies conducted with only *E. coli* indicate that initial product temperature significantly impacts log reduction of this organism in orange juice (P<0.05), while this is not the case for apple juice (P=0.256). Storage time post-HPP significantly impacted log reduction of *E. coli* in both products, with significantly more reduction observed the longer the product was stored (P<0.05).

Significance: Results indicate *E. coli* is more pressure-resistant than *Salmonella* and *L. monocytogenes* in apple and orange juices, and that there is a certain tolerance with regard to initial product and post-HPP storage temperature and the impact of these critical factors on the log reduction of *E. coli* in these products.

P1-108 Evaluation of Adaptive Response in *E. coli* O157:H7 to Light and Gallic Acid-Based Antimicrobial Treatments

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❖ ***Developing Scientist Entrant***

Introduction: Ability of bacteria to develop cross-protection to various stresses is well-known. However, exploration into cross-protection between conventional physiological stresses and emerging antimicrobial treatments is limited.

Purpose: Investigate of whether prior exposure to sub-lethal stresses can increase the resistance of *E. coli* O157:H7 towards two GA and UV light-based treatments, and whether repeated exposure to these two treatments selects for bacterial subpopulations cross-resistant towards heat, acid, and oxidative challenge.

Methods: Stationary phase *E. coli* O157:H7 were exposed to sublethal heat, acid, NaCl, and H_2O_2 before being treated by either GA and UV-A light simultaneously (GA+UVA), or UV-C light post-irradiated GA (UVC-GA). The cultures were also subjected to repetitive cycles of exposure to either UVA+GA or UVC-GA treatment to evaluate whether a more resistant sub-population can be identified.

Results: Prior exposure to heat or acid stress increased (P<0.05) the resistance of *E. coli* O157:H7 towards UVA+GA treatment, while osmotic stress increased (P<0.05) their sensitivity to that treatment. Interestingly, only heat stress showed protective (P<0.05) effect to subsequent UVC-GA treatment, while acid or osmotic stress increased (P<0.05) the sensitivity. Prior exposure to oxidative stress had no effect (P>0.05) on the sensitivity of the bacteria to either of the treatment. Repeated exposure to UVA+GA or UVC-GA treatment selected for subpopulations that demonstrated higher (P<0.05) resistance to these two treatments as well as heat or acid challenge. Further experiments showed that increased synthesis of enzymes such as superoxide dismutase and upregulation of RpoS were likely to be associated with the development of cross-protection.

Significance: Results help optimize the two techniques to improve their efficiency for bacterial inactivation while avoiding unexpected resistance development, and for a better selection of hurdles during food processing. The possible underlying mechanism behind the phenomenon of cross-protection was also elucidated for a better understanding and control of these emerging techniques.

P1-109 Ensuring Food Emergency Response Network Laboratory Preparedness for Detecting *B. anthracis* and *Y. pestis* from Food

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Introduction: *Bacillus anthracis* and *Yersinia pestis* are considered Category A agents by the Centers for Disease Control (CDC), posing a significant public health risk. If an intentional or unintended food adulteration event occurred, laboratories need to be ready to detect such agents. Therefore, it is important to assess the proficiency of the Food Emergency Response Network (FERN) laboratories through FDA's ISO/IEC 17043 accredited proficiency testing (PT) program.

Purpose: To evaluate FERN laboratories' proficiency, capability and capacity for detection and isolation of *B. anthracis* and *Y. pestis* from various food matrices.

Methods: PT samples were prepared using 1000 and 2000 CFU/g of *B. anthracis* inoculum in sweet potato purée. *Y. pestis* inoculum levels were 10 and 100 CFU/g in chicken and vegetable-based baby food. PT samples were prepared in bulk and tested for the presence of the organisms over a 10 to 15-day period. *B. anthracis* samples were tested using the FERN Screening Method for *Bacillus anthracis* in Foods. *Y. pestis* samples were tested using the FERN *Yersinia pestis* Screening Method. Thirty-four *B. anthracis* sets and 27 *Y. pestis* PT sample sets were sent to FERN laboratories. Results were analyzed to determine how many laboratories across the United States are prepared for testing of this nature.

Results: Assigned values were determined by consensus agreement according to ISO standards. *B. anthracis* was homogenous and stable for 10 days in sweet potato purée and 34 laboratories correctly detected *B. anthracis* in inoculated samples. *Y. pestis* was homogenous and stable for 15 days in chicken and vegetable-based baby food and 27 laboratories correctly detected *Y. pestis* in inoculated samples.

Significance: One hundred seventy samples inoculated with *B. anthracis* and 108 samples inoculated with *Y. pestis* were correctly identified by participating FERN laboratories; furthering FDA's mission to protect the public health, demonstrating laboratory proficiency and FERN's capability during a food adulteration event.

P1-110 Evaluation of Freeze-drying Conditions for Extension of Bacteriophage Shelf Life

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Introduction: Incidence of foodborne pathogens such as *E.coli* O157:H7 is of concern for military personnel. Commercial bacteriophage preparations have been shown effective as a means for pathogen reduction in foods, but in current liquid formulas do not fit military logistics. Expanding the logistical use of bacteriophage cocktails can be achieved by developing shelf-stable, dry cocktails.

Purpose: To identify candidate storage buffers to optimize freeze dried bacteriophage stability for one year at room temperature.

Methods: EcoShield bacteriophage (commercial mixture of three different *Myoviridae* bacteriophages) was mixed in equal parts with different excipient formulas containing either storage media (SM) or sodium glutamate (SG), with the addition of sucrose (S), trehalose (T), gelatin (G), polyvinylpyrrolidone (PVP), or polyvinyl alcohol (PVA). Mixtures were freeze dried in duplicate and stored at 25°C for 30 weeks. Plaque assays were performed in duplicate at each time point using *E. coli* O157:H7 with lysogeny broth/agar and resulting data was collected to determine survivability over time. A Student's t-test utilizing the log values of counts was used to evaluate the statistical significance of phage loss over time.

Results: SM+PVP+S and SM+PVP+T showed no bacteriophage loss throughout the freeze drying process. All other samples showed significant loss (P<0.05) of bacteriophage post freeze drying. During storage SM+G+PVA, SM+G+PVA+S, SM+G+PVA+T, SG+PVP+T, SM+PVA+S, SM+PVA+T, SM+PVP and SM+PVP+T retained the most bacteriophage (0.1119 to 0.4720-log loss) with no significant differences among the eight samples. All remaining samples demonstrated significant bacteriophage loss over 30 weeks storage.

Significance: These data suggest within the 15 sample sets examined, the addition of polymers and sugars to storage buffers, specifically SM buffer with trehalose, may aid in bacteriophage stability throughout freeze drying and prolonged storage.

PAO #: U19-852

P1-111 Food Safety Modernization Act Subpart M: An Evaluation of Pathogen Testing Requirements

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❖ ***Developing Scientist Entrant***

Introduction: The United States Food and Drug Administration (US FDA), in recognition of unique foodborne disease risks, proposed additional minimum standards for sprouts production in Subpart M of the Food Safety Modernization Act (FSMA). Subpart M requires producers to test sprouts or spent sprout irrigation water for *Salmonella* spp. and *Escherichia coli* O157:H7.

Purpose: The following epidemiologic analysis was conducted to determine appropriateness of FDA's pathogen testing requirement, and therefore its potential to minimize known or reasonably foreseeable hazards in sprouts production.

Methods: The Centers for Disease Control and Prevention (CDC) National Outbreak Reporting System (NORS) database was used to identify sprouts-related outbreaks between 1998 and 2017. Data on pathogen genus and serotype, commodity subtype and outbreak detail (e.g., dates, illnesses, hospitalizations) were organized into descriptive statistics. Results were compared with FDA's published rationale and requirements for pathogen testing in FSMA Subpart M.

Results: Between 1998 and 2017, CDC reported 57 sprouts-related outbreaks, of which 40 (70%) were attributable to *Salmonella* spp., 10 (18%) to *E. coli*, 3 (five percent) to *L. monocytogenes*, one (twp percent) to Norovirus, and three (five percent) had unknown etiology. Five (50%) of the outbreak-associated *E. coli* had O157:NM (H-) serotype, three (30%) non-O157 serotypes and two (20%) O157:H7 serotype. Among non-O157-associated outbreaks, there were three implicated serotypes: O26 (outbreak in 2011), O145 (2012) and O121 (2014). The last sprouts-related outbreak of *E. coli* O157:H7 occurred in 2003.

Significance: Analysis of sprouts-related outbreaks in the US covering 1998-2017 indicates that O157:H7 is not the most epidemiologically relevant *E. coli* serotype for this commodity—a deviation in trend from other produce commodities, such as leafy greens. Testing sprouts or spent sprout irrigation water for only the O157:H7 serotype would miss a majority (80%) of historical *E. coli* outbreaks, and therefore brings into question the requirement's capacity to prevent future illness.

P1-112 North Central Region Pre- and Post-Grower Training Knowledge Assessment

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❖ ***Developing Scientist Entrant***

Introduction: A requirement for the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) is that at least one member of a produce farm/organization must take an approved FDA food safety course, such as, Produce Safety Alliance (PSA) Grower training.

Purpose: To assess whether produce growers knowledge of the FSMA PSR improved after receiving PSA Grower Training in the North Central Region of the United States.

Methods: At the beginning of training (pretest) and after the training (post-test), trainers distributed a knowledge assessment to participants. The assessment contained a 25-question multiple choice test established from the seven modules in the course developed by Dr. Amy Harder. Responses were collected by trainers and given to the NCR FSMA evaluation team and entered into Qualtrics from trainings in eight states and 40 trainings. Analysis was performed through SPSS.

Results: Findings indicated that participants (*n*=767) mean total pretest score was 65% while the mean post-test score was 82% out of 25. Participants' pretest food safety knowledge indicated a good understanding of worker health, hygiene, and training (Module 2; 96% correct) but poor understanding of how to develop a farm food safety plan (Module 7; 44% correct). Post-test scores indicated participants continued to score the lowest (68%) on Module 7 and the highest (98%) on Module 2. Participants also consistently scored low on wildlife, domesticated animals, and land use (Module 4; 76% correct). The module with the largest increase in scores between the pretest and post-test was agricultural water part II: postharvest water (Module 6; 26% change).

Significance: This assessment provides evidenced that instructors of the PSA Grower course need to focus on education related to farm food safety plans and wildlife, domesticated animals, and land use. This finding also provides evidence that more educational materials need to be developed to improve training techniques.

P1-113 Louisiana Wild-Caught Catfish under USDA Inspection

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❖ ***Developing Scientist Entrant***

Introduction: In 2015, the US Department of Agriculture (USDA) published the rule "Mandatory Inspection of fish of the order Siluriformes and products derived from such fish." This rule mandated that all catfish processors had to comply with the USDA inspection program beginning March 2016. This was the first time that regulations of seafood products moved from the Food and Drug Administration (FDA) to USDA.

Purpose: Assess the impact of USDA Siluriformes fish regulation on small Louisiana wild-caught catfish processors.

Methods: USDA identified 10 wild-caught catfish facilities in Louisiana. Nine of these facilities were studied. Pre and post-enforcement surveys were completed to identify whether facilities had established prerequisite programs and record keeping associated with sanitation, hazard analysis and critical control points (HACCP), food defense, and product recall. In addition, we evaluated the sanitary conditions in processing facilities and processors' attitude about the change in regulations. Facility size, species processed, and other demographic information were also recorded.

Results: The facility sizes ranged from 240 ft^2 to 3,200ft^2, the number of employees were <10 and 20 to 49 for 90% and 10% of the facilities, respectively. The average of catfish processed per facility ranged from 10,000 to 2,000,000 lb. per year. At the beginning of the study, only one facility had a HACCP plan developed but not implemented. After one year of full enforcement, all the facilities developed and implemented a HACCP plan to process fresh catfish. Even though USDA presence in these facilities improved safety documentation, processors stated that processing procedures did not change. An 80% of the processors reported a reduction in the catfish amount processed due to limit in hours of operation and loss of fishermen.

Significance: Findings from this study provide the policy makers with valuable information to help improve the Siluriformes inspection program and can assist with future policy changes.

P1-114 Mycoflora and Aflatoxin Levels in Stale Retail Pepper Marketed in Ogun State, Nigeria

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❖ ***Developing Scientist Entrant***

Introduction: Fungi constitute a major problem in the production, storage and processing of agricultural products.

Purpose: Recent concern about the consumption of stale retail pepper in our society necessitated the need to determine the mycoflora and aflatoxin contamination of stale pepper.

Methods: A total of twenty stale pepper samples (*Capsicum annum*) from different markets were analysed using standard microbiological procedures and high performance liquid chromatography (HPLC).

Results: Identification of isolates was carried out based on their morphological and microscopic characteristics. Isolated fungi were *Aspergillus flavus* (40.0%), *Sacharomyces cerevisiae* (26.7%), *Mucor* (13.6%), *Aspergillus fumigatus* (6.67%), *Rhizopus* Spp (6.67%) and *Penicillium* (6.67%). Fourteen of the pepper samples had no detectable aflatoxin and six had aflatoxin content of 0.002 ng/kg, 19.000 ng/kg, 0.005 ng/kg, 0.002 ng/kg, 0.001 ng/kg and 0.002 ng/kg, one of which was above the European Union maximum tolerance level of five ng/kg. Statistical analysis was done using SPSS

Significance: The presence of toxin-producing *Aspergillus flavus* capable of causing food poisoning, as well as the sanitary quality of pepper handlers raises concern over public health risks. Adequate training of pepper handlers to maintain high standards of personal and environmental hygiene, which include proper washing of peppers before consumption, regular washing of hands and effective application of hazard analysis critical control point (HACCP) will help control contamination of products.

P1-115 Food Contact Polymer Safety Vulnerabilities and Use of Failure Mode Effects Criticality Analysis for Effective Worker and Food Safety and Chemo-Bioterrorism Management

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Introduction: The safety of food contact polymers (FCPs) in food packaging and disposable gloves are regulated for safety, with reliable estimates of $16 billion in flexible food packaging and 40 billion pairs of disposable gloves utilized in the United States per year.

Purpose: While integral to the safety of the food supply, failure modes, critical control or defense points for food packaging and disposable gloves have seldom been compared to evaluate the adequacy of the safety/regulatory framework involved in their production and use.

Methods: Scenario modeling employed failure mode effects criticality analysis and root cause analysis to understand the various failure modes and their resulting effects and criticality. Following data collection, deductive failure analysis was employed with multiple accidental and intentional events evaluated to identify possible improvements of current approaches and targets for mitigation.

Results: When materials involved, production processes, facility type/geographic location, work force implications and quality/safety assurance standard operating procedures are reviewed, the two product categories have differing risk profiles. The risk priority numbers indicate that negative impacts for food packaging and disposable gloves that could be avoided/reduced in ascending order consist of: foodborne illness, food spoilage, worker safety/efficiency/turnover and chemo-bioterrorism. The latter is seen to reach a potential impact of one to two trillion dollars. For food packaging, FCPs are varied, production automated, and 87% produced in the United States, with frequent inspection and shelf-life testing. Poor manufacturing, storage or use of food packaging may result in worker injury, and physical, chemical and microbiological contamination. For disposable gloves, all factories are in southeast Asia where labor conditions are poor and bioterrorism risks are extensive. Root cause analysis indicates that employee security maintenance programs and third-party auditing are key to of risk mitigation.

Significance: The data developed suggests that for food packaging risks are being managed or are low based on circumstances, but that for disposable gloves there is in an accumulation of chemical, microbiological, and user safety/efficiency risks where control mechanisms are lacking.

P1-116 Inactivation of *Enterococcus faecium* and *Salmonella* in Fried Potato-based Snacks

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Introduction: Potato-based snacks are a popular food item consumed worldwide. *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 10% moisture level when subjected to thermal treatment in oil at 90, 94 and 98°C.

Methods: Product was inoculated with *E. faecium* or a cocktail of *Salmonella* to achieve about six to seven log CFU/g. Inoculated samples were stored overnight for culture adaptation at 4°C. *E. faecium* samples were submersed into a hot oil bath at temperatures of 90, 94, and 98°C for 12.5 min, five min or 100 s, respectively. *Salmonella* samples were submersed at 85, 92, or 98°C for five min, 2.5 min or 50 s, respectively. Inoculated 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 a *D*-value of 3.65 min at 90°C, 1.45 min at 94°C, and 0.63 min at 98°C. *Salmonella* had a *D*-value of 2.11 min at 85°C, 0.80 min at 92°C, and 0.34 min at 98°C.

Significance: *E. faecium* was approximately two times more heat resistant than *Salmonella* at 90°C and 3.7 times at 98°C. 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.

P1-117 An Assessment of Food Safety Training Needs and Preferences among Ohio Food Processors of Various Sizes

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❖ ***Developing Scientist Entrant***

Introduction: Employee training is an important component of food safety system implementation. Determining how facility size impacts training needs and preferences may improve the development of future Extension curriculum.

Purpose: The purpose of this study was to identify self-reported food safety training needs and preferences among small to medium sized food manufacturers in Ohio.

Methods: An anonymous survey was distributed through an email-embedded link to Ohio food processors through existing Extension listservs (IRB exemption 2018E0654). Respondents were characterized by food production in a licensed facility inspected by the Ohio Department of Agriculture's Division of Food Safety, although specific regulatory requirements varied. Respondents were categorized into one of four groups depending on total number of employees (one to two employees, three to 10, 11 to 30, or >30). Descriptive statistical analyses were performed based on responses from 75 food processors to a series of forced choice, open response, and select-all type questions regarding food safety practices and preferences among training delivery style and content.

Results: While both "time" and "cost" were most frequently identified (>25 and >19%, respectively) as barriers to implementing on-going employee training across respondents, small facilities (one to two employees) increasingly identified direct costs as limiting. Additionally, respondents from the smallest facilities preferred online training (89.2%) compared to respondents from the largest facilities (>30 employees) who expressed preference for classroom trainings (61.9%). Finally, 50% of small facility respondents did not report requiring continuing food safety education. Respondents from facilities with 11 to 30 employees (27.3%) and >30 employees (52.4%) required annual employee training.

Significance: Development of future food safety Extension training addressing preferences and perceived needs among food processors may increase the implementation of continuing food safety education among small facilities with limited resources.

P1-118 Exploring Food Safety Practices Related to Food Intolerance and Food Allergy in Campus Foodservices

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❖ ***Developing Scientist Entrant***

Introduction: Food allergy and food intolerance are more common in young people, especially among college students. Since most students consume foods at campus dining services, it should be noted that increasing food safety awareness among dining employees who provide foods for college students is essential. However, limited research has been conducted to investigate the food safety practices of food intolerance and food allergy among campus dining employees in the U.S.

Poster

Purpose: The study examined knowledge and attitudes of campus dining employees and identified barriers of accommodating students with food intolerances and food allergy.

Methods: The study was conducted using both paper and internet-based surveys. The study obtained 103 responses from University dining employees in the U.S., who are at least 18 years of age.

Results: Descriptive statistics provided the demographic profiles of participants. Male (*n* = 49, 47.6%) and female (*n* = 49, 47.6%) were equally distributed. Most participants were Caucasians (*n*= 51, 49.5%), and in the range of 21 to 30 years old (*n*= 37, 35.9%). Linear and multiple regressions indicated that the knowledge (4.45 ± 1.10, p = 0.002) and the attitudes (3.86 ± 1.23, p = 0.000) were significant predictors of food safety practices (4.77 ± 1.68). Respondents were not knowledgeable when asked about the best way to treat a food allergic reaction (n= 30, 29.1%), identify cause of food intolerance (n= 41, 21.4%), and common food intolerance (n= 27, 26.2%). Unable to label allergen information and provide regular trainings due to time constraints were the top two barriers to accommodate students.

Significance: Employees' knowledge and attitudes towards food intolerance and food allergy were positively related to food safety practices on campus. Results can be used by university dining services to develop better strategies to accommodate students with food intolerance and food allergy.

P1-119 Evaluation of Hydrocooling with Two Different Sanitizers in Reducing Microbial Load and Shelf Life for Whole Corn

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❖ ***Developing Scientist Entrant***

Introduction: Growers typically pack corn in to crate and hydrocool quickly with chilled water to remove field heat. Efficacy of hydrocooling without sanitizer or with 150 ppm free Cl (FC) or 80 ppm peroxyacetic acid (PAA) as sanitizer was tested in reducing microbial load from whole corn.

Purpose: Compare the efficacies of hydrocooling with FC and PAA in reducing microbial load from whole corn and effect these treatments have on their shelf life.

Methods: Corn was hydrocooled for ~60 min with plain water, whereas unwashed corn was used as a dry control. Water was amended with 150 ppm FC or 80 ppm PAA, respectively. Three trials (*n*=3) were run for each hydrocooling experiment. Water was analyzed for sanitizer concentration, pH, total dissolved solids (TDS), turbidity, temperature, and chemical oxygen demand (COD). Corn and water were analyzed for aerobic plate count (APC) and yeast and mold (Y and M). Dry control and hydrocooled whole corn were incubated at 5°C for 21 d. Representative samples were taken on days three, seven, 14, and 21 d for microbiological analysis.

Results: APC (log CFU/corn) were 9.58±0.18, 9.40±0.21, 8.64±0.15, and 8.72±0.29 for dry corn, plain water, FC, and PAA per ear of corn, respectively. Y and M counts (log CFU/corn) on dry corn, plain water, FC, and PAA corn were 8.22±0.20, 8.12±0.26, 7.03±0.10, and 7.54±0.07, respectively. Water in FC trails had no detectable microbes during the first two trials. Water in PAA trials had greater than three log CFU/corn of microbes in all trials. At day 21, APC and Y and M were the lowest on FC corn.

Significance: Both FC and PAA reduced initial microbial load from whole corn. FC would be beneficial in preventing cross contamination as no microbes survived in the water. FC, compared to PAA, resulted in better microbiological quality of whole corn during storage.

P1-120 Advanced Environmental Sampling and Testing Methods for Outbreak Investigations

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Introduction: Environmental investigations during foodborne and waterborne outbreaks help identify potential sources of contamination and provide information needed to facilitate remediation and prevention efforts. During environmental investigations, the use of advanced water collection methods, collection of complementary environmental samples, and use of targeted sample testing methods can improve detection rates for pathogens and fecal contamination indicators.

Purpose: To describe advanced environmental sampling and testing methods used in environmental investigations to help elucidate fecal contamination sources and inform remediation and prevention efforts.

Methods: Dead-end ultrafiltration (DEUF) is a field-deployable large-volume water sample collection method that can decrease microbial detection limits by several orders of magnitude compared with traditional grab sampling methods. Submerged sediment samples can be collected from soft-bottomed bodies of water to detect microbes within this environmental niche that may represent historical contamination events. The use of microbe-specific elution buffers allows screening large quantities of sediment. Microbes persisting in surface biofilms can be captured using specialized swabs. Detection of pathogens and indicators from these sample matrices is further improved by microbe-specific detection methods developed and validated for a wide variety of environmental matrices and microbial species.

Results: The use of these targeted sampling and testing methods has resulted in increased detection rates of pathogens and fecal indicators from environmental samples collected during outbreak investigations. These data help to inform the potential contamination sources, design follow up studies into the sources and routes of contamination, and develop potential remediation efforts and strategies to prevent future contamination or illness.

Significance: Advanced environmental sampling and testing methods can be deployed during outbreak investigations to improve data generation and inform measures for improved public health.

P1-121 Bactericidal Effect of Non-Thermal Plasma Against Foodborne Pathogens on Diverse Foods

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Introduction: The public demand for minimally processed foods with low preservatives and better food quality has increased. Non-thermal food processing technologies could be promising and alternative methods for minimally processed foods. Non-thermal gas plasma treatment (NTP) is an emerging technology for enhancing food safety.

Purpose: The purpose of the study was to evaluate the bactericidal effect of non-thermal plasma against foodborne pathogens in various food samples (fruits, vegetables, nuts and powdered foods) and observe the relationship between pathogen inactivation and surface properties.

Methods: *Escherichia coli* O157:H7 spot-inoculated on food samples were exposed to plasma treatment surface dielectric barrier discharge (SDBD) for 20 min. To compare the resistance of different foodborne pathogens, three samples (apple, cabbage and red pepper) were spot-inoculated with culture cocktail of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* (except red pepper) and treated with non-thermal plasma up to 20 min. Hydrophobicity and surface roughness of food samples were measured using optical tensiometer and a noncontact 3D surface profiler, respectively. All experiments were replicated three times.

Results: Non-thermal plasma for 20 min treatment reduced *E. coli* O157:H7 by 0.90 to 5.49 CFU/cm^2 (fruits), 0.35 to 5.16 CFU/cm^2 (vegetables), 0.90 to 1.58 CFU/g (nuts) and 0.76 to 2.66 CFU/g (powdered foods), respectively. As treatment time increased, microbial reduction also increased. After non-thermal plasma treatment for 20 min pathogens on apple, cabbage, and red pepper gradually reduced from 3.65 to 4.94 CFU/cm^2, 2.53 to 3.37 CFU/cm^2 and 2.40 to 2.47 CFU/g, respectively. There was no significant difference (P>0.05) in resistance between foodborne pathogens. The results also showed that surface roughness was more important factor than hydrophobicity correlated to bacterial inactivation by SDBD non-thermal plasma gas treatment.

Significance: These data concluded that roughness is an important factor for SDBD non-thermal plasma treatment. Low surface roughness food samples showed higher inactivation rate using SDBD non-thermal plasma gas treatment.

P1-122 Comparing Efficacy of Hydrocooling with Different Concentrations of Free Chlorine in Reducing Microbial Load from Whole Corn

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Introduction: Growers hydrocool corn with chilled water to reduce the field heat and maintain its freshness. Efficacy of hydrocooling with or without free chlorine (FC), using sodium hypochlorite as asanitizer to reduce microbial load from whole corn, was tested in a mobile hydrocooler.

Purpose: Evaluate the efficacy of hydrocooling with FC to reduce the microbial load from whole corn.

Methods: Corn was field-packed into wooden crates on pallets and hydrocooled for ~60 min with plain water and with 75 or 150 ppm FC (*n*=3). Water samples were collected from the front, middle, and back of the cooling tank at the start and end of each run. Water samples were analyzed for FC, pH, total dissolved solids (TDS), turbidity, temperature, and chemical oxygen demand (COD). Corn samples from top, middle, and bottom layers of the pallet were collected before and after each run. All corn and water samples were analyzed for aerobic plate count (APC) and yeast and mold (Y and M) counts.

Results: The COD in water increased from 53 ppm to 135 ppm when hydrocooled with 75 ppm FC and had a final TDS of >1700 ppm and oxidation-reduction potential of >890. With 150 ppm FC, the water had an initial COD of >270 ppm, TDS of >3000 ppm and oxidation-reduction potential of >830. Unwashed corn had APC and Y and M counts (log CFU/corn) of 9.75±0.29 and 8.40±0.14, respectively. APC and Y and M counts remained unaffected by plain water but were reduced to 7.06±0.36 and 6.53±0.29 log CFU/corn, respectively by hydrocooling with 75 ppm FC. Reduction of microbes from corn was less by hydrocooling with 150 ppm than with 75 ppm FC.

Significance: Plain water showed no effect on APC and Y and M counts, while FC reduced microbial load from corn. The lower reduction seen in higher FC could be due to excessive COD and TDS in the water.

P1-123 WITHDRAWN

P1-124 A Novel Simulation Approach to Improving the Effectiveness of Sampling for Bulk Food Products

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❖ ***Developing Scientist Entrant***

Introduction: Sampling for bulk food products has been studied by sampling in a grid and the effectiveness of sampling has been evaluated by classical statistical theories. Developing a model that takes samples in a continuous space and evaluating it by simulation may offer an alternative view to sampling in realistic food safety scenarios.

Purpose: This study intends to improve sampling effectiveness by better understanding the impact of relevant factors (prevalence, sampling strategy, sample size) on sampling performance.

Methods: A coordinate-based model was constructed in R to simulate random point-contamination, sampling, and lot rejection using attribute plans. The model was applied to a 10 by 10 field with contamination at random locations and predicted the ranges of probability of detection using the Monte Carlo technique for 10,000 iterations. Linear regression analysis was conducted to compare sampling performance using different combinations of input parameters (prevalence: 0.1, 0.5, 1, 2, 5, 10%); sampling strategies: simple random sampling (SRS), stratified random sampling (STRS), *k*-step systematic sampling (SS); sample size: five, 10, 15, 20, 30, 60). Two-way interactions between the three parameters were also tested.

Results: The probability of detection was positively correlated with the prevalence (P<2×10^{-16}) and the sample size (P<2×10^{-16}). Of all the interaction terms, only the interaction between prevalence and sample size was significant (P<2×10^{-16}). Comparison among the three sampling strategies across all combinations of prevalence and sample size suggested that neither STRS nor k-step SS had significantly different performance than that of SRS (P=0.89 and 0.55 respectively).

Significance: SRS, STRS, and k-step SS are equally effective for detecting random contamination at a relatively low prevalence range. Further work will extend this model to applications such as sampling in produce fields or corn in bins.

P1-125 WITHDRAWN

P1-126 Deep Cleans Alone Do Not Reduce *Listeria monocytogenes* Persistence in Retail Delis with Known High Prevalence

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❖ ***Developing Scientist Entrant***

Introduction: Retail deli environments can be persistently contaminated with *Listeria monocytogenes*. We hypothesized that deep cleans and improved SSOPs in delis with known high *L. monocytogenes* prevalence would reduce prevalence and persistence.

Purpose: This study compared *L. monocytogenes* strains before, immediately after, and follow-up from a deep clean intervention in stores with high *L. monocytogenes* prevalence.

Methods: Seven delis were identified to have high (≥10%) *L. monocytogenes* prevalence. Deep cleans were conducted in collaboration with corporate sanitarians and food safety managers. Environmental samples were collected from 20 sites before, after, and longitudinally post-deep clean. PFGE was performed on isolates to define strains; PFGE patterns were analyzed in BioNumerics (Applied Maths, v. 6.6) using an unweighted pair group-matching algo-

rithm and Dice correlation coefficient. Isolates were mapped by store and time; a pulsotype was considered persistent if it was observed for three months or longer.

Results: Over the period of the study, a total of 243 isolates representing 103 pulsotypes were recovered from delis from three U.S. states. Six pulsotypes, five of which were transient, were found in more than one deli, typically in the same state. All the seven delis exhibited distinct *L. monocytogenes* pulsotype cohorts. Overall, six pulsotypes were persistent in four delis, one of which had a single pulsotype detected in 11 of 19 months sampled. Four delis had a pulsotype detected both before and after deep cleans; in three of these four delis, at least one pulsotype persisted for the duration of the study.

Significance: Deep cleans did not control or reduce *L. monocytogenes* prevalence and persistence in delis with known high *L. monocytogenes* prevalence. Our finding indicates that there are factors other than SSOPs that significantly impact food safety dynamics in retail deli environments warranting further studies exploring factors beyond sanitation.

P1-127 Microbiological Risk Assessment of *Staphylococcus aureus* in Ready-to-Eat Lettuce in Taiwan

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Introduction: In Taiwan, *Staphylococcus aureus* is a major foodborne pathogen causing a high number of illnesses linked to the consumption of RTE lettuce products, particularly those that are noncompliant with Taiwan's Certified Agricultural Standards (CAS).

Purpose: This study was to assess the probabilities of illnesses caused by *S. aureus* in CAS and non-CAS RTE lettuce in Taiwan and to identify the critical points for control to reduce the risk.

Methods: The data collected for the quantitative risk assessment included the prevalence and initial levels of *S. aureus* in RTE lettuce, the time and temperature profiles during lettuce processing, transportation, and storage, and the consumption patterns. Laboratory experiments were performed to develop temperature-dependent growth models of *S. aureus* in lettuce. Five modules were constructed to quantify the growth and infection risk of *S. aureus* in lettuce under time and temperature conditions that the products are likely to be exposed to from the processing plants to before the consumption.

Results: The probability of illnesses caused by *S. aureus* in CAS lettuce was estimated to be at 2.40×10^{-6} per serving (95% CI: 1.58×10^{-5} to 4.03×10^{-7}). The probability was higher for non-CAS lettuce at 3.86×10^{-4} (95% CI: 1.07×10^{-4} to 7.72×10^{-4}), indicating non-CAS lettuce products pose a significantly higher *S. aureus* infection risk to the consumers. It is estimated that approximately 160 more cases of staphylococcal illnesses would be caused by non-CAS lettuce than CAS lettuce. The sensitivity analysis showed that the consumer storage conditions, the initial contamination level of *S. aureus* in lettuce, and the retail storage temperature were the most significant factors influencing *S. aureus* risk of RTE lettuce in Taiwan.

Significance: The findings demonstrated that the microbial food safety measures in CAS are effective in reducing the risk of illnesses caused by *S. aureus* in RTE lettuce and identified the critical control points in lettuce processing to consumption to increase the microbiological safety of RTE lettuce.

P1-128 Development of a QMRA Model to Evaluate Health Risks for *Escherichia coli* O157:H7 in Cilantro

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❖ ***Developing Scientist Entrant***

Introduction: Cilantro was implicated in a 2016 *Escherichia coli* O157:H7 outbreak sickening over 100 people in the United States. Cilantro is sold as a raw agricultural commodity and typically added uncooked to foods. Quantitative microbial risk assessments (QMRAs) have proved useful to inform risk management options for control of such hazards.

Purpose: The study aims were to develop a QMRA model to evaluate the public health risks associated with consumption of cilantro contaminated with *E. coli* O157:H7 in the United States, and to evaluate production and post-harvest factors affecting illness numbers.

Methods: Cilantro was modeled from infield production through harvest, processing, transportation, storage, and consumption. Using Visual Basic for Application (VBA) macros and @RISK software, a risk model was developed for exposure and health outcomes. The model was simulated using Latin Hypercube sampling for 100,000 iterations to estimate the number of consumption associated illnesses.

Results: Assuming 40 to 45 days to harvest, the model predicts the average *E. coli* O157:H7 concentration at harvest as 3.2×10^{-4} CFU/g. With a prevalence of 0.1% contamination for harvested cilantro, the model predicts the average concentration increases to 5.2×10^{-2} CFU/g at the time of consumption. Using a dose-response model the average number of illnesses per the United States population is estimated to be 164 illnesses per year. Sensitivity analysis results indicated that transportation temperatures and quality of irrigation water had the largest impact on the number of illnesses per year. Scenario testing for varying risk factors demonstrated the importance of limiting cross contamination along the production chain, especially at higher initial prevalence levels and preventing temperature abuse during transportation from farm to retail when reducing the overall risk of illness.

Significance: The risk assessment model can characterize and estimate the health risks associated with *E. coli* O157:H7 in cilantro to evaluate potential hazard mitigation strategies.

P1-129 Comparison Between Random Forest and Gradient Boosting Machine Methods for Predicting *Listeria* spp. Prevalence in the Environment of Pastured Poultry Farms

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Introduction: Foodborne pathogens such as *Listeria* spp. contain the ability to survive and multiply in poultry farming environments, which provides a route of contamination for poultry processing environments and final poultry products. An understanding of the effect of meteorological variables on the prevalence of *Listeria* spp. in the farming environment is lacking.

Purpose: The purpose of this study was to develop models that can accurately predict the presence of *Listeria* spp. in farming environments based off weather data, and to compare random forest and gradient boosting machine models in a food-safety context.

Methods: Soil and feces samples (*n*=1537) were collected from 11 pastured poultry farms in the southeastern United States from 2014 to 2017 and evaluated for *Listeria* spp. presence. Random forest (RF) and gradient boosting machine (GBM) predictive models were generated to describe and predict *Listeria* spp. prevalence in feces and soil samples based on meteorological factors at the farming location. Meteorological factors of interest included temperature, wind speed, gust speed, precipitation, and humidity.

Results: *Listeria* prevalence was significantly greater ($P<0.05$) in the spring than in the summer and fall. Both feces models performed very well, with area under the curve (AUC) values of 0.905 and 0.855 for the random forest and gradient boosting machine models, respectively. The soil gradient boosting machine model outperformed the random forest model with AUCs of 0.873 and 0.700, respectively.

Significance: The developed models can be used to predict the prevalence of *Listeria* spp. in pastured poultry farm environments based on weather data and should be of great use to poultry farmers, producers, and risk managers. Furthermore, this study proves the usefulness of the gradient boosting machine modeling approach in a food safety context.

P1-130 Risk Estimation of *Clostridium perfringens* from the Consumption of Hamburger and Sandwich Products Available in Retail Markets Using Probabilistic Modeling

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Introduction: *Clostridium perfringens* was the third leading causative bacterial agent for foodborne illness in Korea in 2018. RTE foods including hamburgers and sandwiches are a convenient meal for consumers, but the risk for foodborne illness associated with RTE foods is high. In order to develop risk management strategies, risk assessment of *C. perfringens* in RTE foods is needed.

Purpose: The objective of this study was to develop the probabilistic risk model and to estimate the risk of *C. perfringens* from the consumption of hamburger and sandwiches in Korea.

Methods: A predictive model for the survival of *C. perfringens* in hamburgers and sandwiches was developed using Baranyi and Davey models. Data on microbial contamination in initial samples and the time/temperature during retail markets to transportation were monitored using the method provided by the Food Code in Korea and data logger, respectively. Consumption patterns were adopted from the MFDS 2011 study and Exponential model was used as a dose-response model. The probabilistic risk model was developed based on the collected data using the @Risk program.

Results: The developed predictive model showed that the required time for the first decimal reduction was increased as temperature decreases. The estimated initial contamination level of *C. perfringens* was -3.2 log CFU/g. As a result of Monte Carlo simulation, the estimated probability of infection (P_{inf}) caused by *C. perfringens* from the consumption of hamburgers and sandwiches was 1.78×10^{-13} per person per day. The sensitivity analysis highlighted the distribution of consumption frequency and prevalence of microbial contamination as the most influential input variables on the P_{inf}.

Significance: The results indicate that the risk of *C. perfringens* associated with hamburgers and sandwiches is very low, however, *C. perfringens* survived well in foods at refrigerated temperature, care must be taken to avoid initial contamination of *C. perfringens* during the manufacturing process.

P1-131 Quantitative Assessment of Listeriosis Risk from Domestic Cheese Consumption in Korea

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Introduction: *Listeria* infection can be serious or even life-threatening, particularly for pregnant women, their babies, and elders. Various cheeses are involved in an outbreak of listeriosis worldwide due to high consumption and prolonged refrigerated storage.

Purpose: This study estimated the risk of infection by *L. monocytogenes* due to consumption of cheeses produced in Korea.

Methods: Various cheeses (*n*=520) including natural and processed cheeses from raw or sterilized milk were purchased from on-off line markets and were monitored for contamination with *L. monocytogenes*. To estimate the level change of contamination stepwise from markets to home, the growth model of *L. monocytogenes* in cheeses was developed as a function of temperature (four, 10, 17, 25 and 36°C) using a modified Gompertz equation. The daily consumption amount and frequency of cheese were investigated with 1,500 residents in major provinces in Korea. Finally, Monte Carlo simulation analysis was run for the worst scenarios using @RISK.

Results: The simulated initial concentration of *L. monocytogenes* in cheeses was -4.366 log CFU/g. The daily consumption amount and frequency of cheeses were 18.5 g and 3.78% per person, respectively. The maximum probability of an outbreak of listeriosis by the consumption of cheeses in Korea was 6.182×10^{-8} per person per day. Results of sensitivity analysis show that the frequency of intake was the highest correlation, followed by storage temperature in the markets.

Significance: Considering the frequency of consumption, vulnerable groups such as pregnant women and elders should be educated on the risk of listeriosis. In addition, proper time and temperature management from commercial cheese markets, especially online, should be emphasized.

P1-132 Quantitative Microbial Risk Assessment of *Listeria monocytogenes* in Smoked Salmon from Retail Market to Home

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Introduction: High water activity and neutral pH of smoked salmon are a good environment for the growth of *Listeria monocytogenes* at refrigeration temperature. Because of its high fatality rate, contamination with *L. monocytogenes* in smoked salmon leads to serious risks to consumers. Thus, it is necessary to assess the microbiological risk of smoked salmon.

Purpose: The objective of the study was to conduct a quantitative microbial risk assessment of *L. monocytogenes* in smoked salmon from the retail market to home.

Methods: The initial contamination levels of *L. monocytogenes* in smoked salmon (*n*=375) at retail were monitored. To predict the change of *L. monocytogenes* populations from the market to home during transportation, a predictive model of *L. monocytogenes* in smoked salmon was developed as a function of temperature (four, 10, 17, 25 and 36°C). Data on daily consumption amount and frequency of smoked salmon were collected on 1,000 individuals in Korea. A simulation model was developed and the probability of foodborne illness by the consumption of smoked salmon was estimated with @Risk.

Results: *L. monocytogenes* was detected in two smoked salmon among 375 samples, and the contamination level of *L. monocytogenes* was -3.57 log CFU/g. The growth model of *L. monocytogenes* in smoked salmon was inputted into the simulation model. Daily consumption amount and frequency of smoked salmon were 45.34 g and 1.23% per person, respectively. An exponential dose-response model of *L. monocytogenes* was used and the probability of illness by *L. monocytogenes* of smoked salmon was 5.95×10^{-10} per person per day.

Significance: *L. monocytogenes* is a serious threat to the safety of refrigerated and frozen smoked salmon. Once the contamination of *L. monocytogenes* in smoked salmon occurs, the risk becomes high due to the long shelf life of smoked salmon at the retail market. Thus it is important to implement HACCP and control cross-contamination from raw fish and equipment in the processing facility.

Poster

P1-133 Model Development for Survival and Growth of *Vibrio parahaemolyticus* in Tuna Sashimi as a Function of Temperature

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Introduction: *Vibrio parahaemolyticus* outbreaks have been associated with RTE sushi and sashimi. Although consumption of tuna sashimi is rising in Korea, there is a lack of research on *Vibrio parahaemolyticus* associated with tuna sashimi.

Purpose: The objective of this study was to develop growth and survival models for *Vibrio parahaemolyticus* in tuna sashimi as a function of temperature.

Methods: Tuna sashimi was inoculated with the cocktail of pathogenic (ATCC 27519, 43996) and nonpathogenic *V. parahaemolyticus* (ATCC 17802, 33844) and stored at four, 10, 12, 14, 15, 20, 25 and 27, and 30°C. A modified Gompertz and Weibull primary model was used to obtain growth and survival kinetics, respectively. For secondary models, lag time (LT), specific growth rate (SGR) and *D* values (times for the first decimal reduction of bacteria population) were graphed as a function of temperature.

Results: The growth of *V. parahaemolyticus* was not observed in tuna belly sashimi (toro) due to the level of fat (39.9%). The growth of *V. paraheamolyticus* was observed in tuna sashimi (akami) at 15°C up to 27°C, while survival of *V. paraheamolyticus* in tuna sashimi was observed at below 14°C and *D* value increased from 24.40h at 4°C to 48.01h at 14°C. The highest survival ability of *V. paraheamolyticus* in tuna sashimi was observed at 14°C. Concave shape of curves (p>1) were observed in all survival models, indicating that *V. parahaemolyticus* populations in tuna sashimi decrease slowly in the beginning of storage.

Significance: The results may be useful for microbial risk assessment in tuna sashimi products.

P1-134 Quantitative Risk Assessment for *Clostridium perfringens* in Pickles and Kimchi

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❖ ***Developing Scientist Entrant***

Introduction: Pickles and kimchi are major side dishes in Korea, and those are prepared with various spices. Thus, *Clostridium perfringens* spores can be introduced into pickles and kimchi. In addition, there were recalls for *C. perfringens*-contaminated kimchi.

Purpose: The objective of this study was to assess the risk of *C. perfringens* in pickles and kimchi.

Methods: Radish kimchi was inoculated with a mixture of *C. perfringens* strains at four log CFU/g. The kimchi samples were fermented at 7 to 35°C. The *C. perfringens* cell counts were enumerated on tryptose sulphite cycloserine agar. The cell counts were used to develop predictive models with the Weibull and a polynomial model. To prepare a simulation model with @Risk program, probabilistic distributions for initial contamination and distribution conditions were prepared, and consumption data and a dose-response model were collected. The probability of illness/person/day for *C. perfringens* was calculated through the simulation.

Results: Of 118 samples, *C. perfringens* were detected in eight pickle and kimchi samples (6.8%), and the initial contamination level of *C. perfringens* was estimated, using RiskBeta (9,111). δ values (h) for the pathogen decreased as temperature increased. Temperature during transportation and temperature during market display showed a Weibull (1.3219, 2.8404, shift 3.1093, truncate (0, 40)) distribution and uniform (1.0753, 21.525) distribution. The consumption amount and frequency were 78.95 g and 60.3%, respectively. Exponential dose response model was induced in the simulation model. The simulation with all data showed that the probability of illness per person per day was very low.

Significance: The results indicate that the risk of *C. perfringens* in pickles and kimchi is very low in Korea.

P1-135 Microbial Risk Assessment of *Vibrio parahaemolyticus* in the Salted Seafood Jeotgal

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❖ ***Developing Scientist Entrant***

Introduction: Jeotgal are salted seafoods, and they are prepared and consumed without heating. *Vibrio parahaemolyticus* foodborne outbreaks have occurred due to raw seafood consumption in Korea. Therefore, *V. parahaemolyticus* foodborne illness may occur from jeotgal consumption.

Purpose: This study estimated the risk of *V. parahaemolyticus* from jeotgal consumption in Korea.

Methods: The project consisted of the microbial risk assessment steps (hazard identification, exposure assessment, hazard characterization, and risk characterization). For hazard identification, the effects of *V. parahaemolyticus* on human health were surveyed through literature. For exposure assessment, contamination levels of *V. parahaemolyticus* in different types of jeotgal (squid, oyster, clams, pollack roe, and octopus) were monitored, and the distribution environment, consumption amount and consumption frequency were also surveyed. In addition, predictive models for *V. parahaemolyticus* growth were developed. For dose-response, a dose-response model for *V. parahaemolyticus* was investigated. With the all data collected, a simulation model was prepared in @Risk to calculate the probability of *V. parahaemolyticus* foodborne illness.

Results: Beta distribution for *V. parahaemolyticus* prevalence in jeotgal showed that the mean initial contamination level was -3.2 log CFU/g. The developed predictive model showed that the pathogen levels gradually decreased under the investigated distribution conditions (mean temperature: 20.5°C, mean time: 16.32 h). Jeotgal consumption amount and frequency was 13.89 g and 0.8%, respectively, and the β-Poisson model [risk=1-(1+dose/1.18×10^5)$^{-0.17}$] was the appropriate dose-response model. The simulation with the collected data showed that the probability of *V. parahaemolyticus* foodborne illness by jeotgal consumption was 1.46×10^{-14} per person per day.

Significance: This result indicates that the risk of *V. parahaemolyticus* in jeotgal is very low in Korea.

P1-136 Knowledge, Attitudes and Practices of Hygiene and Food Safety in Health Professionals in a University Hospital of Lisbon

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Introduction: In hospital food service systems, hygiene and food safety issues assume a particularly relevant role, as they serve a population more vulnerable to microbiological and nutritional risk.

Purpose: Assess the level of knowledge, attitudes and practices in hygiene and food safety of health professionals (HP) in a hospital unit.

Methods: In a sample of 318 HP, 111 allied health professionals (AHP) and 207 nurses (N), data were collected through a questionnaire, with 43 questions, covering five major sections (general information, knowledge, common practices, and others).

Results: Results reveal insufficient hygiene and food safety knowledge, with higher incidence in allied health professionals, with over 75% having insufficient knowledge in comparison to almost 50% of nurses, mainly due to misunderstanding of maintenance and food conservation temperatures. Only 25% of both HP groups reflect full knowledge of best practices in control and prevention of food contamination.

Data reveals positive associations between age (*rpb*=0.137; *P*=0.049), professional experience (*rpb*=0.148, *P*=0.033) and the self-assessed attitudes reported in the nurses' group, indicating that the best standards tend to be observed by older and more experienced professionals. Another positive association in the nurses' group is found for elements with previous hygiene and food safety-specific training (*rpb*=-0.324; *P*=0.019) to the adoption of best practices regarding nourishment and personal hygiene and its associated risks (*rp*=0.304; *P*<0.01).

Significance: Results reinforce the need to engage all HP, namely allied health professionals and nurses, in HFS training programs, as these professionals are frequently involved in the reception, manipulation and distribution of food to patients and other users of these catering systems. Such an approach and the awareness of the risks and transmission patterns will allow them to act as educational agents and as promoters of best practices and standards, playing important roles in the prevention and control of foodborne diseases.

P1-137 Evaluation of Food Defense in Hospitality

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Introduction: Food defense is the protection of foodstuffs from threats of contamination and adulteration that may cause harm to public health and/or economic damage. In Europe, it is still a relatively new topic, since the standards of food security existing in the hotel industry are based mainly on HACCP. With the increase of tourism, it is important to ensure overall security and maintain external trust and interest by raising food safety standards

Purpose: Evaluate and assess the preparedness level of the Portuguese hospitality businesses for the prevention of intentional contamination acts.

Methods: There were audits in eight five-star hospitality businesses in Portugal's southern region and food defense plans were formulated for each one of them, using the American software Food Defense Plan Builder (FDPB), created by the FDA

Results: According to FDPB methodology, the visited units scored from 87.7% to 93.1% with an average of 90.6% conformity. In the vulnerability/accessibility assessment, the result from all sites ranged from 7.1 to 11, with an average of nine, on a scale where zero is invulnerable/inaccessible and 20 vulnerable/accessible. The registered nonconformities represented 6.6% and from those, 95.5% scored a medium to low priority. From the 18 FDPB prerequisite question subgroups, eight scored a conformity rate of 100% and in the vulnerability/accessibility evaluation, none of the average values reached the acceptable threshold.

Significance: Results revealed an extremely positive level of preparedness on food defense, even more for being such a rare methodology to be used in the food sector, excluding the United States of America. This work can be used to expand the expertise and certification of food sectors businesses in Portugal in food defense.

P1-138 Risk Assessment of *Clostridium perfringens* in Salted and Fermented Squid (Squid Jeotgal)

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❖ ***Developing Scientist Entrant***

Introduction: Squid jeotgal is fermented squid that is salted and seasoned with spices, followed by fermentation. Because spices may have *Clostridium perfringens* spores and anaerobic condition are created by fermentation, there is a possibility for *C. perfringens* growth in squid jeotgal.

Purpose: The objective of this study was to assess the risk of *C. perfringens* in squid jeotgal.

Methods: *C. perfringens* in squid jeotgal, collected from markets, were enumerated on *perfringens* agar base agar. Temperature and time data for distribution and display were collected. Predictive models were developed with the Weibull model (primary model) to calculate δ (time required for first decimal reduction) of *C. perfringens* at seven to 35°C and *ρ* (shape of curves), and the parameters were analyzed with a secondary model (polynomial equation). Consumption patterns for squid jeotgal were surveyed, and a dose-response model was also searched. With all collected data, a simulation model was prepared, and the simulation was conducted with @Risk to calculate the probability of *C. perfringens* foodborne illness.

Results: One hundred seventeen samples were analyzed, and six samples were *C. perfringens* positive. Thus, initial contamination level of the pathogen was estimated to be -2.3 log CFU/g by the exponential distribution. δ values decreased 225.30 to 18.69 h as storage temperature increased, and the developed secondary model was appropriate with R^{2}=0.905. Annual consumption amount and frequency were 13.89 g and 0.8%, respectively. The exponential model [Risk=1-exp (-r× dose) (r=1.82× 10^{-11})] was selected as an appropriate dose-response model. Subsequently, the simulation with the collected data showed that the probability of foodborne illness by *C. perfringens* per person per day through the consumption of squid jeotgal was 2.53×10^{-14} per person per day.

Significance: This result indicates that the risk of *C perfringens* in squid jeotgal is low in Korea.

P1-139 Risk Assessment of *Clostridium perfringens* in Paste-type Fermented Sauces

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illness caused by *Clostridium perfringens* is continuously reported in Korea, and there is high probability of *C. perfringens* contamination in paste-type fermented sauces.

Purpose: The objective of this study was to analyze the risk of *C. perfringens* infection from consumption of paste-type fermented sauces.

Methods: The prevalence of *C. perfringens* in paste type fermented sauces was investigated by plating the samples on tryptose sulfite cycloserine agar. Data about storage temperature and time of paste type fermented sauce were collected, and probabilistic distributions for the data were determined, using @Risk program. Predictive models for describing the fate of *C. perfringens* in Cheonggukjang (model sauce) were developed, using the Weibull model, and the amount and frequency of consumption were surveyed. A dose response model was searched. Subsequently, these data were used to estimate the risk of *C. perfringens* foodborne illness caused by the intake of paste type fermented sauces through a simulation with @Risk.

Results: Of 1,097 paste-type fermented sauce samples, 74 samples (6.7%) were contaminated with *C. perfringens*. The initial contamination level of the sauces was estimated to be 0.3 log CFU/g by exponential distribution (RiskExpon(RiskExpon(0.95716,RiskShift(0.99913))). The predictive models showed that the cell count levels gradually decreased through the investigated distribution conditions. An exponential distribution showed that consumption amount was 12.12 g and frequency was 41.3%. Exponential model [p=1-exp(-1.82×10^{-11}×dose)] was the appropriate dose response model. The result of the simulation,

Poster

with @Risk showed that the probability of *C. perfringens* foodborne illness was2.84×10^{-9} per person per day for raw consumption and 5.91×10^{-10} for heat cooking consumption in Korea.

Significance: This result indicates that the risk of *C. perfringens* in paste type fermented sauce is low in Korea.

P1-140 Effect of Packaging on the Risk of *Clostridium perfringens* in Ready-to-Eat Lunch Boxes Sold at Convenience Stores

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Introduction: The demand for RTE lunch boxes sold at convenience stores has recently increased in Korea. The most popular main dishes in the lunch box are beef, pork, and chicken etc., which are suitable for the growth of *C. perfringens*. Due to their popularity, it is necessary to evaluate the risk of *C. perfringens* in RTE lunch boxes.

Purpose: The objective of study was to investigate the effect of packaging method on the risk of *C. perfringens* in RTE lunch boxes called "Dosirak" sold at convenience stores.

Methods: The types and main menus of lunch boxes were investigated at four major convenience stores. The initial contamination levels of *C. perfringens* in RTE dosirak were monitored. A predictive survival model of *C. perfringens* was developed. A Weibull model was fitted well to survival behavior of *C. perfringens* as function of temperature and packaging. The δ and *P* values were calculated. The daily consumption amount and frequency of RTE lunch boxes were investigated with 1,000 residents in eight major provinces in Korea. The probability of *C. perfringens* outbreak was calculated by simulation with @Risk.

Results: Ninety-two different kinds of lunch boxes were sold at four major convenience stores. The most popular main menu of dosirak was Jeyuk-bokkeum (stir-fried pork), followed by bulgogi (stir-fried beef) and teriyaki chicken. *C. perfringens* was not detected (<0.5 log CFU/g) in RTE lunch boxes. Higher δ values of *C. perfringens* in anaerobic packed dosirak were observed than in aerobic packed dosirak. An exponential model was used as a dose-response model. Subsequently, the probability of foodborne illness by *C. perfringens* per person per day with consumption of dosirak was 5.62×10^{-11} in aerobic packed dosirak and 1.14×10^{-9} in anaerobic packed dosirak.

Significance: This result suggests that the risk of *C. perfringens* in ready-to-eat lunch box is very low, regardless of packaging method.

P1-141 Quantitative Microbial Risk Assessment of *Vibrio parahaemolyticus* from the Consumption of Ready-to-Eat Foods Containing Seafood Available in Retail Markets

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Introduction: RTE foods containing seafood (RTES) can be contaminated with *Vibrio parahaemolyticus* and have been implicated in foodborne outbreaks. In order to develop the risk management strategies, risk assessment of *V. parahaemolyticus* in RTE foods is essential.

Purpose: The objective of this study was to develop the probabilistic risk model along the food chain and to estimate the risk of *V. parahaemolyticus* from the consumption of RTES in Korea.

Methods: The risk model consists of different modules, including contamination of *V. parahaemolyticus* in RTE foods at production, transportation to retail markets, selling at retail markets, transport to home and consumption stages. Data on the time and temperature at each stage and microbial contamination were monitored using data logger and the method provided by Food Code in Korea. A predictive model to describe the growth of *V. parahaemolyticus* in RTES as a function of time and temperature were developed with Baranyi, Davey and quadratic model. Data on consumption patterns were adopted from the MFDS 2011 study and Beta Poisson model was used as a dose-response model. The probabilistic risk model was developed based on the collected data using the @Risk program.

Results: Prevalence and contamination levels of *V. parahaemolyticus* in RTES were 1.9% and -3.01 log CFU/g, respectively. As a result of Monte Carlo simulation, possible microbial growth was estimated during transportation of RTE foods from retail markets to home under currently available data. The estimated probability of illness due to *V. parahaemolyticus* from the consumption of RTES was 7.65×10^{-9} per person per day. The sensitivity analysis highlighted the distribution of consumption frequency and prevalence of microbial contamination as the most influential input variables on the probability of infection predicted.

Significance: The developed risk model and risk outputs provided scientific background regarding risk management options to control the risk of *V. parahaemolyticus*.

P1-142 Quantitative Risk Assessment Modeling Techniques in Managing Microbiological Food Safety Risks: Risk-based Hazard Analysis and Critical Control Point Plans

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Introduction: Hazard Analysis and Critical Control Point (HACCP) is an internationally recognized system to assure the safety of food products and the foundation of global food safety programs. However, its success is limited by its inability to relate stringency to measurable public health impacts due to its inherent qualitative nature.

Purpose: The aim of this research was to incorporate quantitative microbiological risk assessment (QMRA) techniques into HACCP to develop risk-based HACCP (RB-HACCP) plans.

Methods: The researchers hypothesized that the Critical Control Points (CCPs) step in the process that can be identified using risk assessment modeling techniques such as sensitivity analysis (SA) and what-if scenario analyses can be used to more objectively evaluate Critical Limits (CLs). QMRA models were developed to identify potential risk-based CCPs (RB-CCPs) for *Listeria monocytogenes* for two food products: frankfurters and cold-smoked salmon (CSS). The former has a definitive inactivation step while the latter has a series of partial control steps. Steps of the processes were prioritized using SA to determine steps that most contribute to control of *L. monocytogenes.* What-if scenario analyses were subsequently used to quantitatively determine the consequences of system deviations, thereby allowing risk-based CLs (RB-CLs) to be set and the most-effective risk mitigation strategies to be identified.

Results: This conceptual framework, combined with relevant plant-specific data, was used to identify RB-CCPs and RB-CLs, thereby producing RB-HACCP plans that are linked with public health goals to achieve lower risk of listeriosis. This allowed a direct comparison between industry HACCP plans for frankfurters and CSS with RB-HACCP plans derived from the risk assessments.

Significance: The comparison suggests that the use of RB-HACCP plans may offer advantages in developing the preventive controls risk management food safety plans required under the FDA Food Safety Modernization Act of 2011.

P1-143 Comparison of Linear and Non-linear Models to Describe the Inactivation Kinetics of Vegetative Pathogens during Oil Roasting of Sunflower Kernels

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Introduction: *D*- and *z*-values are widely used to determine the heat resistance of microorganisms. Calculation of *D*- and *z*-values assume first-order kinetics. However, factors such as strain variability, starting inoculum, matrix, and other environmental factors may cause the microorganisms to display non-linear kinetics. Under such circumstances, the utilization of *D*- and *z*-values may result in erroneous conclusions. Hence, alternative approaches to linear models must be considered.

Purpose: The purpose of this study was to compare linear and non-linear models to describe the inactivation kinetics of *Salmonella* spp., *Listeria monocytogenes*, Shiga toxin-producing *E. coli*, and *Enterococcus faecium* in sunflower kernels during oil roasting.

Methods: Sunflower kernels were inoculated with multi-strain *Salmonella* spp., *L. monocytogenes*, and *E. coli* cocktails as well as *E. faecium*. Sunflower kernels were oil roasted at 240, 250 and 260°F for up to five min based off ABC Guidelines for Oil Roasting Processes. Following treatments, samples were immediately cooled and enumerated for the inoculated pathogens using scientifically valid microbiological testing procedures. All the experiments constituted three replicates. Linear and non-linear regression models were fit to log-transformed data using Proc REG procedure in SAS software. Models were statistically deemed fit at an α=0.05.

Results: Data analysis indicated that non-linear regression models described inactivation kinetics in *Salmonella* spp., *L. monocytogenes, and E. coli* better compared to linear models. The R-square values ranged from 0.93 to 0.98. Linear models were found to be a better fit for *E. faecium*. The R-square values ranged from 0.91 to 0.98.

Significance: Data analysis indicated that non-linear regression models described inactivation kinetics in *Salmonella* spp., *L. monocytogenes, and E. coli* better compared to linear models. The R-square values ranged from 0.93 to 0.98. Linear models were found to be a better fit for *E. faecium*. The R-square values ranged from 0.91-0.98.

P1-144 Monetizing the Impact of Food Safety Recalls on the Low-moisture Food Industry

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Introduction: Food Safety and Modernization Act (FSMA) Preventive Controls Rules require that food producers validate that their processes sufficiently reduce the risks from microbial pathogens. However, food safety technology decisions are inherently business decisions, and there currently are no quantitative tools for monetizing the benefits of those investments.

Purpose: The objective was to quantify financial loss from Class I recalls of low-moisture foods, with the ultimate goal of justifying food safety technology investments.

Methods: Financial impacts of low-moisture food recalls on 13 publicly-held food companies were analyzed by computing Cumulative Abnormal Returns (CAR) in stock values over the recall event period. Using GRETL software, returns for a stock under non-recall conditions were predicted. The abnormal return (AR) was calculated as the difference between predicted and actual returns during the event period. Abnormal returns were aggregated to compute CAR over a 20-day period, starting on the day when the recall was announced. The CAR was multiplied by the pre-recall market capitalization for a company, to compute lost value due to the recall.

Results: The regression equations for predicted returns yielded *P* values < 0.001 for all 14 cases. CAR values (20 days post-recall) were -26.5 to 8.4% (mean of -5.3%). Assuming a 0.007 annual recall risk, the average loss 20 days post-recall was $3.3M, with the range of outcomes varying from a loss of $20M to a gain of $3.1M. Assuming implementation of a food safety technology that reduces recall risk by 0.001, mean financial loss would be reduced to $478k per event.

Significance: Class I recalls cause major financial losses for publicly-held manufacturers of low-moisture foods. These loss estimates provide an incentive for the industry to invest in improved food safety technology, and the results provide concrete financial justifications for such investments.

P1-145 Creating a Risk Model for Nosocomial Listeriosis in Cancer Patients Who Consume Ready-to-Eat Salad

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✦ ***Undergraduate Student Award Entrant***

Introduction: Listeriosis is a foodborne illness with relatively low incidence, but substantial mortality rates. Risk of listeriosis is greater in immunocompromised populations, particularly cancer patients, because their treatments compromise several of the barriers against infection. Therefore, nosocomial foodborne listeriosis remains problematic for these patients, with consumption of RTE fresh salads raising particular concern.

Purpose: The objective of this study was to develop a data-driven risk model for *Listeria monocytogenes* in cancer patients due to RTE salads.

Methods: Risk of listeriosis from salad consumption was assessed using an exponential dose-response model with rate constant *r* of 1.79E-10, adopted from a 1997 model that conservatively estimated risk of listeriosis for immunocompromised individuals. Food consumption data were collected from 100 patient surveys, with salad being defined as green lettuce, raw spinach, and/or raw tomatoes. Risk was calculated using Monte Carlo simulation in CrystalBall software. Calculated risk was multiplied by a scale factor of 4.9, as cancer patients have been shown to be 4.9 times more likely to develop listeriosis than individuals with other immunocompromising afflictions. The hypothetical efficacies of two risk management strategies, washing treatments (stirring in water for two minutes, and immersion in sodium hypochlorite for 15 minutes followed by a tap water rinse) and storage (at five and 15°C), were also assessed.

Results: The maximum risk of listeriosis due to salad consumption during one cycle of chemotherapy was 0.0051 (0.51%). Ninety percent of risk values were less than 0.00001. Storing at 5°C and rinsing with tap water substantially reduced the dose of *L. monocytogenes* per risk period.

Significance: Few consumption data-based risk models exist for this subpopulation. Data-driven risk models for listeriosis in cancer patients could provide a justification for existing dietary restrictions.

P1-146 Heterologous Stress Adaptation to Gentamicin in Four Strains of *Listeria monocytogenes* after Sublethal Adaptive Response to Quaternary Ammonium Compound (QAC)

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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 survival of *Listeria monocytogenes* cells adapted to sublethal QAC in lethal concentrations of gentamicin.

Methods: Four strains of *Listeria monocytogenes* were exposed to gradually increasing sublethal concentrations by spiking at hourly intervals for five h to reach a final concentration of two ppm QAC in TSBYE and then the cells were allowed to grow at that sublethal concentration for 19 h until OD_{600nm} reaches 0.9 at the end of each day for five cycles at 22°C. Three methods were used for observing differences in QAC adapted and non-adapted cells for changes in survival patterns in gentamicin: i) Short-range MIC of gentamicin was determined for QAC adapted and non-adapted cells by broth dilution assay; ii) Changes in growth rate and lag time of QAC adapted and non-adapted cells was determined in 50% MIC of gentamicin by OD_{600nm}; and iii) Survival of QAC adapted and non-adapted cells was determined by concentration-to-kill assay in lethal gentamicin (four µg/ml) in an agar model. All experiments were repeated thrice. Log-transformed counts were analyzed using one-way ANOVA in completely randomized block design and means were separated by Fisher's protected LSD when $P<0.05$.

Results: Three major findings were observed: i) No significant changes in short-range MIC of gentamicin was observed between QAC adapted and non-adapted cells; ii) however, growth rate of QAC adapted cells was significantly higher ($P<0.05$) for up to 16 h than non-adapted cells in 50% MIC of gentamicin by OD_{600nm}; and iii) Survival of QAC adapted cells was higher by approximately one log CFU in concentration-to-kill assay in lethal gentamicin in TSA compared to non-adapted cells for one of the four strains but these differences were not statistically significant.

Significance: These findings illustrate the potential for generation of gentamicin tolerant phenotypes of some *L. monocytogenes* strains as a result of a sublethal adaptive response to QAC in conditions where QAC may be used widely.

P1-147 Distribution of Toxin Genes and Antimicrobial Resistance Genes among Staphylococci Isolated from Clinical and Food Samples in Algeria

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Introduction: Staphylococci are responsible for nosocomial/community-acquired infections and foodborne illnesses worldwide. These bacteria remains a persistent opportunistic of the respiratory tract and skin, either for human or animals and constitutes a contamination source in food or health-care.

Purpose: The aim of this study was to determine the occurrence of toxin genes carried by strains of *Staphylococcus aureus* and coagulase negative staphylococci (CNS) in two provinces of Algeria, Médéa and Ain-Defla.

Methods: A total of 96 *Staphylococcus* spp. isolates were used (*Staphylococcus aureus* and CNS). Fifty-one (53.13%) isolates were obtained from food matrices, and 45 (46.88%) were isolated from human clinical samples.

A multiplex PCR assay was used for screening genes encoding for classical enterotoxins (SEs) (*sea* to *see*), exfoliative toxins (*eta, etb*) and toxic shock syndrome toxin-1 (*tst*). By using Simplex PCR, the slime production genes (*ica)* and antiseptic resistance genes (*qac*A/B and *qacC)* were detected. The antibiotic resistance genes *(tet*M/K, *bla*Z, *aac*A-*aph*D, *erm*C, *erm*A and *mec*A) were also investigated.

Results: More than half of food strains (52.94%) possessed at least one of SEs genes; where *S. aureus* appears to be potentially more enterotoxigenic than CNS (68.18% vs 41.37%). From all the SEs genes amplified (27); *sed* gene, 19 isolates (70.37%) was the most frequently detected. In clinical isolates, only six (13%) *S. aureus* harbored at least one SEs genes. However, 55.55% of clinical isolates (*S. aureus* or CNS) possessed the *tst* gene for Toxic shock syndrome toxin-1.

Significance: This study showed a high frequency of SEs genes in food isolates and *tst* genes in clinical isolates; our findings provide updated data on staphylococcal toxin carriage in Algeria. More in-depth investigations and national monitoring programs need to be put in place.

P1-148 Homologous Stress Adaptation in Four Strains of *Listeria monocytogenes* to Quaternary Ammonium Compounds after Sublethal Exposure

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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 *Listeria monocytogenes* cells in lethal quanternary ammonium compounds (QAC) after sublethal adaptation.

Methods: Four strains of *L. monocytogenes* were exposed to gradually increasing sublethal concentrations by spiking at hourly intervals for five h to reach a sublethal concentration of QAC (2 ppm) and then the cells were allowed to grow at that sublethal concentration for 19 h until OD_{600nm} reaches 0.9 at the end of each day for five 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: i) Short-range MIC of QAC was determined for adapted and non-adapted cells by broth dilution assay; ii) Changes in growth rate and lag time of adapted and non-adapted cells was determined by OD_{600nm} in 50% MIC of QAC; and iii) Survival of adapted and non-adapted cells was determined by concentration-to-kill assay in lethal QAC (five ppm) in an agar model. All experiments were repeated three times. Log-transformed counts were analyzed using one-way ANOVA in completely randomized block design and means were separated by Fisher's protected LSD when $P<0.05$.

Results: Three major findings were observed: i) Short-range MIC of QAC was significantly increased for three strains by 1 to 2 ppm ($P<0.05$); ii) Growth rate of QAC adapted cells was faster for all strains in 50% MIC of QAC by OD_{600nm} which was significantly higher than the non-adapted cells ($P<0.05$) at eight h for up to 24 h; and iii) Survival of QAC adapted cells was increased significantly by three to six log ($P<0.05$) for all four strains in concentration-kill-assay in lethal QAC 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 food processing environments.

Poster

P1-149 Application of Metagenomics to Define Microbiomes and Detect *Listeria monocytogenes* in Smoked Fish and Ice Cream Facilities

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Introduction: Current environmental sampling practices involve targeted culturing procedures. Metagenomics can provide accurate and unbiased analyses that reveal the entire microbiome including pathogens and commensals.

Purpose: This work investigated the microbiomes of environmental swab culture enrichments from smoked fish and ice cream manufacturing facilities to assess the efficacy of culture-based environmental sampling workflows for *Listeria monocytogenes*.

Methods: 16S rRNA gene sequencing was performed on 48 environmental swab cultures, 24 from each food processing facility. Shotgun metagenomic sequencing was performed on seven of the 24 swabs from the ice cream facility and six of the 24 swabs from the smoked fish facility.

Results: Both facilities harbored similar taxa (*Enterococcus*, *Pseudomonas*, *Lactococcus)* but distribution and abundance of these taxa varied across sampling sites. 16S rRNA gene sequencing of swabs from the smoked fish and ice cream facilities demonstrated 16 of 16 and three (60%) of five, respectively, congruence to culture-based detection results for the presence of *Listeria monocytogenes*. Conversely, three (38%) of eight and 10 (52% of 19) samples that were negative for *L. monocytogenes* by culture from the smoked fish and ice cream facilities, respectively, yielded appreciable 16S rRNA reads specific to *L. monocytogenes*, with relative abundances ranging from less than one percent to 14%. However, this may be confounded due to the presence of *L. innocua* in seven ice cream swabs. Comparing whole genome sequencing (WGS) data from isolated colonies of *L. monocytogenes* from each facility revealed that shotgun metagenomic reads resolved to within zero to eight SNPs. *L. monocytogenes* from the ice cream and the smoked fish facilities showed different lineages, I and II, respectively.

Significance: This study demonstrated that current culture-based environmental sampling enrichment methods may under-report *Listeria monocytogenes* contamination in food manufacturing facilities. Additionally, we show that shotgun metagenomic sequencing can be utilized for pathogen subtyping and forensic disposition with an equivalent resolution to isolate WGS given adequate sequencing coverage.

P1-150 Development of Kinetic Models with *Salmonella* Isolates from Poultry to Describe the Kinetic Behavior in Chicken and Duck Tenderloins

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is commonly detected from beef, poultry, and processed meat products. In particular, poultry is a major cause of *Salmonella* foodborne outbreaks due to contamination from intestinal contents and cross-contamination between carcasses.

Purpose: This study developed the mathematical models to describe the kinetic behaviors of *Salmonella* in chicken and duck tenderloins during storage.

Methods: A mixture of 14 *Salmonella* isolates from chicken and duck carcasses were inoculated in chicken tenderloin (25 g), and the cell counts were enumerated on xylose lysine deoxycholate agar during storage at four to 25°C for six days. The Baranyi model was fitted to the *Salmonella* cell counts to calculate lag phase duration (*LPD*; h) and maximum specific growth rate (μ_{max}; log CFU/g/h). For secondary modeling, a polynomial model was fitted to the *LPD* and μ_{max} values as a function of storage temperature. To evaluate the accuracy of the model prediction, root mean square error (RMSE), bias factor (B_f) and accuracy factor (A_f) were calculated by comparing the predicted data and the observed data recovered from both chicken and duck tenderloins.

Results: *Salmonella* cell counts were gradually decreased at 4°C, but the cell counts increased at 10 to 25°C. *LPD*s decreased ($P<0.05$) from 22.96 to 4.55 h, and μ_{max} values were increased (°<0.05) from -0.02 to 0.34 log CFU/g/h as temperature increased. The h_0 values, indicating physiological state, were -0.41, 0.37, 1.64, and 1.75 for four, 10, 15, and 25°C, respectively. The developed secondary models were appropriate to describe the temperature effect on the kinetic parameters with R^2=0.915 to 0.928. Within validation, RMSE values of 0.309 (chicken) and 0.304 (duck) suggested that the model performance was acceptable to describe the kinetic behaviors of *Salmonella* in chicken and duck tenderloins.

Significance: These results indicate that the developed model could be useful in describing the kinetic behaviors of *Salmonella* in chicken and duck tenderloins.

P1-151 Evaluation of Kinetic Responses of Pathogenic *Escherichia coli* in Smoked Duck under Dynamic Conditions

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* shows high contamination levels in poultry products. Smoked duck products are usually served with mild heating or without additional heating.

Purpose: This study was evaluated the kinetic responses of *E. coli* in smoked duck under changing temperature through developing a dynamic model.

Methods: A mixture of *E. coli* strains NCCP14038, NCCP14039, NCCP15661 and NCCP11142 was inoculated in smoked duck slices (25 g) at three to four log CFU/g. The samples were stored aerobically at 10 to 30°C for 48 or 96 h in triplicate, depending on temperature. *E. coli* cell counts were enumerated on 3M Petrifilm *E. coli*/coliform count plate. The cell counts data were used to develop a primary model with the Baranyi model, calculating maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD*; h). These kinetic parameters were further analyzed with a quadratic equation. In accordance with the models, a dynamic model was developed to describe the growth pattern of *E. coli* under changing temperature. To evaluate the accuracy of the model prediction, root mean square error (RMSE) was calculated by comparing the predicted data with observed data.

Results: During storage, μ_{max} values for *E. coli* were 0.05 to 0.36 log CFU/g/h, and *LPDs* were 4.39 to 1.07 h, depending on the storage temperature. Even at 10°C, *E. coli* proliferated after only 4.39 h of *LPD*. The secondary model was appropriate with R^2=0.967 to 0.972 to describe the temperature effect on the kinetic parameters. The model performance was appropriate with 0.130 RMSE, and the dynamic model also simulated the kinetic responses of *E. coli* in smoked duck appropriately at changing temperature.

Significance: This result suggests that *E. coli* can proliferate in smoked duck slices even at low temperature, and the developed models should be useful in describing the kinetic behavior of *E. coli*.

Poster

P1-152 Physiological Characterization of *Listeria monocytogenes* Isolates from Smoked Duck

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* has been a cause of foodborne outbreaks related to ready-to-eat products, especially for sliced products. The consumption of smoked duck has increased, and it is usually served in sliced pieces. In addition, *L. monocytogenes* has been isolated from sliced smoked duck, even though it was smoked at high temperature.

Purpose: The objective of this study was to characterize the physiological properties of *L. monocytogenes* isolates from smoked duck.

Methods: One-milliliter aliquots of *L. monocytogenes* isolates (SMFM201803 SD 1-1, SMFM201803 SD 4-1, SMFM201803 SD 4-2, SMFM201804 SD 5-2, SMFM201804 SD 5-3, SMFM201804 SD 6-2 and SMFM201804 SD 7-1) from smoked duck were inoculated into tryptic soy broth (TSB) with 0.6% yeast extract at 60°C, and the cell counts were enumerated on tryptic soy agar (TSA) with 0.6% yeast extract at zero, two, five, eight, and 10 min. To examine the antimicrobial resistance of the isolates, the optical densities of *L. monocytogenes* cultures were adjusted to a 0.5 McFarland standard. They were then swabbed on the surface of Mueller-Hinton agar (MHA), and discs of nine antibiotics were placed on the plates, followed by incubation at 30°C for 24 h. The cultures (n=4) for *L. monocytogenes* isolates were also streaked on TSA supplemented with 5% sheep blood and cultured at 30°C for 48 h to examine the hemolysis.

Results: Of seven *L. monocytogenes* isolates, five isolates showed heat resistances, compared to the standard strains, and three out of the five isolates showed only 1.5 log CFU/ml reductions (P<0.05). Five out of the seven isolates showed α-hemolytic activity. Also, seven isolates were resistant to penicillin G and three isolates were resistant to clindamycin.

Significance: This result indicates that *L. monocytogenes* isolates from smoked duck have heat resistance, antibiotic resistance, and α-hemolysis. Therefore, the risk of *L. monocytogenes* in smoked duck is high.

P1-153 Predictive Model of Growth of *Listeria monocytogenes* in Queso Fresco Cheese

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a hardy psychotropic pathogen that has been linked to several cheese-related outbreaks in the United States, including a recent outbreak where queso fresco cheese was implicated.

Purpose: The purpose of this study was to develop mathematical models that can predict the growth of *L. monocytogenes* in a Mexican-style cheese known as queso fresco and to validate these models using non-isothermal time-temperature profiles.

Methods: A mixture of five strains of *Listeria monocytogenes* were used to inoculate pasteurized whole milk to prepare queso fresco. Ten grams of fresh cheese samples were vacuum packaged and stored at four, 10, 15, 20, 25 and 30°C. Samples were taken from each incubator at different time points depending on the temperature, diluted in 0.1% peptone water and enumerated on *Listeria* selective Oxoid Modified Oxford Agar with supplement. Growth data from each temperature was fitted using the Baranyi model as the primary model and the Ratkowsky model as the secondary model. Primary and secondary models were combined to develop tertiary models using MATLABâ. These models were validated using the acceptable prediction zone (APZ) method with non-isothermal time-temperature profiles (4 to 20°C for 19 d, 15 to 30°C for 11 d).

Results: The Baranyi model was fitted to the isothermal growth data with acceptable goodness of fit statistics (R^2=0.928; RMSE=0.317). Similarly, the Ratkowsky square-root model was fitted to the specific growth rates at different temperatures (R^2=0.975). The APZ analysis concluded that the models developed in this study were acceptable since greater than 70% of the prediction errors were within the APZ (0.5>Prediction Error>-1.0).

Significance: The model developed in this study will be useful for estimating the growth of *Listeria monocytogenes* in queso fresco. These predictions will help in estimating the risk of listeriosis due to extended storage with temperature abuse scenarios for queso fresco.

P1-154 Modeling the Survival Kinetics of *Campylobacter jejuni* in Simulated Gastric Fluid

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❖ ***Developing Scientist Entrant***

Introduction: Although *Campylobacter* is not highly resistant to various environmental stresses, the number of *Campylobacter* infections has not yet decreased so far. To control and evaluate *Campylobacter* infection effectively, the relationship between *Campylobacter* infection and the number of ingested bacteria (dose) needs to be clarified. As an alternative new concept of dose-response modeling, the key events dose-response framework (KEDRF) has received attention. The KEDRF is a method of stepwise combining bacterial survival probabilities for each biological reaction in the human body during the food digestion process. In the present study, we focused on the first digestive process of the stomach.

Purpose: We aimed to develop a predictive model that enables us to estimate the survival kinetics of *Campylobacter* in a simulated gastric fluid (SGF).

Methods: Eleven strains of *Campylobacter jejuni* were used to take into account the strain variability. The SGF was adjusted to the target pH, ranging from 3.0 to 3.8 and kept at 37°C. The inoculum of *C. jejun*i was put into the SGF and incubated at 37°C for an arbitrary duration. An aliquot of the sample was taken from the inoculated SGF at certain intervals and the survival cell numbers were determined. The relationship between the time of exposure to SGF and the logarithm of survival rate of *C. jejuni* was described by a Weibull model.

Results: The survival kinetics of *C. jejuni* were successfully described by Weibull model regardless of the pH conditions and differences in strain (root mean square error <0.50). In addition, the pH dependency of the scale parameter of the Weibull model was successfully illustrated by a log-linear regression model. These models enabled us to predict survival numbers of *C. jejuni* under a changing pH environment.

Significance: This study showed the possibility for prediction of survival numbers of *Campylobacter* during the actual digestion process in the stomach.

P1-155 Kinetic Behavior of *Salmonella* in Cucumber under Changing Temperatures

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Introduction: *Salmonella* is a major foodborne illness bacteria. They are generally transmitted to humans through consumption of contaminated foods. In the United States, there was an outbreak caused by the consumption of cucumber contaminated with *Salmonella* in 29 states.

Purpose: The objective of this study was to develop kinetic models and a dynamic model to predict the kinetic behavior of *Salmonella* cell counts in cucumber at changing temperatures.

Methods: Prepared cucumber samples were immersed in *Salmonella* inocula at five log CFU/ml for three min, and drained for 10 min to allow cell attachment. The samples were stored at 10 to 30°C for 36 h or 48 h, depending on temperature. *Salmonella* cell counts were enumerated on xylose lysine deoxycholate agar. The Baranyi model was fitted to the cell counts to calculate the kinetic parameters such as maximum specific growth rate (μ_{max}; log CFU/g/h) or lag phase duration (*LPD*; h), and the polynomial equation was then fitted to the parameters as a function of temperature. A dynamic model was then developed to predict *Salmonella* cell counts under changing temperature. Root mean square error (RMSE) was calculated by comparing the predicted data with the observed data to evaluate the model performance.

Results: As storage temperature increased, *LPD* (1.81 to 16.59 h) became shorter and μ_{max} (0.01 to 0.34 log CFU/g/h) increased, indicating that *Salmonella* in cucumbers can grow rapidly at high temperature. A polynomial equation was appropriate to describe the effect of temperature on the kinetic parameters with R^2=0.971 to 0.996, and the developed dynamic model was also appropriate to describe the fates of *Salmonella* under changing temperatures. The RMSE value of 0.367 indicated that the developed model had good performance.

Significance: This result indicate that *Salmonella* can proliferate rapidly in cucumber, and the developed models should be useful in describing the kinetic behavior of *Salmonella* in cucumbers.

P1-156 Development of a Dynamic Model to Describe the Fate of *Escherichia coli* in Diced Cucumbers under Dynamic Temperatures

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Introduction: *Escherichia coli* are detected in various foods, especially salad vegetables such as diced cucumbers. However, it is difficult to control pathogens in these vegetables because they are consumed without cooking.

Purpose: The objective of this study was to develop a dynamic model to describe the kinetic behavior of *E. coli* in diced cucumbers at changing temperatures.

Methods: Cucumber was diced into 25-g portions. The diced cucumbers were dipped into inocula of *E. coli* at five log CFU/ml for three min and drained for 10 min, followed by placing them in a sterile bag. The samples were stored at 10, 20, 25 and 30°C. *E. coli* cell counts were enumerated on 3M Petrifilm. To develop a primary model, the Baranyi model was fitted to the cell count data to calculate lag phase duration (*LPD*; h) and maximum specific growth rate (μ_{max}; log CFU/g/h). A polynomial model was then fitted to the parameters as a function of temperature. Subsequently, a dynamic model was developed in accordance with primary and secondary models. To evaluate the accuracy of the model, the root mean square error (RMSE) was calculated.

Results: The *E. coli* cell counts were increased during storage at 10 to 30°C, and thus, μ_{max} increased as temperature increased. The *LPD* decreased as storage temperature increased. The secondary model well-described the temperature effect on *LPD* and μ_{max} with R^2=0.972 to 0.983. Also, the prediction of the dynamic model was appropriate. Within validation, RMSE values with 0.272 suggested that the model performance was appropriate to predict *E. coli* cell counts in sliced cucumber at changing temperature.

Significance: This result indicates that *E. coli* can grow rapidly in diced cucumbers at high storage temperature, and the developed dynamic model should be useful in describing the kinetic behavior of *E. coli* in diced cucumbers at changing temperature.

P1-157 A Risk Assessment Study of *Staphylococcus aureus* in Pancake Batter

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❖ ***Developing Scientist Entrant***

Introduction: To prevent the growth of *Staphylococcus aureus* to levels where heat-stable enterotoxin may be produced, FDA's 2011 Seafood HACCP Guidance suggests conservative temperature-dependent hold times for generic hydrated batter systems. Per the guidance, twelve hours maximum cumulative exposure time at temperatures between 10 and 21°C, and three hours when greater than 21°C is allowed. A microbial challenge study was conducted to provide production facilities more concise guidance on the allowable hold-time between rinse-downs at a given temperature for a defined batter formulation.

Purpose: The purpose of this study was to evaluate the growth of *S. aureus* in pancake batter over time at various temperatures.

Methods: Pancake batter was inoculated with *S. aureus* strains (five-strain cocktail) at a concentration of one to two log CFU/g. Inoculated samples were incubated at 12, 20, 25, 30 and 35°C. Triplicate 25-g samples were enumerated on Staph Express Petrifilm at initial inoculation and every six hours until 10^5 to 10^8 CFU/g was reached for three consecutive time points. The data was log transformed and growth curves were plotted. A negative binomial linear model was fit to each using PROC GENMOD in SAS software.

Results: The study results demonstrated that *S. aureus* growth exceeds the Food Safety Limit (10^5 CFU/g) with a 95% confidence interval in pancake batter in 114.7 h at 12°C; in 26.4 h at 20°C; in 16.7 h at 25°C; in 11.2 h at 30°C; and in 7.8 h at 35°C.

Significance: The results of this study support Conagra Brands pancake manufacturing facilities by determining maximum run times at a given temperature. Adherence to the time and temperature parameters outlined in this study will prevent the potential for *Staphylococcus aureus* growth to levels of human health concern and will be incorporated into the plant's Food Safety Plan to ensure production of safe, wholesome food.

P1-158 A Risk Assessment Study of *Staphylococcus aureus* and *Bacillus cereus* in Beans Based on the Potential for Product Accumulation during Food Processing

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❖ ***Developing Scientist Entrant***

Introduction: High moisture foods (A_w >0.91; pH > 4.5 and < 9.6) such as beans, may permit growth of *Staphylococcus aureus* and *Bacillus cereus*, and therefore, the potential formation of heat-stable enterotoxin, given the processing conditions. Compliance with FSMA 21 CFR Part 117, Subpart B and Section 117.8 (c)(2) and (c)(3) requires a hazard analysis to be performed to determine if preventive controls are needed.

Purpose: The purpose of this study was to evaluate the potential for the growth of *S. aureus* and *B. cereus* in beans during typical manufacturing conditions.

Methods: Pinto and navy bean samples were collected following washing, soaking and blanching. Samples were inoculated with *S. aureus* strains (five-strain cocktail) or *B. cereus* (six-strain cocktail) with approximately 10^1 to 10^3 CFU/g. Inoculated samples were incubated at 20, 25 and 35°C. Triplicate samples

were enumerated using scientifically valid microbiological testing methods following four, eight, 24, 48, 72, 96 and 120 h storage. The data was log transformed and growth curves were plotted. A negative binomial linear model was fit to each using PROC GENMOD in SAS software. Values within the 95% confidence interval were determined for each bean type and temperature.

Results: Growth of the organisms in bean samples following washing did not exceed the Food Safety Limit (FSL; 10^5 CFU/g) ($\alpha<0.05$) up to 120 h. Bean samples following soaking and blanching both surpassed the FSL. This limit was exceeded at 12.4 hours at 20°C; at 11.9 hours at 25°C; and at 5.0 hours at 35°C.

Significance: The study findings highlight that preventive controls must be administered during bean processing to significantly minimize and prevent the growth of *S. aureus/B. cereus*. In addition, preventive control management components must also be in place in the facility's Food Safety Plan to ensure the effectiveness of the preventive controls.

P1-159 Models for Survival of Foodborne Pathogens in Low-water Activity Foods Using Literature Data

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❖ ***Developing Scientist Entrant***

Introduction: Microorganisms can survive for long periods of time in dry foods; however, the factors that control this survival are not well understood.

Purpose: The purpose of this research was to understand and model factors that influence the survival of foodborne pathogens in low water activity foods based on data extracted from the published literature.

Methods: Peer-reviewed journal articles (n=49) meeting the inclusion criteria and related to the survival of *Salmonella*, *Listeria monocytogenes*, and pathogenic *Escherichia coli* were found through university library databases and Google Scholar searches. Foods encompassed by the literature search included almonds, pecans, pistachios, flour, and baked goods. Survival data were extracted from tables and graphs using WebDigitizer into Microsoft Excel. Linear regression models were fit to each data set to estimate CFU/day rates of reduction. Multiple linear stepwise regression models for factors influencing survival rate were developed using the software R. Factors included were temperature, relative humidity, water activity, inoculation buffer type, media type, total experimental time, and initial cell concentration. The Akaike Information Criterion was used to select significant model parameters. Subset regression modeling was used to further refine the models.

Results: The initial multiple linear regression models had adjusted R^2 values of 0.80, 0.82, and 0.85 for *Listeria*, *E. coli* and *Salmonella* respectively. Subset regression modeling increased the adjusted R^2 values to 0.82, 0.85, and 0.86 for *Listeria*, *E. coli* and *Salmonella* respectively. Subset regression models showed that *Listeria* survival was significantly influenced by temperature, relative humidity, media type, and length of experimentation time. *E. coli* survival was significantly influenced by temperature and relative humidity. *Salmonella* survival was significantly influenced by temperature, relative humidity, media type, and buffer type.

Significance: These survival models can be used in risk assessments, to guide future experimental design, and to improve food safety management.

P1-160 Determining Food Safety Modernization Act Compliance in Produce Packinghouses in the Dominican Republic

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Introduction: The Foreign Supplier Verification Program (FSVP) requires that importers verify that the food they import into the United States is produced under the same sanitary standards as food is grown or manufactured in the United States. Depending on the nature of their operation, exporting packinghouses may be covered by either the Produce Safety Rule (PSR) or the Preventive Controls for Human Foods Rule (PCHFR).

Purpose: A "Food Safety Modernization Act (FSMA) Readiness" team evaluated nine packinghouses in the Dominican Republic (La Vega province) to determine their compliance with either the PSR or the PCHFR, in addition to the FSVP.

Methods: A one to 10 scale was developed to determine FSMA adherence. Packinghouses were scored using interviews with management, mock facility inspections, microbial quality for water contacting produce and zone one surfacse, and a review of available food safety records.

Results: On a one to 10 scale, scores ranged from zero to 2.6. Out of the nine packinghouses examined, no facility had a complete food safety plan, although a few were in the process of writing one and had hired PCQIs. Generic *Escherichia coli* was detected in zone one contact water, including produce wash water, at four of the facilities. No facility had completed any requirements for the FSVP, and no facility had a documented plan for how to deal with an FDA inspection. Major deficiencies identified included overall poor hygiene in production areas, lack of employee handwashing, lack of suitable employee break areas, poor to non-existent water monitoring and defense protocols, poorly maintained facilities and equipment, and overall lack of food safety education at the employee and management levels.

Significance: If Dominican packinghouses are consistent with similar facilities throughout the Caribbean and Central America, concerted FSMA and food safety education efforts are necessary to improve the safety of produce imports.

P1-161 Growth of *Clostridium perfringens* in Cooked Chicken during Cooling: One-step Dynamic Inverse Analysis, Sensitivity Analysis, and Markov Chain Monte Carlo Simulation

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Introduction: *Clostridium perfringens* is a major foodborne pathogen found in cooked meat and poultry products.

Purpose: The objective of this study was to determine the kinetic parameters and apply Markov Chain Monte Carlo (MCMC) simulation to predict the growth of *C. perfringens* from spores in ground chicken meat during dynamic cooling.

Methods: Inoculated samples were exposed to various cooling conditions to observe dynamic growth. A combination of four cooling profiles was used in one-step inverse analysis with the Baranyi model as the primary model and the cardinal parameters model as the secondary model. Six kinetic parameters were estimated.

Results: With initial inoculum of ~ four log CFU/g, the estimated T_{min}, T_{opt}, and T_{max} were 14.8, 42.9, and 50.5°C, respectively, with an optimum specific growth rate of 5.25 ln CFU/g per h (or h^{-1}). Analysis of scaled sensitivity coefficients showed that both Q_0 (-2.92) and Y_{max} (19.3 ln CFU/g, or 8.4 log CFU/g) are independent parameters, while other parameters are mild to strongly correlated. Although it may be difficult to estimate highly correlated parameters using a single temperature profile, one-step analysis with multiple different temperature profiles helped estimate them successfully.

The estimated parameters were used as the prior information to construct the posterior distribution for Bayesian analysis. MCMC simulation was used to predict the bacterial growth using six dynamic temperature profiles for validation of the accuracy of the predictive models. The MCMC simulation results showed that treating each parameter as a random number in Bayesian analysis produced more accurate predictions of bacterial growth during cooling than the deterministic method, with residual errors within ±0.25 log CFU/g.

Significance: Bayesian analysis is recommended for predicting the growth of *C. perfringens* in cooked chicken meat during cooling.

P1-162 Growth of Non-toxigenic *Clostridium botulinum* Mutant LNT01 in Cooked Beef: One-step Kinetic Analysis and Comparison with *C. sporogenes* and *C. perfringens* during Dynamic Cooling

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Introduction: *Clostridium botulinum* is a dangerous foodborne pathogen. Understanding its growth kinetics is necessary for assessing the safety of meat products. Due to safety concerns, it is difficult to use and test *C. botulinum* directly in foods in laboratories.

Purpose: The objective of this study was to investigate the growth kinetics of *C. botulinum* LNT01, a non-toxigenic mutant of *C. botulinum* 62A, in cooked ground beef.

Methods: The spores of *C. botulinum* LNT01 were inoculated to ground beef and incubated anaerobically under different temperature conditions to observe growth and develop growth curves. A one-step kinetic analysis method was used to analyze the growth curves to minimize the global residual error. The data analysis was performed used the USDA IPMP-Global Fit.

Results: The results of data analysis showed that the minimum, optimum, and maximum growth temperatures of this mutant are 11.5, 36.4, and 44.3°C, and the estimated optimum specific growth rate is 0.6 ln CFU/g per h or 0.3 log CFU/g per h. The maximum cell density is 7.84 log CFU/g. Both isothermal and dynamic growth curves were used to validate the growth models and kinetic parameters. The residual errors of validation followed a Laplace distribution, with about 60% of the residual errors within ±0.5 log CFU/g of observations, suggesting that these models could predict the growth of *C. botulinum* LNT01 in ground beef with reasonable accuracy. Comparing with *C. perfringens*, *C. botulinum* LNT01 would grow much slower rates and with much longer lag times. Its growth kinetics is very similar to *C. sporogenes* in ground beef.

Significance: The results of computer simulation using the kinetic models show that, while prolific growth of *C. perfringens* may occur in ground beef during cooling, no growth of *C. botulinum* LNT01 would occur under the same conditions. *C. botulinum* LNT01 mutant could be used as a surrogate for studying the growth kinetics of *C. botulinum*.

P1-163 Predictive Model for Growth of *Clostridium botulinum* from Spores in Beef during Cooling

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Introduction: *Clostridium botulinum* is implicated in outbreaks associated with consumption of improperly temperature-controlled low-acid foods. The contaminating spores can germinate and then vegetative cells multiply. These cells can produce a deadly neurotoxin, the causative agent of botulism. Thus, food safety measures primarily target preventing growth from *C. botulinum* spores to guard against the hazards associated with this pathogen. Improper cooling of cooked products is an important food safety concern.

Purpose: To develop a dynamic predictive model for proteolytic *C. botulinum* in beef over the growth temperature range of 10 to 46°C.

Methods: C. *botulinum* growth was quantified in 20 g beef inoculated with ca. two log CFU/g spores, heat shocked by cooking to 71°C for one h, cooled to below 10°C, and then incubated at isothermal conditions between 10 to 46 °C. Data were fitted into four primary growth models, namely Baranyi, Huang, modified Gompertz, and logistic models. Performances of these models were evaluated using accuracy (A_f) and bias (B_f) factors, the coefficient of determination (R^2), and root mean square error (RMSE). Growth rates generated as a function of temperature were fitted to the modified Ratkowsky secondary growth model.

Results: Out of the four models, the Baranyi model fitted well to growth data in the temperature range of 13 to 43°C. The secondary model fitted well to growth rates in this temperature range (R^2= 0.96). Theoretical minimum and maximum temperatures having zero growth rate were calculated as 10 and 46°C, respectively. The resulting values of the accuracy factor (A_f 1.23) and the bias factor (B_f 0.88) showed good performance of the fitted model.

Significance: The developed growth model can predict *C. botulinum* growth from spores in cooked beef under non-isothermal conditions or during extended chilling of cooked beef. Beef processors can employ this information when validating their food safety plan.

P1-164 Validation of the UltraSnap Surface ATP Test and Ensure Luminometer for ATP Hygiene Monitoring on Stainless Steel Surfaces

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Introduction: The use of rapid ATP tests allows for a quick determination of cleanliness; this study demonstrates the use of dry and wet bacteria from surfaces as a measure of the performance of the device.

Purpose: The purpose of this device is to allow users to verify bacterial cleanliness.

Methods: Cultures of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Saccharomyces cerevisiae* were tested. The bacteria were cultured in tryptic soy broth, yeast in Sabouraud. Cells were harvested by centrifugation, washed with buffered peptone water and resuspended. Each organism was diluted to five contamination levels. Surfaces were tested wet and dry. Replicate samples were spread using 0.25 ml over a 4" by 4" stainless steel coupon. Twenty coupons were used for each dilution. Sterile buffered peptone water was spread over 20 coupons for background. 10 coupons were tested for each dilution while the surfaces were wet, and 10 coupons for each dilution were tested while the surfaces were dry. The surfaces were visibly wet when sampling or stored at room temperature until visibly dry.

Results: *S. cerevisiae* was detected at lower dilutions than the other organisms. Wet, it had a limit of detection (LOD) of 849 CFU. When allowed to dry, it had an LOD of 1,012 CFU, corresponding to 69 RLU and 280 RLU respectively. *B. subtilis* when wet had an LOD of 1.93×10^4 CFU. Dry, it gave an LOD of 3.07×10^4 CFU, corresponding to 36 RLU and 28 RLU respectively. *P. aeruginosa* was detectable at higher dilutions. When wet, it gave an LOD of 1.34×105 CFU. When allowed to dry for two h, it gave an LOD of 7.46×10^4 CFU, corresponding to 37 RLU and 145 RLU, respectively.

Significance: The direct detection of bacteria from surfaces using non-specific ATP tests can be a first line defence for cleaning verification. This study validates this device with significant levels of microorganisms

Poster

Poster

P1-165 Identifying Risk Factors Associated with *Salmonella* Prevalence in Southeastern United States' Pastured Poultry Farms

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❖ ***Developing Scientist Entrant***

Introduction: In recent years, consumer demand has increased for pastured poultry products where the practice involves raising poultry in an open, floor-free area. It is necessary to identify the important meteorological and farm practices and processing factors associated with this type of farming practice since pathogens such as *Salmonella* can be present in the environment.

Purpose: The objective of this study was to develop a model that can identify the specific meteorological and farm management factors contributing to the presence of *Salmonella* in pastured poultry farms.

Methods: Longitudinal study was conducted from 11 pastured poultry farms from 2014 to 2017. Feces, soil, whole carcass rinse for processing and final product samples were collected for *Salmonella* presence. Random forest models were generated for each sample. The meteorological factors included variables like temperature, humidity, precipitation, wind speed, and gust speed. The farm management and processing practices variables included the number of birds, years farming, type of farms, and storage temperature. Models were used to predict the presence of *Salmonella*. The relative important plots and partial dependency plots were generated to interpret the models. The model performances were evaluated using the area under the receiver operating characteristic (ROC) curve values.

Results: The *Salmonella* presence in pastured poultry farm was affected by meteorological and farm management practices. Important meteorological factors were guest-speed, temperature, humidity and seasons. Out of the farm practices variables, years farming, flock age, and feed were identified as the important variables. The models were robust in predicting the *Salmonella* presence. A model with perfect prediction would have the area under the ROC curve value of 1.00. The models in this study had values over 0.83.

Significance: The predictive models developed in this study will provide users a practical and effective tool to make informed decisions with scientific evidence to control the risk of *Salmonella* contamination in poultry farms.

P1-166 A Predictive Model for Cross-Contamination of *Salmonella* in the Poultry Chilling Process

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* cross-contamination in the chilling process is a major concern in the poultry industry. Chilling water is an ideal medium for bacterial transfer, which could lead to an increase of *Salmonella* prevalence. However, a predictive model for describing the change of bacterial incidence after chilling has not been studied well, which is needed in a quantitative microbial risk assessment (QMRA) model for the poultry supply chain.

Purpose: This study was to investigate the cross-contamination of *Salmonella* by simulating chilling conditions in the laboratory, and to develop a model for predicting the bacterial incidence after chilling.

Methods: According to the chilling process in Chinese poultry slaughterhouses, the combined effects of bacterial contamination level (one, two, three four, and five log CFU/g), prechill *Salmonella* incidence (three, 10, 22, 33 and 40%) and chlorine concentration (zero, 20, 50, 80 and 100 mg/l) on post-chill bacterial incidence were evaluated. Response surface methodology based on the central composite design with a total of 20 runs was used to predict the post-chill incidence with JMP10 software, and analysis of variance was used to test the significance and variance of the model. Eight random independent trials were conducted with chicken carcasses to calculate the bias factors (B_f) and accuracy factors (A_f) for model validation.

Results: *Salmonella* incidences after all chilling treatments were 30 to 91.7%. Contamination level and prechill incidence showed a positive effect and chlorine concentration showed a negative effect on *Salmonella* incidence, respectively ($P<0.05$). Among the three factors, the contamination level was the major contributor to *Salmonella* incidence. The developed model showed a satisfactory performance to predict the post-chill incidence as evidenced by statistical indices (pseudo-R^2=0.9; $P<0.0001$; RMSE=6.21) and external validation parameters (B_f = 1.02; A_f = 1.11).

Significance: The developed model could provide input data for QMRA models of *Salmonella* in the poultry supply chain in China.

P1-167 Development of an Agent-based Model for Norovirus Contamination of Berries from Infected Workers on the Farm

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Introduction: Fresh and frozen berries have been linked to viral foodborne outbreaks worldwide, but little is known about how berries become contaminated or the levels found on berries once contaminated.

Purpose: The objective of this project was to develop an agent-based model to simulate the spread of norovirus on a berry farm and to predict the prevalence and concentration of norovirus particles on berries picked. Results from this simulation will be incorporated into a published frozen berry quantitative microbial risk assessment to investigate the impact of worker sanitary interventions on risk of norovirus from frozen berries.

Methods: The model was developed using the software AnyLogic. The model simulates the transmission from hands to berries and the pickers' health status was allowed to change through the simulation from susceptible, exposed, infectious, shedding, recovered to immune. The model predicts the prevalence and concentration of norovirus on one million strawberries picked on a farm over a one-week period, by 100 pickers based on literature data. Handwashing compliance was varied during the simulation, depending on the infection status of the worker. Every simulation started with a single ill worker.

Results: The number of infected workers at the end of one week was normally distributed with a mean of 31±26 infected workers and a median of 17 infected workers. The fraction of contaminated berries ranged from 0.2 to 26.6%. If a berry was contaminated, the concentration of norovirus particles was normally distributed and had a mean log of 3.2±1.3 virus particles per berry. As handwashing increased from two to three washes per day, the concentration of norovirus particles decreased by 1.2±0.8 log virus particles on infected workers hands.

Significance: Our results show the importance and limitations of interventions such as handwashing, and how they impact the safety of hand-harvested berries.

P1-168 Understanding the Cross-Contamination of Melons Via Environmental Matrices Simulating Field Conditions

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Introduction: Foodborne outbreaks involving contaminated melons have occurred multiple times in the past decade. Understanding the sources of contamination will help devise control measures and prevent outbreaks.

Purpose: To investigate soil and dust as vehicles of cross-contamination of *Salmonella enterica* to cantaloupes and honeydew melons.

Methods: Six experimental cantaloupe and honeydew melon varieties grown in seven different locations in six states (Arizona, California, Georgia, Texas-Weslaco, Texas-Uvalde, North Carolina, and Indiana) were sampled. Soil from melon fields in Yuma, Arizona was used. Dust was obtained by passing this soil through a No. 100 sieve. For soil transfer, 10-g rinds were placed on *Salmonella* inoculated soil (nine log) and allowed one h of contact time. *Salmonella* inoculated dust (10 log) was sprayed using a duster on the melon rind in a specially designed chamber. *Salmonella* was recovered in PBS using vortexing for soil and dust samples, and sonication followed by centrifuging for melon rinds. Pellets resuspended in PBS and vortexed soil/dust samples were plated on xylose lysine desoxycholate (XLD) agar. All experiments were repeated three times. Transfer (%) of *Salmonella* from soil and dust was calculated using the formula: Transfer (%) = (population on destination×100) / (population on destination+ population on source).

Results: Transfer (%) of *Salmonella* was greater on cantaloupes than honeydew melons with an average of 0.749%±0.008 and 0.183%±0.003 for soil, respectively. Dust had a higher transfer (%) on honeydews than cantaloupes with an average of 0.068%±0.001 and 0.026%±0.0005, respectively. Transfer (%) of *Salmonella* was the highest on HD150, Infinite-Gold (AZ) and least on HD252, Infinite-Gold (TX-Uvalde) and HD150, DaVinci-SAKATA (IN).

Significance: These results help us understand the risk of *Salmonella* contamination from environmental matrices such as soil and dust to melon crops in field conditions. The data can be used for a science-based risk analysis. Appropriate control measures can be implemented.

P1-169 Impact of a Kiln Intervention on Human Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) in Smoked Fish in Ghana

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❖ ***Developing Scientist Entrant***

Introduction: In Ghana, smoked fish is an important animal protein source. It is available in two forms: smoked-soft and smoked-dry. Both are processed using traditional kilns (Chorkor smoker or metal-drum kiln) fuelled with dried wood. This practice results in high concentrations of carcinogenic polycyclic aromatic hydrocarbons (PAHs) in the products. A novel kiln called FAO-Thiaroye technique (FTT) has been introduced in the country as an intervention.

Purpose: The study used a risk-based approach to evaluate the impact of the intervention on human exposure to PAHs in smoked fish in Ghana.

Methods: Batches of *Sardinella* spp. were processed in the traditional kilns and the FTT into the two types smoked fish, with five replicates per kiln (n=30). The PAH levels in the products were then determined by gas chromatography-mass spectrometry. PAHs of interest were benzo(a)pyrene (BaP) and the sum of chrysene, benzo(a)anthracene, benzo(b)fluoranthene and BaP (PAH4). A cross-sectional survey was conducted to determine the consumption (quantities and frequencies) of the commodity in Ghana (812 respondents). The margin of exposure approach (MoE) was then used to determine the PAH public health concern associated with products from different kilns.

Results: BaP and PAH4 levels in the traditional kiln products were up to 70 µg/kg and 395 µg/kg, respectively, while the corresponding levels in FTT products were up to 1.8 µg/kg and 7.6 µg/kg. Whereas the lowest MoE for traditional kiln products was 572 (indicating high hazard exposure), that for FTT products was 72,238 (indicating low hazard exposure).

Significance: The findings suggest that the FTT has a strongly positive impact on reducing human exposure to PAHs in smoked fish in Ghana.

P1-170 Identification of Sources of Nickel Contamination in Foods and Its Exposure Assessment

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❖ ***Developing Scientist Entrant***

Introduction: The International Agency for Research on Cancer has classified nickel as carcinogenic to humans. Nickel is well known to cause allergenic reactions for sensitive individuals through dermal contact. A risk assessment of the European Food Safety Authority (EFSA, 2015) indicated concerns regarding nickel present in diets for the general population and nickel-sensitive individuals, in terms of chronic and acute effects.

Purpose: To identify food products on the European market contributing to the exposure of the population to nickel as preparation for potential risk management strategies.

Methods: In total 826 samples, including 27 food groups, were analyzed for nickel (microwave digestion followed by ICP-MS determination). Statistical analysis and potential contamination pathways were identified. A probabilistic exposure assessment using the Belgian food consumption survey (2014) with @Risk software was conducted.

Results: The average nickel concentration ranged from below the limit of detection (LOD) (12 µg kg^{-1}) for polyol-containing chewing gums to 6271 µg kg^{-1} for solid tea samples. The food products playing major roles in the exposure of Belgian populations to nickel are as follows: chocolate, beans, hazelnut, lentils, figs, peanut and peanut butter with an average nickel concentration of 4140, 2892, 2383, 1883, 1566, 1356 and 1348 µg kg^{-1} respectively. For all samples collected from the brewery sector (commercially available beers, raw materials and semi-products in the beer production process) low levels of nickel were detected, on average 8.36 µg kg^{-1} for commercially available beers. LOD for beers was 0.04 µg kg^{-1}.

Significance: These data identified food products containing elevated nickel concentrations and their potential contamination routs. The exposure of the Belgian population to nickel through consumption of different food products is relevant and further mitigation strategies are needed to decrease the public health burden.

P1-171 Estimated Daily Intake and Cumulative Risk Assessment of Perchlorate Via Diverse Foods for Taiwanese Populations

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Introduction: Perchlorate (ClO_4^-), recognized as a thyroid-disrupting chemical, is a strong oxidizer and has attracted significant attention due to its reactivity and occurrence and persistence in water, soil, food and even in human urine. Little is known about the occurrence of perchlorate in food and human exposure to perchlorate in Taiwan.

Purpose: Our primary objective in this study was to conduct nationwide monitoring of perchlorate levels in 310 food samples to estimate the level of perchlorate to which Taiwanese are exposed.

Methods: Three-hundred ten food samples of twelve categories were purchased from the market or the township with the highest food production according to the statistics published by the Council of Agriculture Department and National food consumption database in Taiwan. All food samples were extracted by deionized H2O and acetonitrile, purified by Envi-Carb cartridge, and then analyzed by HPLC-MS/MS. QA/QC was consistent with Taiwan FDA regulations. Estimated daily doses (EDIs) of perchlorate were calculated by multiplying the detected levels of target foods by food consumption of the corresponding food items. A detailed risk assessment for sex- and the age-specified population was conducted based on the reference dose (RfD) derived by the National Academy of Sciences and the tolerable daily intake (TDI) established by the European Food Safety Authority and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). For the estimation of the daily intake of perchlorate, food consumption rates were obtained from the Nutrition and Health Survey in Taiwan.

Results: Quantifiable amounts of perchlorate were detected over 65 % detection frequencies. Vegetables had the most abundant perchlorate levels (214 ng/g wet weight), followed by dried tea (189 ng/g wet weight), processed food (mean=37.9 ng/g wet weight), and fruits (27.9 ng/g wet weight). The highest 95th percentile of EDI for perchlorate was found in ≥65-year olds (1.43 and 1.46 µg/kg body weight perday), followed by four to six-year olds (1.40 and 1.30 µg/kg BW/day). The hazard indexes ranged from 2.14 to 5.59 according to the RfD, 1.29 to 2.79 according to the TDI proposed by EFSA, and 0.04 to 0.08 established by JECFA.

Significance: These results indicated that Taiwanese people's current exposure to perchlorate from domestic food consumption was evaluated not acceptable.

P1-172 Risk Assessment for Non Dioxin-like Polychlorinated Biphenyl Exposure from Food Consumption in Taiwan

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Introduction: Non-dioxin like polychlorinated biphenyls (NDL PCBs) are persistent organic pollutants (POPs) that accumulate in the environment and many foodstuffs and are associated with human health effects.

Purpose: The objectives of this project are to investigate the background levels of NDL-PCBs in various foods, complete a risk assessment and risk communication for NDL-PCB, and propose a management policy for NDL-PCBs in foodstuffs.

Methods: We first selected the townships with the highest food production as the sampling locations according to statistics from the Council of Agriculture and Fisheries Department. Second, various food samples were collected in major traditional markets or large stores of selected towns from February to September 2015. Finally, the NDL-PCB level of food was measured. Samples were pretreated for cleanup prior to HRGC/HRMS analysis for qualitative and quantitative measurement of 200 food samples.

Results: Based on per gram fresh weight, the highest 39 NDL-PCB levels were found in fish (2.31 ng/g fresh weight), followed by other aquatic products (0.891 ng/g fresh weight). The NDL-PCB levels in plant foods were much lower than animal foods. The NDL-PCB levels in seafood was significantly higher than the terrestrial animal foods, especially in large predatory fishes.

The highest 95% upper limit of the average daily dose (ADD) of six NDL-PCBs was found in three to six-year olds (7.21 ng/kg/day), followed by zero to three-year olds (6.34 ng/kg/day). The main dietary source of NDL-PCB were fish (44.8% to 75.4%) and pork (13.2% to 29.2%). The highest 95% upper limit of ADDs of NDL-PCB in all age groups are lower than the tolerable daily intake (TDI) reference value, 10 ng/kg/day. Therefore, the current dietary exposure to NDL-PCB in Taiwanese people was unlikely to cause undesirable health effects.

Significance: These results indicated that current exposure to NDL-PCB from domestic food consumption cannot be negligible, and increased inspection of NDL-PCBs in foodstuffs should be going on.

P1-173 Occurrence and Profiles of Phthalates in Processed Food from Taiwan and Their Implications for Human Exposure

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Introduction: Researches reported that intake of contaminated foods is the most important exposure pathway of phthalates for the general population. Phthalates mainly migrate to food from packing materials. Literature had pointed out that increasing exposure to phthalates may affect the thyroid, reproductive and developmental function of a human.

Purpose: The present study aimed to investigate the levels of phthalate esters in domestic commercial processed foods and to model consumers' exposure to phthalates through the selected foodstuffs.

Methods: Ten categories of 500 food samples were collected from the Taiwanese market and nine phthalates – dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DIBP), di-n-butyl phthalate (DnBP), benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP), and diisodecyl phthalate (DIDP) – were analyzed with HPLC/MS/MS. Daily dietary exposures to phthalates were estimated using the food consumption data from the database of the Nutrition and Health Survey in Taiwan (NAHSIT) survey.

Results: The detection rate of nine phthalates in the food items was varied from 1.5% for DMP to 99.3% for DIBP. The highest average levels were found for DINP (0.232 µg/g wet weight), the substitutes of DEHP, and followed by DEHP (0.103 µg/g wet weight). The estimated dietary exposures were dominated by DINP (45.1 to 57.3%) and DEHP (15.0 to 19.5%), and followed by DnBP (5.70 to 7.81%). The highest 95th percentile of EDI in each phthalate was found in three- to six- year olds (0.16 to 7.04 µg/kg/day), followed by zero- to three- year olds (0.12 to 6.58 µg/kg/day) and six to 12 year olde (0.12 to 5.60 µg/kg/day). The 95% integrated HI for reproductive and developmental toxicity ranged from 0.503 to 0.841, and 0.322 to 0.586 for hepatic toxicity.

Significance: The current dietary exposure to phthalates for most Taiwanese was acceptable. To ensure food safety, we recommend continuous monitoring of phthalates levels in foods and packaging materials, especially fresh foods and imported foods.

P1-174 Predictive Microbial Modeling of Baking Inactivation Kinetics

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Introduction: For more than 100 years thermal inactivation kinetics parameters, *D*-values and *z*-values, have been used to predict lethality of pathogens during in-container sterilization. However, these parameters alone are inadequate for predicting lethality during baking due to non-isothermal heating and dynamic moisture loss of the product. Improved predictive microbial models that accurately capture the dynamic nature of baking processes are needed.

Purpose: To assess the performance of multiple dynamic inactivation models for predicting microbial inactivation during baking of cookies.

Methods: Models were developed from previously published data for cookies baked at 135°C and 150°C and at high and low humidities. Performance of four models with parameters estimated for isothermal inactivation, from the dynamic process using cookie internal or external temperatures, and using oven dew point temperature were assessed by root mean square error (RMSE), the corrected Akaike-information-criterion (AIC_c), and a normal distribution of the errors. Significance of inactivation parameters was determined by a generalized linear mixed effect model.

Results: Baking time, product internal and surface temperatures and oven wet-bulb temperature were found to be the most significant parameters contributing to microbial inactivation (P<0.05). The isothermal model had the poorest predictive performance (RMSE of 8997 log CFU/g). Model performance was improved dramatically by including internal product temperature (RMSE of 0.850 log CFU/g and AIC_c=319.04) and external product temperature (RMSE of 0.783 log CFU/g and AIC_c=282.06) into the model. The dew point temperature model, which accounted for time, surface temperature, and wet-bulb temperature was the best predictor (lowest RMSE and AIC_c) for microbial inactivation (RMSE of 0.550 log CFU/g and AIC_c=197.08) and could be applied across the full data set, not otherwise possible without environmental measurements.

Significance: Dynamic inactivation models that include product internal and surface temperatures and oven wet-bulb temperature greatly increase the predictive performance and are important process parameters to monitor in baking processes.

P1-175 The Impact of Free Chlorine Concentration in Fresh-cut Romaine Lettuce Wash Water on *E. coli* O157:H7 Cross-Contamination and Risk of Foodborne Illness in the United States

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Introduction: Maintaining free chlorine concentrations in fresh-cut leafy green wash water is done to minimize microbial cross-contamination during processing. There is conflicting information on the impact of free chlorine levels during washing on *E. coli* O157:H7 cross-contamination and risk of illness.

Purpose: Estimate the change in *E. coli* O157:H7 prevalence on romaine lettuce and risk of illness in the U.S. as a function of the free chlorine concentration in wash water used during processing.

Methods: A previously published FDA model on cross-contamination during leafy green processing was combined with *E. coli* O157:H7 dose-response model to evaluate the impact of free chlorine levels in wash water on *E. coli* O157:H7 cross-contamination and risk of illness. Key model inputs were simultaneously varied to investigate a wide range of what-if scenarios, including, within batch prevalence (0.5-10 %), initial level of contamination on lettuce heads (0-3 log cfu/g), level of free chlorine in the flume tank (0-20 ppm), washing time (5-60 s) and produce-to-water ratio (1/20-1/150 kg/L). The model was developed in R and simulated 20,000 independent wash tanks with 120 batches of romaine lettuce.

Results: Simulated results showed 95% probability of no increase in the average risk of illness per serving when a minimum level of free chlorine in wash water was maintained at 10 ppm for scenarios in which initial contamination levels on lettuce heads, within-batch prevalence, and produce-to-water ratio were limited to 2 $\log_{10}$ cfu/g, 10%, and 1/20kg/L, respectively. For the same model input levels, results showed an increase in prevalence of contamination during the washing process.

Significance: While free chlorine in wash water can reduce the risk of illness from exposure to *E. coli* O157:H7 in leafy greens, incoming batch prevalence and initial contamination levels have a significant impact on the extent of cross-contamination that occurs during the washing process.

P1-176 A Comparative Study of Heavy Metal Exposure Risk from the Consumption of Some Common Varieties of Cultured and Captured Fishes in Bangladesh

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Introduction: Food toxicity and health risks due to exposure to heavy metals through fish consumption have become major concerns in the present era. It has become necessary to assess the content of heavy metals such as iron (Fe), copper (Cu), zinc (Zn), arsenic (As), mercury (Hg), and lead (Pb) in commonly consumed cultured and captured fish in Bangladesh.

Purpose: The purpose of this study was to compare the possibly carcinogenic, non-carcinogenic, and other health risks in cultured and captured fish in Bangladesh.

Methods: Fish samples (n=14) from seven fish species in both captured and cultured categories were collected, washed, separated into flesh and bones, minced, and oven-dried for 12 to 24 hours at 80°C, then ground into a fine powder and made into circular pellet weighing 0.1 g each and 7 mm in diameter. Each pellet was compiled in an x-ray fluorescence spectrophotometer for 1,000 seconds to determine heavy metal content. Risk assessment was performed using formulas in Microsoft Excel and SPSS software.

Results: The assessment revealed that among all other identified metals, Zn was most common in the fish samples, followed by Fe, Cu, and others. The HRI of Pb in almost all the samples exceeded the permissible limit (<1) for both adults and children set by the United States Environmental Protection Agency (EPA) and the Food and Agriculture Organization of the United Nations. For Hg, maximum non-carcinogenic risk was estimated at 859.72 for adults and 7,523.57 for children from the consumption of cultured *Puntius sarana*, which is very alarming. Among the carcinogenic risks of Pb, bones of captured *Anabas testudineus* and flesh of cultured *Puntius sarana* showed maximum values of 1.51×10^{-2} and 1.57×10^{-2}, respectively, in children; this exceeds the allowable risk of 1.00×10^{-6} recommended by the EPA (1999). Summarizing the results, the toxicity scores showed substantial rate of possibility to be exposed to heavy metals through fish consumption, especially cultured fish.

Significance: Human health can be at risk due to chronic exposureto heavy metals through fish consumption over the years.

Poster

P1-177 Introduction to the Calculation and Interpretation of Level of Detection

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Introduction: In many international standards related to validation of microbiological methods, the Level of Detection (LOD) is a fundamental method performance characteristic. Typical questions include "How is the LOD computed and interpreted? What is the meaning of its reproducibility? What is the difference between Level of Detection and Limit of Detection?". These questions, along with others, will be addressed.

Purpose: The aim is to provide insight into a method validation approach which is increasingly being used in Europe and the US. This presentation focuses on the calculation and practical use of LOD. Information on how LOD and its reproducibility can be used in the interpretation of positive/negative test results will also be covered. For instance, if the test result from a 20 g test portion is negative using a method with LOD=five CFU/test portion, what is the probability that a homogenized one kg sample has a 50 CFU contamination level?

Methods: Calculation formulas were derived from the ISO 16140 series based on the complementary log-log model. Simulations were used to calculate the measurement uncertainty of the LOD. The discussion of risk analysis is based on microbiological and biostatistical considerations.

Results: Implications of the Poisson assumption for LOD are clarified with respect to the best possible theoretical LOD value and the relationship between LOD_p for different *P* values. The impact atypical data have on LOD are shown in examples. Calculations based on simulated data showed considerable variation of LOD values beyond systematic influences such as incubation conditions and viability of test samples. It is also shown LOD values can be used within risk analysis, and general principles for establishing performance criteria for the LOD in terms of fitness for purpose are discussed.

Significance: This presentation highlights how LOD values can be used to gain further understanding of measurement uncertainty associated with qualitative microbiological testing and how they can be used in connection with risk analysis.

P1-178 Application of Machine Learning for Food Safety Data Analysis

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Introduction: With the development of new technologies such as next-generation sequencing (NGS) in pathogen detection and surveillance, the amount of data in clinical and public health laboratories is increasing dramatically, including metadata. Major challenges currently exist in the interpretation and analysis of large amounts of data.

Purpose: The purpose of this study is to implement machine learning algorithms in food safety data analysis and test the applications.

Methods: Taking *Salmonella* serotyping as an example, random forest (RF) and supporting vector machine (SVM) algorithms were applied on both NGS data and pulsed-field gel electrophoresis (PFGE) data. Topic modeling was used to reduce data dimension and feature selection.

Results: An NGS data set of 323 *Salmonella* isolates was retrieved from National Center for Biotechnology Information (NCBI) database, 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 accuracy and specificity were obtained in both datasets, with even better performance in the SNPs corpus. We also applied topic modeling on 41,232 PFGE profiles of *Salmonella* outbreak-related isolates. The topic model-derived clustering based on highest topic assignment yielded the most accurate classification results.

Significance: The implementation of topic modeling and other machine learning algorithms provides a new way in food safety data analysis. The machine learning algorithms can be applied on various datasets of different sizes to elucidate genetic information and potential biomarker identification, which is especially useful in big data era.

P1-179 Prevalence of Major Antimicrobial Resistance Mechanisms in Putative Extended Spectrum β-Lactamase *Escherichia coli* Isolated from Beef Production Systems and Humans Using Whole Genome Sequencing

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Introduction: Antimicrobial resistance (AMR) has important implications for the continued use of these additives to control infectious diseases in both beef cattle and humans. Recently, whole-genome sequencing (WGS) has been proposed as a one-step tool for efficient AMR surveillance.

Purpose: This study examined AMR mechanisms in putative extended-spectrum β-lactamase *E. coli* (ESBLs) from beef production systems and humans.

Methods: Putative ESBLs (142) were obtained either by sample enrichment in cefotaxime prior to selective plating or direct plating onto ceftriaxone-supplemented MacConkey agar. Isolates from beef production systems consisted of cattle feces (CF) (*n*=40); catch basins (CB) (*n*=36); surface water (SW) (*n*=29); and processing plant (PP) (*n*=4). isolates from humans consisted of municipal Sewage (ST) (*n*=30) and clinically ill patients (HUM) (*n*=3). Isolates were sequenced with the Illumina MiSeq Platform and the sequences assembled using SPADES. ResFinder database was used to detect mechanisms relevant to antibiotics in various classes (tetracyclines, folate synthesis inhibitors, beta-lactams, aminoglycosides, phenicols and quinolones).

Results: Overall, isolates showed resistance mechanisms for tetracycline (*tetA, tetB, tetC and tetM*), (beef production systems 97%; humans 91%); β-lactams (*blaCTX-M, blaTEM, blaCMY, blaOXA*) (beef production systems 74%; humans 100%); folate synthesis inhibitors (*sul1, sul2, sul3, dfrA12, dfrA14, dfrA17*) (beef production systems 77%; humans 73%), aminoglycoside resistance genes (*aac, ant, aph, str*) (beef production systems 73%; humans 82%), phenicols (*floR, catA1, catA2, catB4, cmlA1*) (beef production systems 60%; humans 45%), and quinolones (*qnrS1*), (beef production systems 21%; humans 45%). All-inclusive, average frequency of isolates harboring resistance genes per source were PP (80%), FC (75%), ST (70%), CB (60%), SW (54%), and HUM (55%). The highest occurring AMR mechanisms in all 142 isolates were tetracyclines (91%) and β-lactams (81.7%) while quinolones occurred the least (18.3%).

Significance: Results showed that resistance determinants in ESBL *E. coli* from cattle production systems and associated with humans were reflective of antimicrobial use in cattle and humans, respectively. Tetracycline resistant determinants were common in isolates, regardless of origin, whereas β-lactam and quinolone determinants were more common in isolates associated with humans.

Poster

P1-180 The GenomeTrakr Database Global WGS Network for Foodborne Pathogen Traceback

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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 the 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 ~300,000 draft genomes with a projected growth of over 1,000,000 draft genomes by 2020. Two new international surveillance efforts were added to collect food, animal and environmental isolates including *Campylobacter* and *Listeria* in Africa. NCBI has released new data analysis tools that improve rapid interpretation. NCBI currently is producing daily clustering results for 27 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 2018. Results demonstrate the global benefits of having an open data model. Understanding root causes of foodborne contamination assist our academic, public health and industry partners to develop preventative controls to make food safer globally.

P1-181 Use of Whole Genome Sequencing, Epidemiologic, and Traceback Data to Link a Multistate *Listeria monocytogenes* Outbreak to Ready-to-Eat Pork Products

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Introduction: Four *Listeria monocytogenes* clinical isolates and 10 Food Safety and Inspection Service (FSIS) isolates from Establishment A, an Asian-style pork products producer, collected from 2014 to 2018, were found to be closely related through whole genome sequencing (WGS).

Purpose: To demonstrate how WGS was used as evidence to link a small outbreak to RTE products and its value is given the difficulty in obtaining epidemiologic and traceback data.

Methods: FSIS investigated the outbreak in coordination with the Centers for Disease Control and Prevention and state and local public health partners. State and local health departments interviewed case-patients or their surrogates with a standard *Listeria* Initiative questionnaire. FSIS and public health partners evaluated available epidemiological and traceback data and used WGS data to assess the relatedness between clinical isolates and isolates collected from Establishment A.

Results: Between June 2014 and October 2018, FSIS recovered two product and eight environmental isolates from Establishment A that were closely related through WGS. Isolates from four patients with isolation dates ranging from July 2017 through October 2018 were closely related to the product and environmental isolates (zero to 10 alleles (median of four)). Further, no other food or environmental *L. monocytogenes* isolates were within 50 single nucleotide polymorphisms in the National Center for Biotechnology Information database. All four patients reported eating Asian-style foods and three reported shopping at stores where pork products from Establishment A were sold. Establishment A voluntarily recalled RTE pork products produced from May through November 2018.

Significance: Outbreaks of *Listeria* infections present unique challenges to the gathering of epidemiological data due to the small number of cases and extended incubation period. WGS results, along with available epidemiological information, linked illnesses and RTE pork products, illustrating the value of a multi-faceted approach during outbreak investigations.

P1-182 Long Read Sequencing for Food Safety Applications

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Introduction: With decades of development, the second- and third-generation sequencing technologies have become indispensable and approachable. The third generation sequencers are particularly captivating with their long read sequencing (LRS) capabilities, rapid and flexible sequencing methods, and real-time data analysis features.

Purpose: This study evaluated the application of LRS for the food safety industry, using Oxford Nanopore MinION sequencer.

Methods: Two *Salmonella enterica* and two *Listeria monocytogenes* strains were selected for genomic DNA (gDNA) isolation and sequencing. A total of 11 LRS runs were conducted, evaluating Ligation-, Rapid-, and Rapid Barcoding- sequencing kits in onsite and remote sequencing modes. Runs included single gDNA and multiplexing of four gDNA sequenced onsite and remotely. MinKNOW and FASTQ WIMP were used for sequencing and real-time analysis. Illumina MiSeq sequencing data was also used to comprehensively evaluate LRS performance.

Results: Long read sequencing demonstrated the purported advantages. Results indicated that when coupled with analyzing tools, LRS identified all the species/serotypes with 100% accuracy using sequencing reads generated either on-site or remotely, within an hour (single gDNA) or two hours (multiplexed gDNA). However, drawbacks were observed during benchmarking. For example, sequencing software was prone to crashing and had high demands on computing power. Moreover, base-calling speed was heavily relied upon and easily hindered by the host computer. Lastly, despite accurate species/serotype classification results, the sequencing error rate of LRS was rather high. The average quality score (QS) generated by LRS was 11 (7.9% error rate); whereas the QS with MiSeq was 30 (0.01% error rate).

Poster

Significance: This study suggested that the rapid and remote sequencing capabilities and accurate classification featured in LRS are promising. With further optimization, there is potential to use LRS for food safety applications in the near future, with enhanced performance advantages as compared to current technologies.

P1-183 Molecular Characterization of Native *Lactobacillus* Strains Isolated from *Vaccinium floribundum* Kunth by Partial Sequencing of 16S rDNA Genes

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Introduction: Berries that grow wild in Peru above 3500 m.a.s.l. have acquired medicinal and commercial importance due to a high concentration of antioxidants, and several species of *Lactobacillus*, which are part of their native flora, could be a valuable tool to increase natural benefits in different foodstuffs.

Purpose: This study aims to isolate and perform molecular characterization of the native *Lactobacillus* strains present in *Vaccinium floribundum* Kunth, a fruit grown in the Andean region of La Libertad, Huamachuco.

Methods: Fresh fruits of *V. floribundum* Kunth (10 kg) were subjected to crushing, followed by serial tenfold dilutions in MRS broth, then inoculation in MRS agar and incubation at 30°C under anaerobic conditions (10% CO_2) for three days. From its morphological characteristics, catalase test and Gram stain, 20 colonies were isolated that were considered presumptive. The molecular characterization required the extraction of genomic DNA in each presumptive, whose genetic material served as a model for the partial sequencing of the 16S rDNA gene. The sequence obtained were analyzed with the database of Genebank genetic sequenced, using the BLAST algorithm.

Results: Sixteen (80%) of 20 isolated colonies were confirmed as *Lactobacillus*, identified as the species *L. plantarum, L. paracasei, L. fermentum and L. pentosus* in five (25%) of 20, two (10%) of 20, eight (40%) of 20 and one (five percent) of 20, respectively. In addition, other lactic acid bacteria were identified such as *Weissella cibaria* in three (15%) of 20 and *Leuconostoc mesenteroides* in one (five percent) of 20.

Significance: The identification of native *Lactobacillus* species will allow their potential use in the production of probiotic functional foods based on *V. floribundum* Kunth.

P1-184 Implications of Mobile Genetic Elements for *Salmonella enterica* Single Nucleotide Polymorphism Subtyping and Source Tracking Investigations

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❖ ***Developing Scientist Entrant***

Introduction: Single nucleotide polymorphisms (SNPs) are widely used for whole genome sequencing (WGS)-based subtyping of foodborne pathogens. Mobile genetic elements (MGEs) are commonly present in bacterial genomes and may affect SNP subtyping if their evolutionary history and dynamics differ from that of the bacterial chromosomes.

Purpose: To survey major categories of MGEs in major *Salmonella* serotypes, including genomes from outbreak clusters and specific sources, and to evaluate the impact of MGE on *Salmonella* subtyping and its utility for source tracking.

Methods: Using *S. enterica* as a model organism, we surveyed major categories of MGEs, including plasmids, phages, insertion elements (IS), integrons, and integrative and conjugative elements (ICEs), in 990 genomes representing 21 major *Salmonella* serotypes. We evaluated how plasmids and chromosomal MGEs affect SNP subtyping with nine outbreak clusters of different serotypes in the United States. We further investigated compositional stability and SNP accumulation associated with plasmid and chromosomal MGEs among isolates from a chronic human infection case and a laboratory cross-contamination case.

Results: Chromosomal MGEs account for a median of 2.5% of a typical *S. enterica* genome. Of the 990 analyzed isolates 68.9% contained at least one assembled plasmid sequence. The median total length of the assembled plasmid sequences was 93,671 base pairs. Plasmids carrying high densities of SNPs were found to substantially affect both SNP phylogenies and SNP distances among closely-related isolates if they were present in the reference genome for SNP subtyping. In comparison, chromosomal MGEs were found to have limited impact on SNP subtyping. We recommend plasmid sequences be identified and excluded from the reference genome prior to SNP subtyping analysis.

Significance: This study provides evidence-based guidance on the treatment of MGEs in SNP analysis for *Salmonella* subtyping, which provides important laboratory evidence and clues in outbreak and source tracking investigations of this pathogen.

P1-185 Microbial Genetics and Clonal Dissemination of Clinical *Salmonella* Javiana in the Southeastern United States

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Introduction: *Salmonella* is estimated to cause over a million infections and ~400 deaths annually in the United States. *Salmonella* Javiana outbreaks have also been linked to different kind of foods as well as animal reservoirs. *Salmonella* Javiana strains (*n*=409) that predominantly originated from the southeastern United States over a six-year period (2003 to 2008) were studied. This period concurred with a rapid increase in the incidence of *Salmonella Javiana* infections in the United States.

Purpose: To better understand *Salmonella* Javiana emergence and to assess its threat to cause human outbreaks.

Methods: Antimicrobial resistance (AR) profiles against 15 antimicrobials was assessed. Pulsed field gel electrophoresis (PFGE) assessed the genetic diversity and distribution of *Salmonella* Javiana strains using *Xba*I restriction. Isolates from each cluster were digested with restriction enzyme *Avr*II. A subset of isolates (*n*=19) with unique resistance phenotypes underwent plasmid and incompatibility type analyses. For confirmation of clonality and epidemiological persistence of isolates, the whole genome sequencing (WGS) of a subset of isolates (*n*=18) from different locations and years has been conducted.

Results: Children under age of 10 displayed the highest prevalence of infections. About ninety-two percent (*n*=375) of the isolates were resistant to at least one antimicrobial. Nine major clusters were identified by PFGE. Isolates with identical *Xba*I and *Avr*II profiles were found to be disseminated in human

populations and persistent for multiple years. Isolates resistant to more than one antimicrobial harbored multiple plasmids (less than three to 165 kb) and possessed 14 virulence genes including *pagC, cdtB,* and *iroN*. Variable conserved genes were detected which were involved in fundamental functions and/or energy transcription, however, there was variable occurrence of genes involved in transport, acquisition, motility, virulence, and AR.

Significance: This study will provide data on *Salmonella* Javiana that may be valuable to understanding potential future emergence of other serotypes as prominent human foodborne pathogens.

P1-186 *Pseudomonadaceae* and *Dipodascaceae* Were Associated with Persistent Occurrence of *Listeria monocytogenes* in a Longitudinal Microbiome Monitoring of Three Apple Packinghouses

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Introduction: A multistate outbreak and recalls due to *L. monocytogenes* contamination of apples or apple products in recent years demonstrated the need for improved pathogen control in the apple supply chain. Apple processing facilities have been identified in the past as potential sources of persisting *L. monocytogenes* contamination. However, the composition of facility environmental microbiota that may affect the ability of *L. monocytogenes* to survive and persist in these environments has not been investigated to date.

Purpose: The purpose of this study was to elucidate the potential association between the composition of the microbiomes and mycobiomes and the occurrence of *L. monocytogenes* in the built environment of three apple packinghouses located in the northeast United States.

Methods: *L. monocytogenes* was detected in environmental sponge swabs by following the FDA Bacterial Analytic Manuel (BAM) protocol for enrichment of *Listeria monocytogenes*. The facility microbiomes and mycobiomes were characterized through 16S rRNA and ITS2 amplicon sequencing.

Results: Analysis of 117 samples from the three investigated apple packing facilities (F1, F2 and F3) showed that facility F2 had a significantly higher *L. monocytogenes* occurrence compared to F1 and F3 ($P<0.01$). Furthermore, facility F2 had distinct microbiomes compared to facilities F1 and F3. The mean microbial and fungal alpha diversity determined using Shannon index were both significantly different ($P<0.01$) in F2, compared to F1 and F3. Higher relative abundance of *Pseudomonadaceae* was observed in facility F2 which had a higher occurrence of *L. monocytogenes*. Furthermore, fungal family *Dipodascaceae* was found exclusively in facility F2.

Significance: Our results show associations between microbiome composition and the *L. monocytogenes* occurrence in apple packinghouses. These results provide a data baseline needed for further in-depth investigation of microbial interactions between non-pathogenic and pathogenic microorganisms found in food processing environments that may lead to optimized pathogen control strategies.

P1-187 Associations between Microbial Ecology, Environmental Factors and Microbiological Quality of Surface Waters Collected in the Northeast United States

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❖ ***Developing Scientist Entrant***

Introduction: Recent outbreaks demonstrate that surface water used for irrigation can act as a key source of preharvest produce contamination by foodborne pathogens. Several studies indicated that current indicators of foodborne pathogen contamination are not reliable under all relevant environmental conditions. Hence, alternative or supplementary indicators would be beneficial.

Purpose: The purpose of this study was to investigate the associations between the surface water microbiome, sampling sites, sample types, and the presence of foodborne pathogens.

Methods: We characterized the composition of the bacterial and fungal communities in 68 water samples collected from six streams in New York between May and August 2017. Samples were separated into suspended sediments and water fractions. Microbial communities were determined by NGS sequencing of PCR-amplified 16S rDNA V4 and ITS2 sequences. Sequences were classified into operational taxonomic units (OTUs) and assigned taxonomy to investigate sample biodiversity. Beta diversity indices were used for PCoA clustering and permutational multivariate analysis of variances (PERMANOVA). Random forest (RF) was used to test whether microbiome composition can be used for prediction of pathogen presence.

Results: Microbial communities differed between water fractions and suspended sediments according to the PCoA, and PERMANOVA ($P<0.01$). Pair-wise PERMANOVA indicated that microbial communities differ significantly between the sediment fractions from different sampling sites ($P<0.01$). Although we found evidence of an association between certain microbial families (e.g., *Kallotenuaceae*, *Flavobacteriaceae*, and *Sericytochromatia*) and *Salmonella* presence, the RF had low accuracy (AUC=0.55), which may be due to the small sample size (*n*=68 samples).

Significance: This study took a novel approach combining metagenomic, statistical and machine learning approaches to provide baseline data describing the surface water microbiome composition and the relationships between microbiome composition and cultured pathogen occurrence.

P1-188 Organic Amendments Influence the Rhizosphere and Phyllosphere Microbiota Profiles of Collard Greens Grown in Southeast Texas

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Introduction: Organic soil amendments are applied to soils to provide crops with nutrients and improve soil water infiltration and water-holding capacities.

Purpose: The objective of this study was to assess the response of rhizosphere and phyllosphere microbiota composition to various organic amendments (chicken manure, cow manure, or milogranite fertilizer) at four rates (control: zero kg/ha, low: 168 kg/ha, med: 336 kg/ha, high: 672 kg/ha) under collard greens grown in southeast Texas conditions.

Methods: Each treatment was replicated three times resulting in a total of 36 plots. Six soil and leaf samples were collected from each plot. DNA was then extracted and V4 region of bacterial 16S rRNA gene was amplified and sequenced. DNA sequence analysis was performed using the QIIME pipeline.

Results: Samples from treatments with high organic amendment rates yielded higher bacterial populations. A significant increase in α-diversity was observed with amended soils compared to the control based on the observed OTUs ($P\leq0.05$). Principal coordinates analysis of the weighted UniFrac distance metric showed a clear separation between samples that were driven by the amendment type. γ-proteobacteria populations significantly increased in the rhizosphere and phyllosphere of collard greens grown in the amended soils ($P\leq0.05$).

Poster

Significance: In conclusion, rhizosphere and phyllosphere bacterial communities were modulated by different organic amendments that might increase potential bacterial pathogens on collard greens leaves.

P1-189 Diversity of the *Escherichia coli* O145:H28 Accessory Genome Including Shiga Toxin-converting Prophages Originating from a Leafy Greens Growing Region in California

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) includes a group of genetically and phenotypically diverse strains that cause foodborne disease. The STEC core genome has been widely used to deduce the strain lineage, whereas the accessory genome has been suggested as encoding functions that can be used to predict the virulence potential of environmental isolates.

Purpose: The goal of this study is to reveal the composition and putative functions of the STEC O145:H28 accessory genome and to test if virulence potential can be predicted from it.

Methods: The genomes of 12 STEC O145:H28 strains isolated from a major leafy greens-growing region in California were sequenced using PacBio RSII. Comparative genomics analyses were performed to reveal the core and accessory genomes. Production of Shiga toxin was quantified by ELISA.

Results: The genomes of environmental strains vary from 5,432,556 bp to 5,576,061 bp. The number of prophages in each genome varies from 14 to 17. All environmental strains carry the core virulence determinants of enterohemorrhagic *Escherichia coli* including LEE island and hemolysin-encoding plasmid. The accessory genome contains nearly 2000 genes. Six and five strains carry a Stx1a- and Stx2a-converting prophage, respectively; whereas one strain carries two Stx2a-converting prophages. The Stx2a-converting prophage identified in strains linked to the 2010 romaine lettuce-associated outbreak in the United States was only detected in one environmental strain. The insertion sites of Stx1a-converting prophages included *wrbA*, and between the genes *yccE* and *ydeH*. The insertion sites of Stx2a-converting prophages included *znuB*, *ompW*, and between the genes *mlaA* and *dsdX*. Interestingly, the prophages using the same insertion site were clustered together and exhibited a similar level of Shiga toxin production following mitomycin C treatment.

Significance: The data suggest that genotypically indistinguishable strains can be typed using the accessory genes and the virulence potential can be predicted using accessory function including the prophages insertion sites.

P1-190 Food Component Influence on the Water Activity and Net Isosteric Heat of Sorption for Low-moisture Foods at Elevated Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: Recent research suggested exponentially increased thermal resistance of *Salmonella* at a reduced water activity (a_w) in thermal treatments. Information on a_w change as affected by food components at high temperatures is limited.

Purpose: The objective of this project was to quantify the influence of major food components on a_w changes in low-moisture foods with increased temperature.

Methods: Corn starch, soy protein, coconut milk powder, and cheddar cheese powder were selected as high carbohydrate (~100%, dry basis), high protein (~100%), high fat (~66%), and intermediate (~33% each for carbohydrate, protein, and fat) products. The four products were vacuum dried at 25ºC at a pressure of 10 kPa. Dried samples were conditioned in jars containing saturated salt solutions to different water activity/relative humidity (11.3, 22.5, 32.8, 43.2, 52.9, 65.8, 75.3, 84.3%) at room temperature (~25°C). The a_w of food samples in sealed containers were measured at 25, 30, 40, 50, 60, 70, and 80ºC by a specially designed high-temperature cell. The moisture content of all samples was determined by a moisture analyzer. Data was fit into the Clausius-Clapeyron equation to obtain net isosteric heat of sorption parameters.

Results: For a given initial a_w at room temperature, high carbohydrate products had larger a_w increase than high protein and high-fat samples with increasing temperature. The net isosteric heat of sorption increased from high-fat to high-protein to high-carbohydrate food at the same moisture content. Based on the quantitative relationship between a_w and thermal resistance of bacteria, cells could be more easily inactivated in high-carbohydrate products than in high-protein and high-fat foods.

Significance: Understanding the correlation between food components and a_w change at elevated temperatures could be a new approach to predict the thermal resistance of bacteria in low-moisture foods.

P1-191 Evaluation of Genetic Relatedness and Plasmid-mediated Virulence of *Salmonella S*chwarzengrund Strains Isolated from Food and Clinical Sources

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Introduction: *Salmonella enterica* is one of the leading bacterial pathogens that cause foodborne illnesses in the United States. Mobile genetic elements, such as plasmids, can contain genes that contribute to antimicrobial resistance and increased virulence. Both characteristics have been associated with plasmids of incompatibility group (Inc) FIB and may contribute to the pathogenicity of the strains that harbor them.

Purpose: This study assessed the virulence potential of a group of *Salmonella* Schwarzengrund strains isolated from food and human sources.

Methods: Nineteen isolates collected from foods and 36 clinical isolates were included in this study. Whole genome sequencing (WGS) was performed on an Illumina MiSeq. Plasmid types were analyzed by PlasmidFinder and the genetic relatedness of isolates was examined by single nucleotide polymorphism (SNP)-based phylogenetic analyses. To assess virulence of the food isolates, invasion and persistence assays were performed using human intestinal epithelial cells (Caco-2). Conjugation experiments were performed using plate and/or broth mating approaches to determine the transferability of the plasmids.

Results: Nine food isolates and three clinical isolates contained IncFIB plasmids. SNP-based phylogenetic analyses showed that IncFIB-containing food and clinical isolates clustered within the same clade, which was separated from the other isolates that lacked IncFIB plasmids. All food isolates examined were able to invade Caco-2 cells. Bacterial counts were higher in a majority of the food isolates at one hour (invasion) as compared to 48 h (persistence) post infection. All nine IncFIB plasmids from food isolates were able to be conjugally transferred from Schwarzengrund strains into the recipient *E. coli* J53. Conversely, none of the IncFIB plasmids from the clinical isolates (*n*=3) were transferred, likely indicating that IncFIB plasmids from food isolates differ from clinical isolates in this study.

Significance: The study is important to further aid in understanding the virulence potential of *Salmonella* Schwarzengrund as a foodborne pathogen.

P1-192 Whole Genome Sequences of Potentially Toxigenic Fungi from Walnuts, Peanuts, and Selected Fruits

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Introduction: Foods of plant origin, such as tree nuts and fruits, are known to foster the growth of toxigenic and pathogenic fungal species. It is important to identify fungal contaminants in fresh fruits and nuts because some fungi can grow and produce mycotoxins on these commodities and can cause infections or allergies.

Purpose: The purpose of this study was to develop a rapid means of identifying fungal contaminants in foods by generating high quality whole genome sequences; this will provide reliable data for evaluating whether the identified fungus/fungi pose a health concern for consumers.

Methods: All the isolates were tested for the presence of live fungi by direct plating on DG18 agar as described in the Bacteriological Analytical Manual (BAM). The DNA was extracted with the AllPrep Fungal DNA/RNA/Protein kit following the manufacturer's instructions. Whole genome sequencing was performed using a Nextera XT DNA Library Prep Kit with 2×150 bp paired-end sequencing on an Illumina NextSeq Sequencer.

Results: Twenty-two fungal isolates from moldy seedless grapes, walnut halves, apples, and peanuts from the Washington, D.C. area were sequenced. The sequences were identified to the species level, using an MLST and custom kmer database, as *Aspergillus, Alternaria*, and *Fusarium species.* The raw sequenced data and some of the draft genomes were submitted in NCBI under BioProject PRJNA482816.

Significance: There are only a few publicly available fungal genome sequences from food isolates. High quality newly generated whole genome sequences can provide a quick and accurate answer to epidemiological questions and can be used as a tool for identification determination of their pathogenic potential.

P1-193 Enterotoxigenic Profile Characterization of *Bacillus cereus* Using Targeted RNA Sequencing

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Introduction: Toxin-producing *Bacillus cereus* is an environmentally ubiquitous pathogen causing diarrheal syndrome and emesis, with spores that can persist in harsh conditions.

Purpose: The purpose of this study was to develop an accurate and effective multiplex probe panel to detect and characterize toxin profiles and expression in *Bacillus cereus* using sequencing data.

Methods: Twelve *Bacillus cereus* strains isolated from foods and patients were selected. Total RNA was isolated by using the RiboPure Bacteria Purification kit. A novel work flow was employed that combined the Illumina TruSeq RNA Exome kit and Twist Bioscience Custom Panel protocol. RNA was converted to cDNA. The amplified cDNA was then hybridized to customized probes for genes encoding targeted toxins, including hemolytic enterotoxin hemolysin BL (*hbl*), nonhemolytic enterotoxin (*nhe*), cytotoxin K (*cytK*), and emetic toxin (*ces*). The post-capture library was amplified and sequenced using a MiniSeq sequencer. Analysis of the raw reads was performed using CLC Genomics Workbench 10 with PCR results being used as reference.

Results: RNA-seq data of *ces*, *hbl*, and *nhe* matched PCR results. All strains contained gene cytK whereas the number of samples was five in the PCR assay. Expression of *nheB* was higher than *nheA* and *nheC* in all 12 samples. Expression of *hblA* and *hblD* were higher than *hblB* and *hblC* in the four samples with positive results of *hbl*. Expression of cytK ranged from 0.21% to 54.19% in each tested strain.

Significance: We initiated a novel targeted RNA-seq workflow using a customized multiplex probe panel to characterize toxin profiles in *B. cereus*. The work flow is capable of specifically detecting target toxins and elucidating protein expressions, providing an accurate and valuable assay to identify *B. cereus* and its toxins in food safety programs.

P1-194 Distribution of Antimicrobial Resistance Genes Across Nontyphoidal *Salmonella enterica* Isolates from Various Foods

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Introduction: Antimicrobial resistant (AMR) bacteria represent a major and increasingly vexing public health problem. Of particular importance within food safety is the prevalence of AMR genes in nontyphoidal *Salmonella*, a leading cause of foodborne disease.

Purpose: We characterize the prevalence of AMR genes across a very large number of *Salmonella* isolates (*n*=106,634) with further analysis on those collected from 17 different food sources.

Methods: We identified AMR genes with the National Center for Biotechnology Information (NCBI) AMRFinder tool. After binning the AMR genes into food categories, we used random forest modeling to determine the degree to which the distribution of AMR gene classes within an isolate could predict the food category of that isolate

Results: Approximately 60% of all isolates and 75% of food isolates harbored at least one AMR gene. The prevalence of the genes varied according to food source and serovar. Isolates from beef, chicken, turkey, and pork had the highest frequency of AMR genes. However, isolates recovered from other foods, such as fruit, nuts and seeds, and vegetables (> 20%), also contained AMR genes that were most commonly associated with aminoglycoside and fosfomycin resistance. Based on a random forest approach, AMR gene profiles could be used to correctly predict food source approximately 70% of the time, but success varied according to food source.

Significance: The results presented here illustrate the degree to which AMR genes are found in *Salmonella* isolated from a variety of foods. We describe the frequency and distribution of these genes across food categories, which further contribute to our understanding of the breadth of the AMR public health issue and may help inform approaches to addressing this problem. NCBI affords a wealth of *Salmonella* isolates for further study and understanding of antimicrobial resistance.

P1-195 Comparative Genomic Analysis of Acinetobacter Isolated from Fresh Produce and Clinical Isolates

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Introduction: There are an estimated 45,000 cases of *Acinetobacter* infection in the United States per year, frequently seen in hospital facilities. These infections cause bacteremia, pneumonia, and septicemia. Although *Acinetobacter* can be found on fresh produce, little is known about how these isolates compare to clinical isolates.

Purpose: The objective of this study was to isolate *Acinetobacter* from fresh produce and genomically compare the isolates to clinical isolates.

Poster

Methods: *Acinetobacter* were isolated on *Acinetobacter* CHROMagar from frozen stocks of fresh produce (32 isolates) and soil (two isolates) that had been enriched for either *E. coli* or *Salmonella*. Confirmation was performed by PCR using primers designed for the *Acinetobacter* genus along with an oxidase test. Whole genome sequence data from the 34 isolates was analyzed for species identification, plasmid presence, and antibiotic resistance genes. For comparison to clinical isolate genomes obtained from GenBank, BLAST score ratio analysis was used for virulence gene and core gene SNP analysis was used for whole genome phylogenetic comparisons.

Results: The most abundant *Acinetobacter* species isolated were *A. pittii* (32.4%), *A. baumannii* (26.5%), and *A. oleivorans* (20.6%), and phylogenetic analysis demonstrated that produce and clinical isolates clustered together. Unlike what is seen in clinical isolates, no plasmids were identified in the fresh produce isolates. However, 31 (91%) of 34 of the isolates carry chromosomally encoded β-lactamase resistance genes (bla_{OXA}). The virulence profiles of the *A. baumannii* produce and clinical strains were similar, including the presence of siderophores and *csu* genes encoding a pilus associated with biofilm formation. Some *A. pittii* produce isolates also carry many virulence genes found in clinical isolates.

Significance: The results of this study reveal the close genomic relationship between *Acinetobacter* found on fresh produce and clinical strains, including presence of virulence genes, and demonstrate that the major difference is absence of plasmids in the food isolates.

P1-196 Development of Next-Generation Sequencing and Metagenomics for Detection of Foodborne Viruses Within Oysters

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Introduction: Shellfish are filter feeders that concentrate various viruses present in surrounding water within their tissues. Thus, viral contamination of shellfish poses a risk for foodborne illnesses. Metagenomics offers new opportunities for detection, identification of viruses, and investigating of human enteric virus profiles in shellfish in an unbiased way. However, the protocols of the sample preparation, next-generation sequencing (NGS) and data analysis required are complicated, need to be developed and optimized.

Purpose: The purpose of this study was to develop NGS and metagenomic approaches for investigating foodborne virus profiles present in oyster samples.

Methods: Seeding samples with approximately 10^7 PFU of Tulane virus, two separate strategies were applied to optimize the sample preparation protocol. The first was partial viral particle purification from homogenized digestive diverticula by differential ultracentrifugation, and the second utilized virus extraction from whole homogenized oysters with the GPTT protocol. Treatments with ribonuclease and deoxyribonuclease before RNA isolation were also evaluated. Viral RNA was isolated and followed by RNA-based library generation. Libraries were sequenced on the MiSeq platform (Illumina) generating paired-end reads. CLC Genomic Workbench, CosmosID and in-house tools were applied for metagenomics analysis on the NGS data. The performance of each strategy was assessed based on the reads number, the percentage of total viral reads, and the percentage of the positive control Tulane virus reads.

Results: The highest percentage of viral reads and Tulane virus reads were obtained from the samples prepared with the ultracentrifugation strategy. After the viral particle enrichment, DNase and RNase treatment did not significantly increase the percentage of viral reads. Virus identification and abundance profiles present in oyster samples were successfully obtained with our protocols.

Significance: Detection of viruses in foods is a major public health concern. This joint FDA/USDA study will help to provide a scientific basis for regulations ensuring the safety and security of our nation's food supply.

P1-197 Frequency of Multi-Locus Sequence Types in FSIS-regulated Ready-to-Eat Products

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Introduction: FSIS began performing whole genome sequencing (WGS) in parallel with pulsed-field gel electrophoresis (PFGE) for *Listeria monocytogenes* in fiscal year 2013. In coordination with PulseNet, FSIS suspended PFGE for *L. monocytogenes* and as of January 15, 2018, generates *L. monocytogenes* characterization through WGS only. FSIS uses many tools to analyze WGS information including multi-locus sequence typing (MLST), which results in both a public sequence type (ST) and an allele code.

Purpose: This study assesses trends in the frequency of MLST designations in FSIS regulated RTE meat and poultry products.

Methods: FSIS field staff collect RTE products, food contact surfaces, and nonfood contact environmental samples from the post-lethality environment at establishments, and FSIS laboratories analyze the samples for *L. monocytogenes.* This study analyzes the results from this testing.

Results: Between January 1, 2012 and November 15, 2018, FSIS tested more than 150,000 samples. Of these 1,012 isolates confirmed positive for *L. monocytogenes* (0.67%) and, of those, 512 isolates sequenced using WGS were available for analysis. The 512 isolates represented 62 STs and 129 allele codes when comparing the first four digits. The three most common STs are ST321 (*n*=90), ST5 (*n*=86), and ST9 (*n*=60). The three most common allele codes when comparing the first four digits are LMO1.0-32.1.1.1 (*n*=91), LMO1.0-18.1.1.13 (*n*=36), and LMO1.0-13.1.2.6 (*n*=28). Approximately two-thirds of allele codes (*n*=87) were identified in more than one result when comparing the first four digits.

Significance: When determining relatedness, FSIS considers whether two or more isolates match by ST and at least the first four digits of the allele code. Similar to PFGE patterns, FSIS also considers allele code frequency. Identifying two or more isolates collected during the same sampling event (potential cross-contamination) or over time from the same establishment (potential harborage) with matching rare allele codes strengthens FSIS' confidence they share a common source.

P1-198 Taxonomic and Functional Shifts in Sprout Spent Irrigation Water Microbiome Due to *Salmonella* Contamination of Alfalfa Seeds

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Introduction:: Sprouts have been associated with numerous outbreaks caused by various pathogens, including *Salmonella* and *Escherichia coli*. Little is known about the underlying molecular mechanisms between *Salmonella* and sprouts during the sprouting process.

Purpose: The purpose of this study is to examine the dynamics and functional activity of the bacterial community in sprout spent irrigation water (SSIW) during sprouting of *Salmonella* contaminated alfalfa seeds by shotgun metagenomics and metatranscriptomics.

Methods: Alfalfa seeds were contaminated with *Salmonella enterica* serovar Cubana at varying levels and sprouted in Easy Sprouters. Genomic DNA and total RNA from filtered or non-filtered SSIW at various time points were extracted. Shotgun metagenomic / metatranscriptomic sequencing was performed on both Illumina Miseq and Nextseq platforms after library preparation. All cDNA sequence data were imported into CLC Genomic Workbench (v. 9.0) and MG-RAST, respectively for functional analysis. All the DNA and cDNA sequence data were profiled at the taxonomic level using CosmosID, MetaPhlAn and a privately curated database.

Results: Comparable metagenomic and metatranscriptomic profiles were found in terms of microbial species. A core SSIW microbiome was observed comprising few bacterial genera dominated by *Klebsiella*, *Enterobacter*, *Pantoea*, and *Cronobacter*. It is interesting to note that *Tatumella* appears at a significant relative abundance in the metatranscriptomic profile while extremely low in the metagenomic profile, suggesting this species may be relatively low in abundance but highly active in the SSIW. Across all sampling points and inoculation levels, two KEGG metabolic pathways were identified with genes significantly upregulated in *Salmonella*: pathways involved with biosynthesis of secondary metabolites and the metabolism of cofactors and vitamins. Functional analysis also revealed the 10 most abundant gene families with function in stress resistance and adaption, cell division, RNA turnover, and virulence.

Significance: The data suggests a dynamic, functional interaction between *Salmonella* and the microbial community in SSIW.

P1-199 Dynamics of Microbiome Composition during Enrichment of *Campylobacter* in Poultry Samples

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❖ ***Developing Scientist Entrant***

Introduction: Isolation of *Campylobacter* from complex food samples is often challenging due to its fastidious growth requirements and the difficulties associated with food microbiome competitive exclusion or inhibition.

Purpose: The purpose of this study is to investigate the dynamics of microbiota that are naturally present in poultry meat and able to grow in *Campylobacter* selective media during its enrichment.

Methods: Fresh chicken breast samples were spot-inoculated with ~one CFU/g of *C. jejuni* ATCC 33560, with negative controls tested in parallel to detect potential natural *Campylobacter* contamination. After 12 h storage at 4°C for culture stabilization, chicken samples were enriched for *Campylobacter* using both FDA BAM and USDA MLG protocols. The enrichments were streaked on selective agars and collected for DNA extraction every four hours during the enrichment incubation. Nextera XT shotgun metagenomic libraries were constructed, pooled and sequenced on Illumina MiSeq. Relative abundances of microbial species in the samples were analyzed by CosmosID.

Results: Using both USDA and FDA methods, the first *Campylobacter* culture positive results were obtained after 32 hours of enrichment, which was congruent with the increased relative abundance of *Campylobacter* as detected by the shotgun metagenomics (P<0.05). After 32 hours, the relative abundance of *Campylobacter* obtained from the two protocols was not significantly different *(P*=0.07). High abundance of other bacterial taxa was also identified during enrichment, including *Chlamydia psittaci*, *Pseudomonas fragi*, *Pseudomonas fluorescens*, *Pseudomonas* spp., *Acinetobacter* spp., and *Acinetobacter harbinesis*. Chicken DNA contributed 26±12% (*n*=48) of the total DNA extracted, indicating the need for development of methods for host DNA removal.

Significance: By identifying the microbiota present and able to grow during *Campylobacter* enrichment, as judged based on the increased relative abundances of their sequences*, Campylobacter* enrichment media may be improved by targeted suppression of these off-target microorganisms.

P1-200 A Retrospective Study Using Whole Genome Sequencing to Characterize *Listeria monocytogenes* Strains Found in Domestic and Imported Cheeses from 2000–Present

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Introduction: Listeriosis is the third leading cause of death from infections caused by foodborne pathogens. This infection occurs through ingestion of foods contaminated with *Listeria monocytogenes*. This pathogen has a wide distribution in different environments and a strong capability to survive under various stressful conditions. Over the last 13 years, there has been an increase in *L. monocytogenes* outbreaks in soft cheeses, which continues to pose a threat to public health safety.

Purpose: The purpose of this study was to compare whole genome sequences from our culture inventory of *L. monocytogenes* isolated from domestic and imported cheeses spanning 19 years.

Methods: Whole genome sequencing was performed on 80 *L. monocytogenes* strains isolated from soft, semi-soft and hard cheeses. Genomic DNA was extracted using the QIAcube instrument and genomic libraries were prepared using the Illumina Nextera XT kit and sequenced on the Illumina MiSeq instrument (2×250bp paired-end). Fastq files were uploaded to the NCBI national database and the Pathogen Detection pipeline was used for strain comparison and SNP analysis.

Results: *L. monocytogenes* strains were isolated from imported and domestic cheeses from 2000-2018. Of these cheeses, 60% were imported from 11 different countries while the other 40% were domestic. The *L. monocytogenes* strains were found in 24 different SNP clusters according to the NCBI Pathogen Detection pipeline. Of the SNP clusters, 75% contained clinical isolates while the other 25% (two to four strains/cluster) were environmental and food. Fifty-eight of 80 (72.5%) of the *L. monocytogenes* strains were associated with clinical isolates ranging from zero to 39 SNPs. Of those 58 strains 15 are related to clinical isolates from zero to two SNPs (clusters; PDS000024625.3, PDS000003335.20 and PDS000001093.23).

Significance: Our data shows the majority of our *L. monocytogenes* strains are related to clinical isolates and that *L. monocytogenes* continues to be a public health concern for domestic and imported cheeses.

P1-201 Global Gene Expression Analysis of *Salmonella* Contaminating Low-moisture Foods

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is the leading foodborne pathogen associated with low-moisture foods (LMF) but the mechanisms underlying its survival in LMF are not fully understood.

Purpose: To characterize the transcriptome of *Salmonella* contaminating LMFs.

Methods: *Salmonella* Enteritidis PT30 was grown on TSA-YE agar (37°C, 24 h), washed twice in sterile DI H_2O and spotted (20 ml) on individual fragments of in-shell pistachios, cornflakes, and dried apples. Inoculated LMFs were placed in petri dishes, air-dried for three h and stored (25°C, dark). The population of *Salmonella* on the LMFs immediately after inoculation, after drying for three h and periodically during storage was enumerated on TSA-YE and on XLD agar

Poster

Poster

(37°C, 24 h). Total RNA of *Salmonella* in the inoculum suspension, on LMFs at three h and one, five, and 12 weeks were isolated (in two independent trials), ribo-depleted, sequenced and analyzed. *Salmonella* on dried apples was visualized using confocal microscopy after staining with the LIVE/DEAD BacLight stain.

Results: The population of *Salmonella* on all tested LMFs was approx. nine log CFU/fragment after inoculation with no significant reduction after drying for three h. The population of *Salmonella* on cornflakes and pistachios gradually decreased to approx. seven log CFU/fragment during 12 weeks. *Salmonella* was undetectable in dried apples after 42 d even with enrichment, although *Salmonella* transcripts were detected from dried apples at 12 weeks. Confocal imaging revealed that a large proportion (ca. 60%) of *Salmonella* remained viable on dried apples despite not growing under standard laboratory conditions. Several differentially expressed genes were identified in all tested LMFs at all times, while some were upregulated only in specific LMF-time combinations. Examples of genes upregulated in all LMFs were an ATP-dependent RNA helicase (*SEN_RS16195*) and an Lrp/AsnC family transcriptional regulator (*SEN_RS02260*).

Significance: Findings revealed novel *Salmonella* genes that are upregulated in LMFs and may be mediating the survival of *Salmonella* on LMFs.

P1-202 Identification of the Lowest Lethality Zone in Wheat Flour Treated with Radio-Frequency Heating and Natural Cooling

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❖ ***Developing Scientist Entrant***

Introduction: Radio-frequency (RF) pasteurization has been identified as a potential technology to pasteurize low-moisture foods. It is reported that RF heating to 80 to 85°C followed by a 10 to 25 min natural cooling introduces 2.5 to 3.7-log reduction of *E. faecium*, a valid surrogate for *Salmonella*. However, most studies monitored the reduction of target microorganism at limited locations, assuming a fixed lowest lethality zone in the whole process. But a natural cooling in the process may lead to the relocation of the lowest lethality zone, which could fail in predicting the worst scenario.

Purpose: To monitor the post-processing microbial populations of *E. faecium* at multiple locations in wheat flour, and thus identify the right location to reflect the worst scenario of RF pasteurization.

Methods: Post-processing microbial reduction of *E. faecium* was tested at 15 locations (evenly distributed in top, middle and bottom layers) in a 1.8-kg wheat flour (water activity 0.45±0.02 at 25°C) package heated to 80°C at different RF heating rates (36.0, 11.3, and 5.5°C·min^{-1}), followed by a 20 min natural cooling. Fiber optic sensors monitored the temperature change in the three layers throughout the process. Bigelow model was applied to predict the temperature dependent log reductions of *E. faecium* in each layer. The experiment was conducted in duplicate for each heating rate.

Results: RF heating to 80°C combined with a 20 min natural cooling could achieve an average *E. faecium* population reduction of 1.94 to 3.48 log CFU/g in wheat flour. The lowest lethality zone was located in the bottom layer in all cases based on measured temperature profile and survival data. Fast-heating rate resulted in non-uniformity in terms of temperature and inactivation. The fitting results using the Bigelow model were in good agreement with that from the experiment.

Significance: This study provides a comprehensive study on the lowest lethality zone identification of wheat flour during an RF treatment.

P1-203 Simulated Commercial Baking Validation of Peanut Butter Bars to Control *Salmonella*

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❖ ***Developing Scientist Entrant***

Introduction: Pathogen contamination of flour poses a significant food safety concern. Most baked products go through a thorough cooking step; however, pathogen survivability during thermal process evaluations must consider product-specific characteristics such as fat level and water activity which can impact pathogen lethality.

Purpose: Validate a peanut butter bar baking process for its ability to reduce *Salmonella* populations originating from contaminated flour.

Methods: Flour was inoculated with a seven-strain cocktail of *Salmonella* (8.4 log CFU/g), re-dried, and used to create a peanut butter bar dough (subdivided into 50-g bars of 0.2 cm thickness). Bars were baked at 177°C oven temperature for 13 min, with 15 min of ambient air cooling. *Salmonella* populations were enumerated pre-baking, during baking (one, three, five, seven, nine, 11, and 13 min), and post-cooling. Internal product temperature was continuously recorded and water activity, pH, moisture content, and *Salmonella* counts (composite of internal and external bar components) were determined at the nine sampling points, three replications were performed.

Results: Internal bar temperatures increased from 25°C to 91.88°C during baking. Water activity decreased (0.81 to 0.71), moisture content decreased (16.15% to 9.99%), and pH increased (from 7.07 to 8.65) during the baking process. Final baked product fat content was 21%. *Salmonella* populations were reduced ($P \leq 0.05$) by the end of baking compared to pre-baked dough samples by 2.4 log CFU/g.

Significance: Thermal processes for most bakery products have been shown to achieve over a five-log reduction of *Salmonella*. In peanut butter bars, *Salmonella* demonstrates increased survivability during baking, with only a ~2.4-log CFU/g reduction after 13 min at 177°C oven temperature. This decreased lethality should be considered by food companies producing similar products. Research is being conducted to generate *Salmonella D*- and *z*-values during baking of peanut butter bars to assist the food industry in safe thermal process development.

P1-204 Quantifying the Inactivation of *Enterococcus faecium* during Spray Drying

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❖ ***Developing Scientist Entrant***

Introduction: Spray dried foods such as infant formula and protein powders pose a risk of bacterial contamination and long-term survival of *Salmonella*, as seen from sporadic recalls and outbreaks. However, compared with other unit processes, there is a significant lack of understanding of the microbial inactivation kinetics during the spray drying process, due to its short and complex nature. Therefore, modeling the inactivation of *Enterococcus faecium*, a potential surrogate organism for *Salmonella*, during spray drying will elucidate the risk of contamination of powdered foods.

Purpose: This study aimed to quantify the inactivation of *E. faecium* during spray drying of soy protein.

Methods: A 500 ml suspension of 10% w/w soy protein isolate was inoculated with six ml of tryptic soy broth containing *E. faecium* NRRL-B2354 (10^9 CFU/ml). The entire 500 ml was pumped through a pilot-scale spray dryer (FT80 Tall Form Spray Dryer, Armfield Ltd., Clarksburg, NJ) at 180°C at a rate of seven ml/min. Powder samples were collected from the primary and secondary collectors and from various spray dryer locations. Surviving *E. faecium* were enumerated by plating appropriately diluted samples on modified tryptic soy agar containing esculin, followed by incubation (48 h, 37°C).

Results: Significant reductions ($P<0.05$) in *E. faecium* were observed in powder samples obtained from the primary (2.83±0.38 log, $n=3$) and secondary collectors (1.33±0.08 log, $n=3$), as well as in the powder samples obtained from the walls of the dryer (<less than four log CFU/cm^2, $n=3$) after spray drying at 180°C.

Significance: Based on these findings, *E. faecium* can be used as a *Salmonella* surrogate to assess reductions during spray drying.

P1-205 *Salmonella* and Surrogate Microorganism Behavior in Homemade Play Dough Based on Online Recipes

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Introduction: As social media has become a popular platform to communicate and share information, online users have shared their favorite play dough recipes with over 30 different recipes online. Homemade play dough made may contain flour contaminated with pathogenic bacteria during harvesting, milling, packing, or storage and could make children who handle or eat raw play dough sick.

Purpose: Little is known about microbial behavior on play dough made with flour, so this study assessed the survival of two strains of *Salmonella* and two potential surrogates on homemade play dough based on three different online recipes.

Methods: Online recipes for homemade play dough were collected and analyzed to calculate the ratio of ingredients (flour, water, and salt) in standard units. Three recipes were selected based on differing levels of salt (eight, 21 and 33%). Samples of play dough (20 g) were inoculated with ~7.5 log CFU/g of *Salmonella* Enteritidis PT 30, *Salmonella* Typhimurium PT 42, *Enterobacter aerogenes* or *Pantoea dispersa* and enumerated following storage for up to seven weeks. All experiments were conducted in triplicate.

Results: Native microflora (~three log CFU/g) on play dough samples prepared with different recipes survived at least seven weeks. The growth of mold was observed when play dough was prepared with the recipe with the lowest level of salt and mold populations increased to approximately seven log CFU/g by seven weeks. *Salmonella* Enteritidis PT 30 and *Salmonella* Typhimurium PT 42 on play dough samples showed ~four log CFU reduction over seven weeks, with non-significant differences between strains and formulations. *Enterobacter aerogenes* showed similar survival kinetics to *Salmonella* strains in play dough samples during storage, while *Pantoea dispersa* showed a ~four log reduction by week five.

Significance: Homemade dough made with contaminated flour does pose a risk but this risk decreases over time, even in differing formulations.

P1-206 Butylparaben Improves the Thermal Inactivation Rate of *Escherichia coli* O157:H7 in Low-Moisture Foods

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❖ ***Developing Scientist Entrant***

Introduction: Heat resistant foodborne pathogens have long been a concern in low-moisture foods and ingredients (LMF) such as meat and bone meal (MBM). Due to the composition and thermal properties of MBM, heat treatment by itself is not very efficient and may cause nutritional loss.

Purpose: This study investigated the enhancement of thermal treatment of MBM by the inclusion of food-grade antioxidant butylparaben (BP) as a processing aid agent.

Methods: Stationary phase *Escherichia coli* O157:H7 was inoculated into MBM at seven log CFU/g with water activity adjusted to 0.4.

Results: Inoculated MBM containing zero (control) or 1000 ppm BP was incubated at different temperatures for up to five h. Synergistic action of heat and BP was observed at 55°C but not at 50°C, while the inactivation rate at 60°C was too rapid to observe synergy. Both treatments at 55°C with or without BP demonstrated higher inactivation rates in the first one hour than the next four hours, indicating the existence of potentially thermal resistant subpopulation. Although there was no significant difference in the *D*-values during the first one-hour treatments, treatment with BP at 55°C presented a significantly lower *D*-value (2.6±0.5 h) than the thermal treatment alone (4.7±0.7 h) in the following four-hour period ($P<0.05$), indicating that the addition of BP helps to eliminate the thermal resistant subpopulation. Interestingly, treatment with BP (*D*-value 4.5±0.5 h) was also more effective than the control (*D*-value 6.0±0.5 h) at 22°C ($P<0.05$). An investigation targeting *Salmonella* Typhimurium is underway.

Significance: These results suggest that the addition of certain food additives can improve the thermal processing efficiency in LMF. Enhancement from other approved compounds will be investigated based on this proof of concept.

P1-207 Evaluation of Methods for Inoculating *Salmonella* into Dairy Powders

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Introduction: Various methods can be used to inoculate low-moisture foods. However, many of these methods may result in undesirable changes to the physical properties of powders, particularly dairy powders that are hygroscopic in nature.

Purpose: This study evaluated four methods to inoculate a *Salmonella* cocktail into dairy powders.

Methods: *Salmonella enterica* serotypes (*Salmonella* Agona 447967, Montevideo 988275, Mbandaka 698578, Tennessee K4043 and Enteritidis PT30) were grown on trypticase soy agar with 0.6% yeast extract (TSAYE). Cultures were harvested in buffered peptone water (BPW) and mixed to form a cocktail.

Four methods were used to inoculate cream, nonfat milk and whole milk powders. The methods were as follows: i) one ml cocktail mixed with 10 g dairy powder, then dried; ii) one ml cocktail inoculated into 1 g of dairy powder, dried, then mixed with an additional 9 g powder; iii) one ml cocktail containing one to two drops Tween 80 inoculated into 10 g dairy powder, mixed, then dried; and iv) 0.1 ml cocktail dried onto sterile borosilicate beads, mixed with dairy powders, then beads removed. Each inoculated dairy powder was enumerated to assess homogeneity and initial inoculation level. Each method was replicated three times beginning with fresh cultures.

Results: All aqueous inoculation methods resulted in clumping and non-homogeneous inoculation, and powders did not return to preinoculation water activity levels when re-dried. Only the bead transfer method resulted in a homogeneous (SD<0.3 log CFU/g for ten one gram subsamples) sample with starting inoculation levels of 8.13±0.1 log CFU/g for all three dairy powders without changing sample water activity.

Significance: The bead transfer method resulted in a high initial inoculum level with homogeneous microbial distribution and had minimal effects on physical properties of the dairy powders. This method should be considered when inoculating highly hygroscopic low moisture foods such as dairy powders.

Poster

P1-208 Validation of a Cracker Baking Process Using Predictive Modeling

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Introduction: The validation of pathogen-reduction processes for low-moisture foods can be challenging due to dynamic temperature and moisture within the product. For example, baked products typically begin as high-moisture dough that drastically changes moisture and structure during baking.

Purpose: The objective of this study was to evaluate the efficacy of model-based predictions of *Salmonella* lethality during cracker baking.

Methods: A simple cracker dough was formulated using wheat flour, sugar, salt, shortening, and water. *Salmonella* Enteritidis PT30 was inoculated into the flour 24 h prior to preparing the dough. Each batch of dough was divided for isothermal inactivation experiments (56, 60, and 63°C) and oven baking. For the isothermal tests, dough samples (~1.3 g) were packed into aluminum test cells and heat-treated in a water bath (n=27 for each temperature and replicate). For the baking tests, dough was rolled to two-mm thickness, cut into 2.5-cm squares, and baked at 177°C in 30 s intervals up to 360 s (n=33 for each replicate), with additional cracker samples for monitoring temperature and measuring a_w. Results from the isothermal experiments were used to estimate a log-linear inactivation model to validate the oven baking portion.

Results: Initial *Salmonella* counts in the dough were 7.78±0.48 log CFU/g. The isothermal results yielded $D_{60°C}$ and z-values of 4.6 min and 4.9°C, respectively. After 360 s, the crackers were ~0.49 a_w, with endpoint core temperature of ~103°C, and *Salmonella* survivors of 1.88±0.33 log CFU/g. When applied to the baking temperature data, the dough-based inactivation model grossly over-predicted the lethality (P<0.001), exceeding a predicted 10-log reduction within 90 s.

Significance: Predicting *Salmonella* lethality in a low-moisture food is complex. Inactivation models developed for the initial state of a baked product may dangerously over-predict the actual achieved lethality, due to declining product moisture causing increased *Salmonella* thermal resistance.

P1-209 Comparative Evaluation of *Salmonella* Recovery from Cinnamon Bark and Oregano Leaves Using Either Aluminosilicate Molecular Sieves in Pre-Enrichment Media or the FDA BAM Method

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Introduction: The detection of *Salmonella* in cinnamon bark and oregano leaves is challenging due to the presence of bactericidal and/or bacteriostatic components.

Purpose: To evaluate the use of aluminosilicate (zeolite) molecular sieves in pre-enrichment media to improve the recovery of *Salmonella* from spices and compare the sensitivity to the method in the FDA Bacteriological Analytical Manual (BAM).

Methods: Commercial spices were spiked with *Salmonella* Montevideo or *Salmonella* Senftenberg at 0.7 to 2.7 log CFU/25 g and left to stabilize for two weeks at room temperature (RT). Quantities of 2.5 or 5 g of zeolite with 225 or 475 mL of pre-enrichment media were added to cinnamon and oregano samples, respectively, and incubated at RT with shaking at 120 rpm for 24 h. The detection sensitivity was compared to the BAM method, which in contrast, requires 2.5 L media. Subsequent enrichment steps were performed as described in BAM and the sensitivity was analyzed statistically using Fisher's exact test with pairwise comparisons. Eight trials with 25 g samples were performed with varying cumulative sample numbers for both serovars ranging from 100 (no zeolite), 116 (zeolite), and 50 (BAM) for cinnamon and 80 (no zeolite), 97 (zeolite), and 44 (BAM) for oregano.

Results: *Salmonella* could not be detected in the absence of zeolite in the lower volumes of pre-enrichment-media. However, with zeolite, 75 (64.6%) and 73 (75.3%) samples tested positive for *Salmonella* in cinnamon and oregano, respectively (P<0.05). The BAM method showed 34 (68%) and 31 (70.5%) positive samples in the cinnamon and oregano groups, respectively. There was no significant difference between the zeolite-added and the BAM detection groups (P>0.05).

Significance: The use of zeolite in the pre-enrichment media resulted in detection limits comparable to the BAM method, while reducing the volume of media, thus making it easier and cheaper to test more spice samples for *Salmonella* contamination.

P1-210 Isothermal Inactivation of *Enterococcus faecium* Nrrl B-2354 in Individual Ingredients and Formulated Cookie Dough

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❖ ***Developing Scientist Entrant***

Introduction: Due to practical resource constraints of conducting process validation tests, often only a single worst-case ingredient is inoculated. D and z values for the test organism in that ingredient are later used to predict process lethality. However, studies have shown that product composition has a significant effect on the thermal inactivation rate of bacteria in low moisture foods. Data are needed to better understand how product composition effects vary the rates of inactivation in individual ingredients versus a dough.

Purpose: To compare thermal inactivation rates of *Enterococcus faecium* inoculated into individual ingredients and when combined into a cookie dough.

Methods: *E. faecium* plate grown culture was inoculated into soy protein powder, wheat flour, milk powder and peanut butter, then conditioned to their native water activity levels (0.30, 0.45, 0.25, and 0.25, respectively). Inoculated ingredients were mixed in a ratio of 3:3:3:11 (soy protein powder: wheat flour: milk powder: peanut butter) for 30 minutes in a relative humidity (RH) controlled glovebox (25% RH). The water activity was measured and the dough was loaded into aluminum test cells for thermal treatment. Isothermal studies were performed at 90, 95 and 100°C. Three subsamples were enumerated at each of six time points on tryptic soy agar with 0.6% yeast extract.

Results: $D_{90, D95}$ and D_{100} were 10.95±0.53, 4.60±0.17 and 2.02±0.08 min, respectively, for *E. faecium* in cookie dough. The calculated z-value was 13.67±0.27°C. When compared with the four ingredients, previously reported D-values of *E. faecium* in cookie dough were significantly lower than peanut butter, higher than wheat flour and milk powder, and there was no significant difference between cookie dough and soy protein powder (P<0.05).

Significance: Comparing inactivation rates in individual ingredients relative to a formulated dough may aid establishing industrial kill-step validations without over- or under-predicting thermal inactivation.

Poster

P1-211 Long-term Survival of *Listeria monocytogenes* on Nuts and Seeds as Affected by Relative Humidity Storage Conditions

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Introduction: Nuts and seeds have been increasingly associated with outbreaks and recall due to contamination with *Listeria monocytogenes*. Contamination of these products can occur at various stages throughout production including harvesting, processing, distribution, and storage. Storage can occur at various relative humidity (RH) conditions for months or years.

Purpose: To assess the survival of *L. monocytogenes* on nuts and seeds during storage at three different RH levels for six months.

Methods: A 40-ml four-strain cocktail of *L. monocytogenes* was inoculated onto 240-g aliquots of pine nuts, chickpeas, black peppercorns, and sesame seeds resulting in an initial population level of 10 log CFU/g. Nuts and seeds were stored at 25°C at 25, 45, or 75% RH for six months. During storage, *L. monocytogenes* was enumerated by mixing one g of sample with nine ml BLEB. Serial dilutions of the homogenate were plated onto BHI agar and stored at 37°C for 48 h. Triplicate samples were analyzed for each time point and two independent experiments were conducted. Population survival rates were quantified based on log-linear kinetics and tested for statistical significance (P<0.05) via ANCOVA.

Results: After six months of storage at 25% RH, *L. monocytogenes* populations were reduced 3.17 to 6.8 log CFU/g. Significantly greater reductions in populations on all nuts and seeds were observed during storage at 45% RH ranging from 3.4 to 7.6 log CFU/g. The greatest overall reduction of *L. monocytogenes* occurred during storage at 75% RH on black pepper corresponding to a survival rate of -0.058±0.003 log CFU/g per d. Overall, regardless of RH level, *L. monocytogenes* survived the best on pine nuts > sesame seeds > chickpeas > black pepper.

Significance: Results of this study can aid in understanding how pathogens like *L. monocytogenes* survive on nuts and seeds during extended storage at different RH levels.

P1-212 Efficacy of a Patented Peracetic Acid-based Sanitizing Solution Against a Shiga Toxin-producing *Escherichia coli* Surrogate during Wheat Tempering

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Introduction: Recalls and outbreaks associated with wheat flour due to Shiga toxin-producing *Escherichia coli* (STEC) necessitate implementation of an intervention method for pathogen control in wheat flour. Adding a sanitizing solution to tempering water during wheat milling provides a potential solution without introducing an additional processing step.

Purpose: Identify a suitable surrogate for STEC for treatment of wheat with a patented peracetic acid-based sanitizer. Determine the efficacy of the sanitizer against the identified surrogate in wheat tempering.

Methods: For the surrogate compatibility test,wheat samples (200 g, n=3) were inoculated with a three percent inoculum of a seven-strain cocktail of STEC (O157:H7, O26:H11, O103:H2, O111:NM, O121:H19, O145:NM and O45:H2), a cocktail of non-pathogenic *E. coli* (NPEC) (ATCC BAA1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, ATCC BAA-1431), or *Enterococcus faecium* NRRL B-2354, acclimatized (22°C, 24 h), sprayed with a total volume 70 ml/kg (three ml sanitizer added to 67 ml deionized water), and enumerated eight h after. For the surrogate challenge test, samplesof wheat (one kg, n=3 by 5) were inoculated with three percent *E. faecium*, acclimatized (22°C, 24 h), treated under two different conditions representing tempering processes used by different mills (20 or 70 ml/kg, both containing four ml sanitizer with either 16 or 66 ml deionized water and held for either 6 or 16 h, respectively) milled and enumerated before and after treatment (5 by 45g/sample)

Results: The sanitizer reduced STEC, NPEC, and *E. faecium* by 2.09, 1.96 and 2.49 log CFU/g, respectively. The two treatment conditions achieved 2.70 and 2.02 log CFU/g reductions (70 mL/kg, 16 h hold and 20 mL/kg, 6 h hold, respectively) on *E. faecium.*

Significance: Both the NPEC strain cocktail and *E. faecium*NRRL B-2354 were suitable STEC surrogates. The sanitizer applied at both treatment conditions representing various tempering processes used by mills gave a >2 CFU/g log reduction for *E. faecium*.

P1-213 Patented Peracetic Acid-based Sanitizing Solution Achieves > 4 log CFU/g Reduction in *Salmonella* and Its Surrogate, *Enterococcus faecium* NRRL B-2354, on Alfalfa Seeds While Maintaining High Germination Rates

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Introduction: *Salmonella* contaminated alfalfa sprouts have frequently caused foodborne outbreaks. Therefore, intervention technologies for alfalfa seeds that maintain high germination rates are needed.

Purpose: The objective of this study was to evaluate the efficacy of a patented peracetic acid-based sanitizing solution against *Enterococcus faecium* NRRL B-2354 as a *Salmonella* surrogate on alfalfa seeds, as well as an impact on germination.

Methods: For the surrogate compatibility test, irradiated alfalfa seeds were inoculated (4 mL/200g; n=2×3) with a six-strain cocktail of *Salmonella* Newport, Senftenberg, Oranienburg, Saintpaul, Typhimurium DT104 and Cubana or *E. faecium* NRRL B-2354, acclimatized (24 h, 22°C), and treated with 20 mL/kg of the sanitizing solution. The enumeration was performed before and after treatment. For the efficacy test, alfalfa seeds (not irradiated) were inoculated (30 mL/kg; n=2) with *E. faecium*, acclimatized (24 h, 22°C), treated with the sanitizing solution (40 mL/kg) and held for one hour, then dried to original % moisture content (140°F). Enumeration (5×45 g/sample) was performed before and after treatment. For germination, separate one kg batches (not irradiated) of untreated and treated alfalfa seeds (same parameters used for efficacy testing) were evaluated for the ability to germinate using AOSA approved methods (n=4; 100 seeds/sample). Student's t-test (α=0.05) was used for statistical analysis.

Results: For the surrogate compatibility test, log reductions for *Salmonella* and *E. faecium* (1.63 and 2.35 CFU/g, respectively) did not differ significantly (P≥0.05). For the efficacy test, treatment at 40 mL/kg achieved a 4.31 average log CFU/g reduction. Germination percentages were 95.00±0.82, and 91.00±1.15 for untreated and 40 mL/kg treated seeds, respectively.

Significance: *E. faecium* NRRL B-2354 was identified as an appropriate surrogate for *Salmonella* for the treatment of alfalfa seeds, enabling future industrial-scale testing. The sanitizing solution demonstrated high efficacy (>4 log) against *E. faecium*. Furthermore, treatment had a negligible impact on germination (4.26%).

Poster

P1-214 Performance Evaluation of a Fluorescence Resonance Energy Transfer-Based Real-time PCR Assay for the Detection of Pathogens in 25 g and 375 g Walnut Samples

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Introduction: Pathogen detection systems such as PCR make up crucial components of the Food Safety Modernization Act (FSMA) implementation strategy and any factors that can influence the performance of these test systems should be evaluated. Tree nuts, including walnuts, present a unique challenge as it is a globally traded low-moisture commodity that is often consumed raw and can produce leachable PCR inhibitors.

Purpose: The performance of a real-time PCR assay (GENE-UP, GU) was evaluated for the detection of *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 in walnuts at 25 and 375-g sample sizes.

Methods: Thirty unpaired samples of walnuts (25 g) were evaluated (per AOAC guidelines) where *n*=5 and 20 were inoculated with *Salmonella* Typhimurium at a high and low inoculation level, respectively. Five uninoculated samples were also tested. After sample enrichment, unpaired test portions were evaluated by both the GU and the reference method (FDA-BAM Ch5). Walnuts (25 g) from separate sources were also spiked with <10 CFU/sample with either *L. monocytogenes* or *E. coli* (*n*=8 each); two uninoculated samples per microbe were also tested. An additional six samples (five inoculated with <10 CFU/sample, one negative control) were evaluated at a larger size for *L. monocytogenes* (125 g) or *Salmonella* Typhimurium (375 g). *L. monocytogenes* samples were enriched in LPT broth at 35ºC for 24-26h, while *Salmonella* Typhimurium and *E. coli* were enriched in buffered peptone water at 42ºC for 20 to 24 h. All presumptive results were confirmed with culture based methods.

Results: GU demonstrated no significant differences from the reference method: [$dPOD_c$: 0.0] uninoculated, and [$dPOD_c$: -0.10 and -0.20; LCL: -0.37; UCL: 0.28] high and low samples (per AOAC guidelines). Expected results were obtained for the rest, where all spiked samples were reported positive by GU and unspiked were reported negative.

Significance: These data provide evidence for highly sensitive detection of *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 by GU in walnuts at varied samples sizes that is comparable to the traditional culture methods.

P1-215 Evaluating Steam Treatment as a Potential Intervention for Microbial Risk Reduction of In-Shell Pecans

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Introduction: Pecan shelling includes conditioning of pecans to facilitate kernel separation and to minimize kernel breakage. While hot water conditioning has been shown to be an effective intervention strategy against bacterial pathogens, steam conditioning could be an efficient alternative method to achieve greater than five log reduction. Recent studies indicated *Enterococcus faecium* as a potential surrogate for thermal process validation in nut industries.

Purpose: This study evaluated the efficacy of steam treatment of in-shell pecans on inactivation of *Enterococcus faecium.*

Methods: In-shell pecans obtained from Louisiana orchards were inoculated with a nalidixic acid resistant strain of *Enterococcus faecium*. The inoculated pecans (7.28±0.17 log CFU/g) were treated in a custom designed steam apparatus that was injected with steam and maintained at 70, 80 or 90°C for 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 s. Samples obtained after each treatment were processed for both qualitative and quantitative tests. Each experiment was done in triplicate.

Results: At 70°C, *Enterococcus faecium* was reduced by 1.21±0.23 log CFU/g within 30 s, and the reduction further increased to 4.28±1.0 log CFU/g after treatment for 30 s. As the temperature was increased to 80°C, a 2.49±0.52-log CFU/g reduction was achieved within the 30 s of treatment. On further exposure, the reduction significantly increased (P<0.05) to greater than five-log (6.57±1.12 log CFU/g) within 120 s. Levels were below the detectable limit after 120 s but were detected during the qualitative tests. At 90°C, samples were below the detectable limit of the test within the 30 s indicating greater than five-log reduction of the organism. However, qualitative tests showed the presence of the organism until 180 s.

Significance: Steam treatment of in-shell pecans could be an effective and efficient alternative conditioning method useful in maintaining its safety.

P1-216 U.S. Food and Drug Administration's Total Diet Study (TDS): Process and Challenges Faced in Modernizing the Food List

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Introduction: The United States Food and Drug Administration's Total Diet Survey (TDS), a surveillance program ongoing since 1961, is undergoing modernization. This poster presents a description of the process followed and challenges faced in updating the list of foods sampled and analyzed in the TDS program.

Purpose: As part of the TDS modernization effort, the sampling protocol and list of foods regularly analyzed for nutrient and toxic elements, pesticides, and radionuclides were updated to more accurately represent the average American diet.

Methods: Extensive analyses were conducted, and critical decisions were made to shape the new food list, which was last updated in 2003. The food list was updated based on information from various datasets: TDS findings of analyte trends in foods, What We Eat in America (WWEIA)/ National Health and Nutrition Examination Survey (NHANES), EPA's Food Commodity Intake Database (FCID), and Nielsen and IRI Liquid Data market-sales data. Additionally, the team identified which food items would be collected regionally versus nationally. Consideration was given to ensure representation of foods and beverages currently consumed in the greatest quantities. Foods on the previous food list with little consumption and/or historically low levels of analytes of concern were removed. In addition, the food list was changed to include more single-ingredient items rather than mixtures to allow calculation of analyte concentrations based on recipes.

Results: With the improved food list, TDS-based estimates of dietary exposures are likely to be more accurate, because concentrations of nutrients and contaminants in foods reported by WWEIA/NHANES respondents are less likely to be under/over-estimated. The new foods have been collected since the new sampling plan was implemented in September 2017.

Significance: TDS data are widely used by various public and private entities. The updated food list improves the quality of the TDS data and its relevant contributions to food safety and public health.

Poster

P1-217 Rapid Bioluminescence Detection of Bacteria in Cannabis-infused Foods Using Microsnap

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Introduction: Increasing use of cannabis-infused foods, especially in countries and states were the consumption is legal, has led to a need for rapid testing

Purpose: This study demonstrates a rapid bioluminogenic microbiology method for the detection of total vegetative counts, *Enterobacteriaceae* and coliforms.

Methods: Organisms were cultured in tryptic soy broth and cannabis suspensions were prepared by adding 10 ml water to one g of cannabis product. Cultures were then diluted in the cannabis suspension or water. One ml of the dilutions were added to the corresponding MicroSnap enrichment devices. These were incubated at the test method incubation temperature. Enriched samples were tested with the corresponding detection devices hourly after five to eight hours.

Results: Detection of target bacteria panel was determined by an RLU value greater than that of the threshold value. Thresholds were set to the background signal average and three times SD. Low inocula of the coliforms were detected within eight hours at 37°C. The lowest inoculum for cannabis flower and edible was indicative of bacteria presence after just five and six hours. MicroSnap coliform RLU thresholds were determined to be positive at >244 RLU, >53 RLU and edible greater than eight RLU. Detection of the *Enterobacteriaceae* bacterial panel at ≤10 CFU/ml in all tested flower strains occurred within the eight-h incubation period and as early as five h. The lowest bacterial inoculum level of 102 CFU/ml in the cannabis edible was detected by six h. RLU thresholds were determined to be greater than two RLU. Detection of the aerobic bacterial panel at ≤102 CFU/ml was detected within the eight h incubation period for all three strains. The lowest bacterial concentration tested with edible was detected by seven h of incubation. RLU thresholds were determined to be greater than eight RLU and greater than two RLU.

Significance: The use of rapid microbiological methods can be successfully applied to the growing cannabis food industry

P1-218 Is It Safe to Use Drinking Water Treatment Residues from Harmful Algal Bloom-affected Areas for Land Application?

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Introduction: Residues generated from drinking water treatment plants (DWTPs) have been used as soil amendments and fertilizers worldwide. However, in harmful algal bloom (HAB)-affected areas, concern exists that cyanotoxins and toxin-producing cyanobacteria can be present at high levels in water treatment residues. Since studies reported that cyanotoxins accumulate in crops and soils, it is critical to check the fate and impact of cyanotoxins from HAB-contaminated water treatment residues. However, there are hardly any studies pertaining to this important food safety issue.

Purpose: Two objectives were pursued: i) characterize the microbial community and cyanotoxin from water treatment residues; and ii) examine the impact of water treatment residues land application on food safety.

Methods: Water treatment residue samples were obtained from an Ohio DWTP whose water source is impacted by HABs. Cyanotoxin and microbiome of water treatment residues were determined with ELISA and metagenomics approaches, respectively. Water treatment residue land application was simulated in a greenhouse by applying at zero, 20 and 40% of soil weight. Carrots were cultivated with six replicates at each level.

Results: Microcystin, saxitoxin and β-methylamino-L-alanine were detected at 258.7, 0.2 and 575.1 µg/kg in water treatment residues, respectively. *Microcystis* was the most predominant genus of cyanobacteria within the bacterial phyla. Cyanophage of *Planktothrix* accounted for more than 98% of total viruses. Carrots from the water treatment residue-added soil developed thicker roots with higher crop yield (P<0.05). About 80% of microcystin was retained in soil (83.0 to 95.5 µg/kg) while approximately five percent of microcystin accumulated in carrots. The majority of microcystin accumulated in the non-edible part (37.3 to 44.1 µg/kg) of carrots, resulting in estimates of daily intake below the tolerable daily intake.

Significance: For beneficial use of water treatment residues in agriculture, it is critical to know the impact of land application to protect crop quality and food safety. For future studies, in-depth investigations about the accumulation of other cyanotoxins, such as saxitoxin, are recommended.

P1-219 Quantification of Aflatoxin B1 in *Aspergillus parasiticus* and *A. flavus* in Peanuts Treated with Plant-based Antimicrobial Compounds

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Introduction: Infection of peanut crops by *Aspergillus flavus* and *A. parasiticus* is a serious problem in Georgia, and aflatoxin-contaminated peanuts continue to be a serious problem in Georgia. Aflatoxin B1 (AFB1) is the most toxic and dangerous carcinogen to humans and animals. There has been considerable interest in the use of essential oils (EOs) with antimicrobial activities against *Aspergillus* spp. in peanuts.

Purpose: Our previous study has established that clove and cinnamon have shown antimicrobial properties against *Aspergillus* spp. in peanuts. The minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of these oils were determined by the standard plate diffusion method.

Methods: Cultures were prepared by placing a mycelial plug (one cm) in the centre of Rose Bengal plates. A 0.02-ml volume of each of the oils at various concentrations (500, 1000, 1,500, 2,000 and 2,500 ppm) was dropped onto two-cm diameter filter paper discs, respectively. Oil impregnated discs were placed on seven day old cultures and incubated at 28±2°C. Experiments were repeated three times. Zone of inhibition was measured (mm) with calipers. Clove oil showed significant growth inhibiting properties resulting in a maximum MIC and MFC over cinnamon at 2,500 ppm. Following incubation, AFB1 in all samples were detected by thin layer chromatography and quantified by high-performance liquid chromatography.

Results: AFB1 was successfully identified by retention times and UV spectra. We expected the AFB1 concentration to decrease as the concentration of clove oil increased, however, we did not observe this pattern. Fungicidal effect of the oil on growth and development of the mycelium and spores could have been one factor

Significance: Clove oil showed significant growth inhibiting properties. Hence, this EO may offer potential as a biological control agent against *A. flavus* and *A. parasiticus* in integrated pest management program of peanuts in Georgia and other peanut-growing states.

Poster

P1-220 Validation of an ELISA-based Assay for Specific Detection and Quantification of Pecan and Macadamia Protein in Food Matrices, Clean-in-Place Rinse Water and Environmental Samples

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Introduction: Nuts are food allergens that should be declared on product labels to protect allergic individuals. A product containing nuts may be processed utilizing shared equipment, increasing the risk of cross-contact. Executing cleaning process validation, routine verification and analysis of foods are important variables to ensure allergen control management strategies are effective. Thus, effective tools for the specific analysis of food allergens are required in food testing laboratories.

Purpose: To evaluate the performance specific protein ELISA tests for quantification of pecan and macadamia proteins in food, CIP and environmental samples.

Methods: Two ELISA tests were evaluated for accuracy and the limit of quantification (LOQ) and detection for pecan and macadamia proteins. Tests were also assessed for linearity, cross-reactivity (N=35 foods) and capability to quantify proteins from various nut sources (N=5). Foods (n=16) were spiked with sources of pecan and macadamia to determine the protein recovery after sample extraction. Similarly, CIP rinse water and swabs used for environmental samples were spiked and analyzed to assess recovery. The protein extraction procedure was assessed for robustness to determine the effect of temperature and centrifugation before ELISA analysis.

Results: Both, pecan and macadamia protein standards were suitable to construct a 4-parameter logistic curve which allows quantification of pecan and macadamia protein in a range of 0.67-54ppm and 0.33-27ppm respectively with a precision of <10%CV. Both methods showed linearity in samples containing raw and processed nuts. Cross-reactivity of pecan and macadamia kits was determined in other nuts but below the LOQ of the tests. Recovery of both proteins was above 90% except for chocolate cookies and spices. Temperature variations and centrifugation did not affect the performance ($p>0.05$).

Significance: Pecan and macadamia protein-specific ELISA tests showed to be a reliable analytical method for quantification of proteins in a variety of matrices in the food industry.

P1-221 Stability Study of Milk, Egg and Peanut Protein in Swabs Utilized for Environmental Sampling Including Stability during Shipping and Storage after Sample Collection

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Introduction: Food allergies are a growing health problem worldwide. Almost 50% of food recalls are associated with undeclared allergens. Control measures to prevent food contamination with allergenic proteins should be implemented and enforced in the food industry. Verification of allergen control measures like environmental monitoring has become essential in food safety programs. After cleaning shared equipment, swabs are often utilized to collect environmental samples and then sent to a service laboratory for protein quantification. Because there can be a delay between taking the sample and receiving it for testing in the laboratory, the stability of the swab during storage and shipment is critical.

Purpose: To verify with ELISA based assay the recovery and stability of milk, peanut and egg protein on swabs used for environmental monitoring.

Methods: Solutions of milk, egg and peanut proteins were spotted on polyester swabs at 10, two, and six ppm per swab, respectively. Afterward, the prepared swab samples were kept at ambient temperature for 24 h in one ml of phosphate buffered saline solution and kept at two to 8°C for 48, 72 or 120 h (n=3 swabs per protein per time). Control swabs were spotted with protein and analyzed immediately. All swabs were analyzed with specific protein milk, egg or peanut ELISA following 3M Allergen Protein ELISA Kit product instructions.

Results: Recovery of milk, egg and peanut protein varied depending on the target protein analyzed, with recovery between 50 to 150%. In the case of milk and egg, no significant difference (P>0.05) was determined among the time points analyzed. Recovery of peanut was significantly different after 120 h (P<0.05) when compared to the rest of the time points with an overall decline in protein concentration at 120 h.

Significance: Service laboratories analyzing swabs for protein content may enable sample analysis up to 48 h after swabs have been shipped without compromising the stability of the sample.

P1-222 Enzymatic Treatment to Reduce the Allergenicity of Almond Milk

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Introduction: Food allergy has been a serious health risk for a significant amount of the population over the past several decades. Almond is one of the common potential hazards that may cause allergic reactions.

Purpose: The purpose of this study is to reduce the allergenicity of almond by enzymatic treatment.

Methods: Deskinned almond was disintegrated in a blender and then filtered to obtain almond milk. Almond milk samples were treated with different concentrations (50 to 300 U/g) of papain at 50°C for various hydrolyzing times (zero, 10, 30, 60, 90 and 120 min). SDS-PAGE was carried out to determine the molecular weight of polypeptidic fragments. The immunoreactivity of enzyme treated almond milk was compared with raw and thermally processed almond milk using an ELISA kit. Free sulfhydryl content was determined by Ellman's method. All data are expressed as mean±SD. ANOVA was used to compare the significance of difference for more than two groups.

Results: After hydrolyzing with papain (50 U/g) for 60 min, the band intensity of the 41 and 39 kDa amandin polypeptides decreased and low molecular weight polypeptides appeared in SDS-PAGE. The ELISA result demonstrated the immunoreactivity of almond milk was reduced by 79.08±2.8%. It was significantly (P<0.05) higher than 23.0±1.3% reduction obtained by heat treatment (100°C, 10 min). The free sulfhydryl content of enzyme treated sample (0.56±0.034 µmol/g) was significantly (P<0.05) lower than raw almond milk (0.97±0.078 µmol/g). This may indicate the decrease of sulfhydryl is responsible for the observed almond milk immunoreactivity reduction.

Significance: The results of this study may provide some important implications for the development of almond based food and beverage that were safe to consume.

Poster

P1-223 Western Blot Analysis of Fermented-Hydrolyzed Foods Utilizing Gluten-specific Antibodies Employed in a Novel Multiplex-Competitive ELISA

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Introduction: Lack of appropriate calibration standards poses a major barrier to accurate quantitation of gluten in fermented-hydrolyzed foods. A novel gluten-based multiplex-competitive ELISA was recently developed with the goal to identify appropriate calibration standards of comparable digestion and similar peptide composition as the gluten found in various fermented-hydrolyzed foods. The multiplex ELISA recognized the protein/peptide-profile differences and classified fermented-hydrolyzed foods based on the type and degree of fermentation. However, non-specific responses were observed with soy-based sauces.

Purpose: Western blot analysis was performed to confirm and characterize the peptide profiles indicated by the multiplex-competitive ELISA and to evaluate the soy-based sauces that generated non-specific responses.

Methods: Gluten specific antibodies (G12, R5, 2D4, MIoBS and Skerritt), HRP-conjugates from nine commercial gluten ELISA test kits previously utilized in the multiplex-competitive ELISA, were employed in the western blot analyses to evaluate 65 fermented-hydrolyzed foods belonging to different categories (12 wheat beers, 11 barley beers, six gluten-reduced barley beers, 24 soy-based sauces, six vinegars and six sourdough breads). The epitope recognition profiles were compared by including intact gluten as a standard in the western blot. Cluster analysis of the estimated gluten concentration values was performed by hierarchical clustering using Ward's Minimum clustering method and the cluster patterns were compared with that observed with the multiplex-competitive ELISA.

Results: Western blot analyses were able to distinguish between non-specific inhibition of antibody binding observed in the multiplex-competitive ELISA with soy-based sauces and the presence of residual antigenic peptides (as observed with four soy-based sauces and one vinegar sample). With few exceptions, cluster analysis results showed that the western blot binding pattern was consistent with the protein/peptide-profile patterns generated by the multiplex-competitive ELISA.

Significance: The improved reliability in the peptide profile detection by western blot analyses improves the reliability used to select appropriate calibration standards by the novel multiplex-competitive ELISA. The selection of appropriate calibration standards is required for accurate quantitation of fermented-hydrolyzed gluten.

P1-224 Growth Temperature and Salt Affect Thermal Resistance of Potential Hepatitis A Virus Surrogates *Staphylococcus carnosus* CS 299 and CS 300

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❖ ***Developing Scientist Entrant***

Introduction: Thermal inactivation studies utilize two parameters, D and z-values, to maintain food safety. Increasing growth temperature and salt can induce higher thermal resistance in *Staphylococcus carnosus* strains CS 299 and CS 300, potential hepatitis A virus (HAV) surrogates for use in validation studies.

Purpose: This study compared the thermal resistance of *S. carnosus* (CS 299 and CS 300) grown at 37°C and 42°C in tryptic soy broth (TSB) containing four percent NaCl.

Methods: Overnight *S. carnosus* CS 299 and CS 300 cultures grown at 37°C and 42°C in TSB with and without four percent NaCl were washed, resuspended in phosphate buffered saline in two milliliter sterile glass vials, heated in a circulating waterbath at 56, 60, 65 and 68°C for zero to 15 min, ice-cooled, plated on TSA and incubated for 24 h at 37°C. Data from three replicates were statistically analyzed using the linear model.

Results: D-values at 56 to 68°C for *S. carnosus* CS 299 and CS 300 grown at 37°C in TSB ranged from 5.43±0.18 to 0.33±0.05 min and 6.18±0.25 to 0.54±0.12 min, respectively and for TSB with four percent NaCl, D-values ranged from 6.74±0.27 to 0.44±0.02 min and 8.02±0.19 to 0.48±0.01 min, respectively. D-values of *S. carnosus* CS 299 and CS 300 at 42°C in TSB ranged from 6.96±0.37 to 0.5±0.05 min and 7.29±0.15 to 0.59±0.06 min, respectively and in TSB with four percent NaCl ranged from 7.24±0.17 min to 0.50±0.07 min and 8.13±0.19 min to 0.68±0.14 min, respectively from 56 to 68°C. $D_{65°C}$ for CS 300 in TSB with four percent NaCl was 1.43±0.11 min, requiring 7.25 log for equivalent 6D HAV reduction.

Significance: D-values of CS 299 and CS 300 grown in TSB containing four percent NaCl at 42°C were significantly higher (P<0.05) when compared to 37°C without four percent NaCl, making them suitable HAV surrogates for use in validation studies.

P1-225 Antibiotic Resistance Profiles and Detection of Enterotoxin Genes in *Staphylococcus epidermidis* Isolates from Pork Production

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Introduction: *Staphylococcus epidermidis* is one of the most frequently isolated coagulase-negative staphylococci in human- and animal-origin samples. Although coagulase-positive *Staphylococcus aureus* has mainly been recognized for its pathogenicity and ability to cause food poisoning, information on antibiotic-resistance profiles and enterotoxigenicity of *S. epidermidis* in livestock-associated environments is very limited.

Purpose: The purpose of this investigation was to determine antibiotic resistance profiles and enterotoxigenic potential of *S. epidermidis* isolates from pork production in Korea.

Methods: A total of 1009, 311, and 267 swab samples (swine farms, slaughterhouses, and retail meat, respectively) were subjected to analysis for isolation of *S. epidermidis*. Using a total of 89 *S. epidermidis* strains, we examined: (i) genotypes of the strains with multilocus sequence typing (MLST); (ii) presence of *mecA* gene and SCC*mec* type; (iii) susceptibilities to 11 different antibiotics using disk diffusion or microdilution methods; and (iv) carriage of staphylococcal enterotoxin genes (*sea-see, seg, seh, sei, selj, sek, sem, seo, tst1, sel, sen, sep, seq, ser, selu*).

Results: Almost all the *S. epidermidis* isolates (98%) exhibited resistance to at least one of the antibiotics tested and 84 (94%) of 89 isolates showed multidrug resistance. Importantly, 23 (26%) of 89 *S. epidermidis* isolates were resistant to methicillin, positive for *mecA*, and had oxacillin MICs ranging from four to 128 µg/ml. The major genotype of methicillin-resistant *S. epidermidis* was ST100 with SCC*mec* type V. One or more classical and/or newly described staphylococcal enterotoxin genes were found in 40.4% of *S. epidermidis*. The most prevalent combination of staphylococcal enterotoxin genes (*tst1*, *sem, sen,* and *seo*) was found in 13 of 89 *S. epidermidis* isolates.

Significance: Our results demonstrated that there are relatively high levels of antimicrobial resistance among *S. epidermidis* isolates in pork production in Korea. Of note, *S. epidermidis* isolates from pigs/pork may serve as a reservoir for antibiotic resistance and staphylococcal enterotoxin genes.

Poster

P1-226 Wide Host Range Phages of the Genus *FelixO1virus* are Potential Candidates for *Salmonella* Infantis Biocontrol

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Introduction: *Salmonella* Infantis is a globally emerging serovar of *Salmonella* causing human illness. Bacteriophages offer a potential biocontrol solution, however, it is not understood what type of resistance in *Samonella* Infantis will be selected for by phage biocontrol.

Purpose: We compared the ability of two phages with different host ranges (wide and narrow) to select resistant mutant bacteria (RMB) and to reduce the growth of *Salmonella* Infantis.

Methods: We performed 12-hour independent challenges using two models of phages of the *FelixvirusO1* genus infective against *Salmonella* Infantis: a phage with wide host range (WHR) and a phage with narrow host range (NHR). Challenges were conducted at a MOI of 0.01 in tryptic soy broth at 37°C and 100 rpm. Every hour we sampled the mixture and measured optical density and relative frequency of RMB. Additionally, 10 RMB isolates were collected, and phage susceptibility was assayed by efficiency of plating (EOP) using the phage wild type (WT) for each model. Four replicates were conducted. WT isolates obtained at hour 12 and the control were sequenced and genomic variants were identified.

Results: There was a significantly higher ($P<0.05$) reduction in *Salmonella* Infantis OD in the WHR model than the NHR model. The selection of RMB was observed in both models in hour one. The RMB frequency was variable in time, with values between 1×10^{-6} and 9.7×10^{-8}. The EOP with WT phages showed higher susceptibility to phages in the WHR model. Variant calling identified potential phage receptor targets, including SNPs in flagellar structures (*fliC* and *fleN)*, LPS, and *ompA*.

Significance: This study shows that WHR phages may be more effective against *Salmonella* Infantis than NHR phages, and provides insight into the emergence of phage resistance. This knowledge will aid in the development of long-term biocontrol solutions for *Salmonella* Infantis.

P1-227 Determinants of Specificity of the *Escherichia coli* O157:H7 Bacteriophage PhiV10

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Introduction: PhiV10 is a temperate bacteriophage and a member of the *Podoviridae* family and is the precursor of the luminescent reporter phage PhiV10 nanoluc for detection of viable *Escherichia coli* O157:H7. Previous screening of STEC *E. coli* libraries showed the phage plaques all *E. coli* O157:H7 isolates. However, thirteen O157, non-H7 and ten non-O157, H7 isolates showed negative results suggesting the necessity of the presence of the O157 and H7 antigens for successful PhiV10 infection.

Purpose: The aim of this study was to determine the role of the O157 and H7 antigens in the infection of *E. coli* O157:H7 by PhiV10.

Methods: The thirteen O157, non-H7 and ten non-O157, H7 *E. coli* strains were subjected to both binding and lysogeny assays. For binding assays, strains were incubated with 10^4 PFU PhiV10 per ml at 20 to 21°C. After ten minutes, unbound phages were isolated from bound phage using a 0.2-micron filter and enumerated by plaque assay with *E. coli* O157: H7, plaque counts similar indicating lack of binding. For lysogeny assays, strains were incubated with PhiV10 nanoluc containing a kanamycin resistance marker for twenty minutes at 20-21°C. Lysogens were enumerated on kanamycin plates (50 µg/ml) and confirmed with luminescence assays.

Results: The binding assay showed all strains expressing the O157 antigen successfully bound PhiV10 as they displayed similar PFU as the control. However, all ten non-O157 isolates expressing the H7 antigen showed no binding of the PhiV10. Results of the lysogen assays indicated zero percent infection of non-O157: H7 strains, zero percent infection of O157: non-H7 strains.

Significance: The results of the research thus far support the hypothesis that PhiV10 binds to *E. coli* O157:H7 via the use of the O157 antigen and infects by using the H7 antigen, which will help in optimizing PhiV10 as a bioreporter for *E. coli* O157:H7.

P1-228 Population Dynamics of *Listeria monocytogenes* during Rehydration of Dehydrated Potato

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Introduction: Dehydrated plant food products have low water activities and do not support the growth of pathogenic bacteria. During rehydration, the increasing water activity and relatively neutral pH of these foods may require a product assessment to determine the extent to which they support the growth or survival of pathogenic bacteria such as *Listeria monocytogenes* outside of refrigeration temperature control.

Purpose: To determine the fate of *L. monocytogenes* during rehydration when inoculated onto dehydrated potatoes.

Methods: Forty ml of a four-strain cocktail of *L. monocytogenes* was inoculated onto 240 g of dehydrated potato cubes measuring one cm^3 resulting in nine log CFU/g. Potatoes were dried for 24 h, then rehydrated with four volumes of 25°C water for 24 h at 25°C air temperature. Water content was measured by calculating the reduction in weights of duplicate 10-g potato samples after heating at 100°C for 24 h. The *L. monocytogenes* population was determined by mixing one g potato, in duplicate, with nine ml BLEB. Serial dilutions were plated onto BHI agar and enumerated after 24 h at 37°C. Two independent experiments were conducted and a $P<0.05$ was considered significant.

Results: The initial water content of the dehydrated potatoes after drying 24 h was 4.8±1.5%. After only 2.5 min of rehydration, the water content of the potatoes increased significantly to 49±4.2%. The final water content after 24 h was 77.5±0.6%. Prior to rehydration, the population of *L. monocytogenes* on the potatoes was 7.5±0.1 log CFU/g, which decreased to 7.0±0.1 log CFU/g after 2.5 min, yet increased after one h to 8.0±0.2 log CFU/g. The final population was 8.3±0.2 log CFU/g, resulting in an overall growth rate of 0.04±0.01 log CFU/g per h.

Significance: The results of this study aid in understanding how pathogens like *L. monocytogenes* survive and grow during rehydration of dehydrated plant foods.

P1-229 Glove-mediated Transfer of *Listeria monocytogenes* on Fresh-cut Cantaloupes

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Introduction: Recently, fruits have been frequently implicated in foodborne outbreaks. Fresh-cut fruits sold in retail are often processed in store with workers wearing gloves during processing. It is unclear how gloves mediate pathogen transfer and contamination of fresh-cut fruits.

Purpose: To investigate whether glove types, contact pressure and time, and residual liquids on contact surfaces affect *L. monocytogenes* transfer on cantaloupes and develop a quantitative model for risk assessment of glove-mediated transfer of *L. monocytogenes* on fresh-cut cantaloupes.

Methods: Fresh-cut cantaloupes (4.5 by 3.5 by 0.7 cm, 12±1 g/piece) were inoculated with high (10^{6-7} CFU/ml) and low (10^{4-5} CFU/ml) levels of a four-strain cocktail of *L. monocytogenes*. To study fruit-to-fruit and surface-to-fruit transfer via gloves, different types of single-use gloves (nitrile, polyvinyl chloride, polyethylene) were used and different contact pressures (0.05, 0.18, 0.37 psi) and time (two, five, 10 s) were evaluated, meanwhile, transfer from rind surface with or without residual liquids were compared (six replicates). Predictive models were used to quantitatively assess glove-mediated transfer of *L. monocytogenes* between fresh-cut cantaloupe flesh samples (six replicates). The model was validated by extended transfer trials by three volunteers (five replicates in total).

Results: Glove materials, contact time, and contact pressures did not cause a significant difference in *L. monocytogenes* transfer from cantaloupe rind to flesh or flesh to flesh. However, glove materials appeared to affect *L. monocytogenes* transfer from stem scar to cantaloupe flesh ($P=0.0371$). Transfer from rind pieces that had been washed with water was significantly higher compared to transfer from pieces that had not been washed ($P=0.0006$). Predictive modeling and validation analysis showed that glove-mediated *L. monocytogenes* transfer can last for more than 85 touches of cantaloupe.

Significance: The results are useful for adjusting in-store processing practices around glove use and conducting a risk assessment of cross-contamination during fresh cutting.

P1-230 Growth of *E. coli* and *Salmonella* spp. at Low pH and Temperature Levels

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Introduction: Challenge studies are regularly used to evaluate the ability of pathogens to grow in food. The choice of organism for challenge studies is important in conducting useful challenge studies. Although pathogens have been found in low-pH refrigerated foods, there is little data to support the choice of specific American Type Culture Collection (ATCC) or reference strains for use in challenge studies under these conditions.

Purpose: The purpose of this study was to evaluate several different strains of *E. coli* and *Salmonella* spp. for their ability to grow at different pH levels and different temperature levels for possible use in challenge studies.

Methods: Five strains each of generic *E. coli* (ATCC 8739, ATCC 11698, ATCC 25922, ATCC 51739, ATCC 51813), *E. coli* O157:H7 (ATCC 35150, ATCC 43888, ATCC 43895, ATCC 700728, NCTC 12900), and *Salmonella* spp. (ATCC 51741, ATCC 6962, ATCC 9239, ATCC BAA-193, ATCC BAA-712) were inoculated at 100 to 1,000 CFU/ml into tryptic soy broth at several pH levels (3.8, 4.0, 4.2, 4.4 and 7.3). Strains were stored under two conditions, 35°C for seven days and 7°C for four months, and periodically checked for turbidity and enumerated on plate count agar.

Results: When held at 35°C and a pH of 7.3 or 4.4, generic *E. coli*, *E. coli* O157:H7 and *Salmonella* spp. were all able to grow. Several *Salmonella* strains were able to grow down to pH 4.0 when held at 35°C. When held at 7°C and pH 7.3 only two strains each of generic *E. coli* and *Salmonella* spp. were able to grow. When held at 7°C and at a pH of 4.4 or lower neither generic *E. coli*, *E. coli* O157:H7 nor *Salmonella* spp. were able to grow.

Significance: These results suggest that using these strains in challenge studies for a low pH product stored at a low temperature would not be useful.

P1-231 Photocatalytically Enhanced Inactivation of Internalized *Salmonella* Typhimurium and *E. coli* in Fresh Lettuce Using UV with TiO_2

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Introduction: Once pathogenic bacteria such as *E. coli* O157:H7 or *Salmonella* are internalized in fresh produce, it poses a challenging food safety issue as they are not effectively inactivated by conventional rinsing or sanitization.

Purpose: To enhance the food safety and public health, the major objective is to investigate the enhanced inactivation of internalized pathogens in fresh produce using UV with the photocatalytic ability of titanium dioxide (TiO_2).

Methods: Green fluorescent protein-labeled *Salmonella* Typhimurium and *E. coli* O157:H7 were inoculated on the leaf surface area (~10^8 CFU/leaf) of four types of fresh produce (iceberg lettuce, romaine lettuce, spinach, and kale), and different concentrations of TiO_2 suspension (0.5 to 1.50 µg/ml) were applied to the contaminated leaves. UV was applied at 750 to 18000 J/m^2.

Results: Depend on the nature of each vegetable, the internalized bacterial level was different (log 2.1 to 4.8 CFU/g of leaf). UV irradiation (6000 J/m^2) lettuce reduced the internalized *Salmonella* Typhimurium and internalized *E. coli* by 0.8 to 2.4 log CFU/leaf. However, the UV inactivating effects were significantly enhanced by TiO_2, yielding a 1.1 to 3.7 log reduction. The enhanced inactivation was dependent on the TiO_2 concentration (up to 1.50 µg/leaf). These results indicate that TiO_2 enhanced the photocatalytic inactivation of both internalized foodborne pathogens.

Significance: The application of TiO_2 would be most practical before UV irradiation and prior to the distribution chain of fresh produce. This study confirmed a platform for future research on the inactivation of various internalized pathogens for protecting public health and the scaling up of fresh produce treatment by food industries.

P1-232 WITHDRAWN

P1-233 Thermal Inactivation of Extraintestinal Pathogenic *Escherichia coli* Suspended in Ground Chicken Meat and the Effect of Virulence and Antibiotic Resistance Factors

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Introduction: Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a foodborne pathogen responsible for urinary tract infections (UTI), sepsis and neonatal meningitis. Retail poultry meat has been identified as a reservoir for ExPEC. ExPEC-associated UTI (uropathogenic *E. coli*) are responsible for >23,000 deaths annually in the United States as opposed to ~40 for STEC. Meningococcal ExPEC is a foodborne meningitis threat. Recent advances in genomics have made the connection between foodborne ExPEC and disease in humans extremely strong.

Purpose: This study was to determine the thermal inactivation kinetics of ExPEC in chicken meat and examine the possible role of virulence factors and antibiotic resistance on ExPEC thermal resistance.

Methods: Uropathogenic *E. coli*, meningococcal ExPEC and food ExPEC isolates (density of 10^{8-9} CFU/mL) were suspended in ground chicken meat. D_{10} values for the 19 ExPEC isolates were used to examine the connection between virulence factors and antibiotic resistance and ExPEC thermal resistance.

Results: D_{10} values for 55, 60, and 65°C were 7.63, 1.10, 0.05 min, respectively. *Z*-values (4.62 to 5.89°C) were consistent with those for *E. coli* O157:H7 in low-fat meat and poultry. When D_{10} of 19 individual ExPEC isolates were determined at 55°C, the D_{10} averaged 4.43 min and ranged from 1.84 to 7.58 min. Differences in D_{10} (55°C) were found between isolates possessing or lacking the virulence genes *iha*, *fimH*, *fdeC*, *sinH*, *cnf1*, *gad*, and *ompT*, many of which aid in adhesion and biofilm formation. There was no correlation between thermal resistance and antibiotic resistance.

Significance: This research established that thermal inactivation conditions needed to inactivate STEC will also inactivate ExPEC in chicken meat. The D_{10} and *z*-values are also lower than those for *Salmonella* spp. Cooking conditions needed to kill *Salmonella* in poultry meat will kill ExPEC. Information regarding genotype and thermal resistance will help in the development of microbial risk assessments for ExPEC in foods.

P1-234 Inactivation of *Klebsiella pneumoniae* in Ground Chicken Meat by High Pressure Processing, Gamma Radiation, and Thermal Processing

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Introduction: *Klebsiella pneumoniae* is a bacterial pathogen associated with inflammatory bowel disease (IBD) and urinary tract infections (UTI). The association between foodborne *K. pneumoniae* and those diseases in humans is now indisputable. IBD affects more than 1.1 million people in the US annually, while *K. pneumonaie*-associated UTI affects >500,000 annually. These conditions disproportionally affect women. Consumption or mishandling of contaminated meat and poultry could affect the health of these at-risk populations. The cost of sepsis, IBD, and UTI is estimated to be >$20 billion annually. Little data is available regarding the inactivation of *K. pneumonaie* isolates in food.

Purpose: This study was to determine the inactivation kinetics of *K. pneumoniae* in chicken meat.

Methods: A *K. pneumoniae* multi-isolate cocktail containing clinical and food isolates was inoculated into ground chicken meat (GCM) that was 95% lean. The intervention technologies used were high-pressure processing (HPP), gamma radiation (GR), and thermal processing (TP).

Results: For HPP at 4°C, the D_{10} values were 2.60, 5.44, 15.1 min at 500, 400, and 300 mPA, respectively. For GR, the D_{10} values were 0.53 and 0.35 kGy at -20°C and 4°C, respectively. TP D_{10} values were 9.34, 0.59, and 0.08 min at 55, 60, and 65°C, respectively. The conditions needed to inactivate *K. pneumoniae* in this study are similar to uropathogenic *E. coli*, and less than those typically needed to inactivate *Salmonella* spp. or *Listeria monocytogenes* in GCM.

Significance: Foods treated with intervention technologies are recommended for people with pre-existing medical conditions such as IBD and recurrent UTI, which can have a host (human genetic) component. People with these conditions may benefit from the consumption of meat and poultry treated using these interventions to lessen their risk of infection. Consumer and women's health groups will benefit from this study, as well as food companies which can provide safer foods for those target populations.

P1-235 Characterizing the Microbiome of Recycled Bedding, the Environmental Persistence of *Salmonella enterica,* and the Implications for Preharvest Bovine Health

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Introduction: Recycling bedding sand is a common practice in dairy operations. Little attention has been paid to the diverse microbial community in bedding sand and how it might impact dairy cattle health and pre-harvest food safety.

Purpose: The microbiome of recycled bedding sand was evaluated at various stages in the recycling process. As a separate but related project we also determined the ability of bovine-associated *Salmonella enterica* serotype Dublin to persist in recycled dairy bedding sand with the native microbial communities.

Methods: DNA was extracted from recycled sand using phenol-chloroform. The variable regions of 16S rRNA were amplified by PCR, and the products sequenced using an Ilumina (MiSeq). Sequences were processed using Mothur software and analyzed with Rstudio.

To assess *Salmonella* survival, *Salmonella* Dublin was inoculated into bedding sand at approximately 10^5 CFU/g sand. At days zero, three, and seven the CFU/g of *Salmonella* was determined by serial dilution and plating on XLD and blood agar.

Results: Microbiome analysis revealed approximately 5000 operational taxonomic units throughout the sand recycling process. The phylum *Proteobacteria* was in greatest abundance, and the top five genera were *Acinetobacter* spp., *Pseudomonas* spp., *Psychrobacter* spp., *Corynebacterium* spp., and *Paludibacter* spp. *Acinetobacter* and *Pseudomonas* increased in relative abundance with time in recycled sand.

When *Salmonella* Dublin was inoculated into recycled bedding sand there was an approximately 2-log CFU/g decrease during a one-week incubation at 22°C

Significance: Recycled bedding contains a complex bacterial community and could serve as a reservoir for pathogens. Although we did not identify *Salmonella* in our microbiome analysis, *Salmonella* has been recovered by others from recycled bedding sand, from which it might serve as a reservoir for infection of cattle and humans. Our In Vitro studies showed the native microbial community of recycled sand had a suppressive effect on the survival of *Salmonella*.

P1-236 Antibiotic Resistance of Lactic Acid Bacteria Isolated from Dairy Products in Tianjin, China

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Introduction: Antibiotic resistance is an increasingly significant problem worldwide. Lactic acid bacteria (LAB) are commonly involved in the manufacture and preservation of fermented foods and the safe use of LAB has a long history. However, the antibiotic resistance of LAB has received increased attention recently, because they may act as the reservoirs for the antibiotic resistance genes and transfer them to other microorganisms, including human pathogens, through the food chain.

Purpose: We conduct a preliminary study to investigate the antibiotic resistance profiles of some LAB obtained from fermented dairy products in Tianjin, China

Methods: Eight dairy samples were obtained from local markets in Tianjin, China. LAB was isolated and identified to species level using conventional biochemical tests. The phenotypic antibiotic resistance of all isolates to four antibiotics was analyzed by using disc diffusion method, and the corresponding resistance genes were determined by PCR and sequencing.

Results: A total of nine strains (three *Lactobacillus bulgaricus* and six *Streptococcus thermophilus*) were isolated from commercial yogurt and cheese products. Eight out of nine isolates were identified to be resistant to one or more antibiotics and six isolates displayed multi-drug resistance. The occurrence rate of resistant strains to vancomycin, neomycin, gentamycin and streptomycin were 11.1%, 77,8%, 66.7% and 44.4%, respectively. Common genes (*van, aadA2, aph*) encoding resistance to these antibiotics were identified in some of the resistant strains. Sequencing result of *aph* amplicon was 100% identical to the gene encoding neomycin phosphotransferase II (NPTII) described in *Escherichia coli*. Sequences from amplicon of *aadA2* were validated to be identical to the gene of tRNA adenosine deaminases (ADA) in *Staphylococcus epidermidis* with a similarity of 99%.

Significance: This study revealed the widespread antibiotic resistance in LAB used in dairy products in China, thus the use of a starter in fermented food should be closely monitored to ensure food microbiological safety.

P1-237 *Salmonella* Detection from Large Milk Powder Samples Using the Thermo Scientific Suretect *Salmonella* Species PCR Assay

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Introduction: Infant formula, milk powders and their ingredients are susceptible to *Salmonella* and *Cronobacter* contamination. Separate international standard reference methodologies for *Salmonella* and *Cronobacter* detection mean that different sample sizes and separate enrichments must be prepared for testing. A harmonized enrichment strategy would be advantageous for microbiologists to minimize the amount of cost and effort spent during testing for these pathogens.

Purpose: The purpose of this study was to evaluate the *Salmonella* detection capabilities for large sample sizes of milk powders and ingredients using the Thermo Scientific SureTect *Salmonella* spp. PCR Assay in comparison with ISO 6579-1:2017. The same enrichment conditions were applied to the alternative method as the SureTect *Cronobacter* spp. PCR Assay.

Methods: Twelve 300-gram probiotic powdered infant formula (PIF) samples were spiked with *Salmonella* species and enriched alongside four unspiked samples in buffered peptone water with vancomycin, before processing according to the alternative method. The ISO method was performed in parallel to an unpaired study. Thirty-six 375-gram PIF, milk powder and ingredient samples were spiked with *Salmonella* Typhimurium or *Salmonella* Infantis injured by desiccation and tested alongside seven unspiked samples according to the same study design, as a second unpaired study.

Results: From the 300-gram PIF study the alternative method confirmed the presence of *Salmonella* in 12 (100%) spiked samples after 16 hours incubation while the ISO method confirmed 11 (91.7%). From the 375-gram samples study, comparable *Salmonella* detection was also achieved for alternative and ISO methods.

Significance: The data demonstrate the comparable performance of the alternative method for *Salmonella* detection compared to ISO 6579-1:2017 using large sample sizes and enabling one enrichment to be tested for both *Salmonella* and *Cronobacter* using PCR assays.

P1-238 Food Authenticity Testing with Next-Generation Sequencing

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Introduction: The Thermo Scientific NGS Food Authenticity Workflow identifies meat, fish and plant species from foods and feeds. The semi-automated workflow combines DNA extraction, library preparation, Thermo Fisher Scientific Ion Torrent technology and extensive database enabling identification of thousands of species with more than a hundred samples simultaneously analyzed.

Purpose: A complete process was developed to study the species authenticity of food products. The method for a streamlined workflow producing quality results.

Methods: Foods from different categories were tested to challenge the method including heavily processed foods, fresh and frozen foods, ready-to-eat meals, liquid foods and dried food products. After the DNA extraction, the sample libraries were prepared with SGS AllSpecies Kits for meat, fish and plant. Ion Chef Food Protection Instrument and Ion GeneStudio S5 Food Protection System were used for templating and sequencing. The results were analyzed with SGS AllSpecies ID software which maps the sequencing reads for each sample against a regularly updated database producing comprehensive high-quality results in a user-friendly format.

Results: Twenty food products were tested with each kit type. The species identified by sequencing were compared to the ingredient list of food products to evaluate the accuracy of the method. All species from 19 meat samples, 18 fish samples and 10 plant samples were correctly identified. All plant species listed in the ingredients were not detected in 10 samples that were complex samples containing several plant species. However, all declared spice species were detected in spice-type samples.

Significance: The advantage of the next-generation sequencing method is the unmatched capacity to identify species without the need to specifically target only a limited set of species. The method is capable of detecting species from a variety of sample types.

P1-239 Isolation and Genome Analysis of *Lactococcus lactis* Strains Characterized for the Potential Utilization of Allulose

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Introduction: Allulose (D-Psicose) is a monosaccharide sugar known as a low-calorie high sweetener. Recently, researchers have interested in its beneficial and physiological effects for humans responding to consumer's demands for high-quality food products. To our knowledge, few studies have been reported on the screening of lactic acid bacteria for potential usability of allulose.

Purpose: The purpose of this study was to develop and characterize the novel probiotic source of potential lactic acid bacteria to maximize the functional role and application of allulose as a prebiotics in food industry.

Methods: Modified MRS-allulose (1%) was prepared to screen allulose-utilizing lactic acid bacteria. Diluted solutions from kimchi, a traditional fermented food of Korea, were spread on the modified MRS-allulose agar and allulose-utilizing bacteria were selected. These isolates were identified and characterized as a probiotics for further application in food industry and for enhancing the potential ability of allulose as a prebiotics in humans.

Results: Three isolates were identified as *Lactococcus lactis* by 16S rRNA gene analysis and were designated as *Lactococcus lactis* B001, C001 and C002. Morphological, physiological and biochemical properties, growth conditions, and antimicrobial activity of these isolated strains were determined. Whole

genome sequence and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the isolated *Lactococcus lactis* revealed that the metabolic pathway (catabolism) of allulose in the isolated strains was presumed.

Significance: We expect that the isolated *Lactococcus lactis* strains could be applied to the food industry as a functional probiotic enhancing allulose-utilization for human health promotion.

P1-240 Modulation of Gut Intestinal Microbiota during Prevention of Salmonellosis with *Lactobacillus* in BALB/cJ Mice

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Introduction: Gut intestinal microflora comprises an interactive ecosystem possessing considerable microbial diversity. The homeostatic status of this microbial ecosystem is the key in gut intestinal defense. Enteric pathogens can outcompete beneficial microbes during dysbiosis with a loss of microbial diversity and infect the host's gut causing acute/chronic diseases. Probiotics as candidates for prevention of foodborne infections are well known as potential modulators of gut immunity and health.

Purpose: The purpose of this study is to identify the functionality of *Lactobacillus* and its metabolites on gut microflora modulation and prevention of salmonellosis.

Methods: BALB/cJ mice were daily fed 10^9 CFU/mL linoleic acids from highly producing *Lactobacillus casei* strain (LC^{+mcra}) for one week. The hematological changes and body weight of mice were compared between control and LC^{+mcra} supplemented mice. Paired-end (2×300 bp) metagenomic sequencing based on 16S rRNA genes of cecal microbes was performed using MiSeq II. Further, the relative abundance of microbial taxa and microbial species diversity were compared between mice with/without LC^{+mcra} or *Salmonella* Typhimurium-infected mice with/without LC^{+mcra} pretreatment.

Results: One-week ingestion of LC^{+mcra} increased mean body weight of mice by 4.96% with no significant difference (P>0.05) detected in hematological characteristics, indicating non-systemic growth promotion in mice. Significant differences (P<0.05) were found in cecal relative abundance of *Firmicutes* (15.72% higher), *Bacteroidetes* (15.29% lower), and *Proteobacteria* (0.24% lower) from mice provided with LC^{+mcra}. The top bacterial family identified in LC^{+mcra}-supplemented mice cecum included *Clostridiaceae, Bacteroidaceae, Lactobacillales,* and *Sphingobacteriaceae*. Shannon species diversity was also significantly raised (P<0.05) by 2.76% in LC^{+mcra} fed mice. In respect to bacterial infection, 13.33% fewer *Firmicutes*, 0.29% more *Bacteroidetes*, and 14.17% more *Proteobacteria* in cecum were associated with mice during salmonellosis without LC^{+mcra} protection.

Significance: Daily supplementation of *Lactobacillus* might boost gut microbial diversity and improve the composition of intestinal microbiota, thus protecting the host from enteric bacterial infections.

P1-241 Real-time PCR Assay for the Simultaneous Detection of *Lactobacillus* Species by Comparative Genome Analysis

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Introduction: *Lactobacillus* is an important bacteria in food due to its use as a probiotic to control pathogenic bacteria. In relation to the functionality and safety of foods, accurate detection of *Lactobacillus* is very important in the industry. However, using the 16S rRNA gene as a standard marker for differentiation of *Lactobacillus* is very limited because many species share similar sequences.

Purpose: The purpose of this study was to design species-specific primers targeting genetic markers obtained by comparative genome analysis and to develop accurate and rapid detection assay for 37 *Lactobacillus* species using real-time PCR.

Methods: To select genetic markers of *Lactobacillus*, 143 complete genomes were compared by comparative genome analysis using python scripts and were used to develop species-specific primers. The specificity of primers was confirmed by reference strains, and the accuracy of real-time PCR was assessed using serial dilutions of genomic DNAs. The validation test was designed to detect 37 *Lactobacillus* species with real-time PCR in one reaction, and the developed assay was applied to the monitoring of probiotic and dairy products.

Results: We selected 37 primer pairs from *Lactobacillus* genetic markers. All primers were amplified only in the target species without cross-reactivity, the efficiency (%) ranges of all standard curves were 90 to 105. These results demonstrate that real-time PCR provides high accuracy and efficiency. Our assay was applied to 31 products, and we confirmed that the detected species in 16 products were identical to the indicated on their labels. In the remaining products, the species named on the labels were replaced by another species or contaminated with different species.

Significance: Our assay was helpful for confirmation of the reliability of commercial probiotic and dairy product labels. This study will be applied to the rapid monitoring of *Lactobacillus* for human health and food safety.

P1-242 Comparison of Gastrointestinal Tolerance and Antimicrobial Effects of Probiotic Bacteria Isolated from Dietary Supplements

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Introduction: Probiotic bacteria must survive before and after consumption and successfully colonize the human gastrointestinal tract to provide health benefits such as preventing enteric diseases. There is a lack of studies comparing survival and antimicrobial effects of probiotic bacteria in dietary supplements sold in the United States.

Purpose: This study investigated the survival of probiotic bacteria isolated from commercial products before and after exposure to the simulated gastrointestinal environment and their antimicrobial efficacy against common enteric pathogens *Escherichia coli* O157:H7 and *Salmonella.*

Methods: Viable counts of 11 commercial probiotic supplements were determined by plate count method before and after exposure to the simulated gastrointestinal environment. Three probiotic isolates, *Lactobacillus plantarum* (LP-115 and 299v) and *L. rhamnosus GG*, were selected for further antibacterial analysis, including agar well diffusion assay, adhesion to HT-29 cells and antagonistic activities (exclusion, competition, and displacement) against *E. coli* O157:H7 and *Salmonella* on HT-29 cells.

Results: Nine probiotic samples contained 6.3 to 10.4 log CFU/pill while two contained less than one 1 CFU/pill. After exposure to the simulated gastrointestinal environment, viable counts reduced one to 48%. Based on the agar well diffusion assay, none the probiotic culture filtrates showed the antimicrobial effect at pH 6.5. HT-29 cells (2×10^5 cells/well) were inoculated with 10^8 CFU of probiotic bacteria. About 1.5 to 3.5% of the inoculated probiotic bacteria adhered to the HT-29 cells. After adhesion, the three probiotic bacteria reduced adhesion of the pathogens to the HT-29 cells by 62 to 97%. For the competition, two *L. plantarum* reduced about 62 to 88% while *L. rhamnousus* reduced 23 to 33% of both pathogens on HT-29 cells. For displacement, two *L. plantarum* reduced 38 to 82% while *L. rhamnousus* reduced three to seven percent of the two pathogens on HT-29 cells.

Significance: Based on our results, the survival of probiotic bacteria in the dietary supplement as well as in simulated gastrointestinal environment varied among different products. The two *L. plantarum* showed higher antagonistic activities against enteric pathogens than did *L. rhamnousus*.

P1-243 Protective Effects of β-Glucan Extracted from Spent Brewer's Yeast during Freeze-drying and Storage of Probiotic Lactobacilli

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Introduction: Yeast-β-glucans are glucose polymers well-known for their bioprotective properties, such as antigenotoxic and antioxidant activities. Probiotic cultures for food applications are generally supplied as freeze-dried powders. To avoid cell inactivation during freeze-drying and warrant stability during storage, polysaccharides are used as protective compounds. However, the role of β-glucans form yeasts as cryoprotectant of probiotics is still unknown.

Purpose: This study evaluates the protective effects of spent brewer's yeast β-glucan (YβD) during freeze-drying and refrigerated storage of probiotic lactobacilli

Methods: The β-glucan was isolated from the cell wall of brewer's yeast (*Saccharomyces cerevisiae*) discarded as brewery slurry, using sonication and enzymatic treatments. Fresh biomass of *Lactobacillus plantarum* 49 (*Lp*49) and *L. plantarum* 201 (*Lp*201) (10^9 CFU/g) obtained from culture in De Man, Rogosa and Sharpe (MRS) broth was resuspended in saline solution (10 ml) for homogenization of 10 g of YβD and frozen (-20°C) for 24 h. The frozen suspensions were freeze-dried (temperature -55±2°C; vacuum pressure <138 μHg; freeze-drying speed one mm/h) for 40 h, packed in metalized BOPP bags, hermetically sealed, and stored under refrigeration. Lactobacilli without added YβD was assayed similarly. At different storage time intervals (days one, seven, 15, 30, 60, 90 and 120) viable cells were enumerated by serial dilution and plating on MRS agar.

Results: Counts of *Lp*49 and *Lp*201 freeze-dried with YβD did not show any decrease until 60 days of storage, while those freeze-dried without YβD reduced approximately two-log CFU/g in the same period. Over the 120 days of storage, *Lp*49 and *Lp*201 freeze-dried with YβD decreased approximately three and one-log CFU/g, respectively, while those freeze-dried without YβD decreased approximately four-log CFU/g.

Significance: YβD obtained from brewery slurry protects probiotic lactobacilli during freeze-drying and storage, however, the extent of protection may vary with the probiotic strain.

P1-244 Functional Properties and Safety Assessments of *Lactobacillus* Strains Isolated from Selected Traditional Fermented Food Products in Nigeria

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Introduction: This study was aimed at isolating potential starter cultures of *Lactobacillus* strains from *nono* (fermented milk product), *wara* (African soft cheese) and *ogi* (fermented cereal gruel).

Purpose: To select strains with functional properties as starter cultures

Methods: One hundred and four LAB strains were isolated and previously identified, of which 19 belonged to the *Lactobacillus* group. They were assessed for probiotic potentials, which include acidification, resistance to bile salts, gastric acidity, adhesion to extracellular matrices (human collagen Type IV, human fibronectin and human plasma fibrinogen) and safety assessments.

Results: Eleven were *L. fermentum*, six were *L. plantarum,* and two were *L. pentosus* strains. *L. fermentum* and *L. plantarum* strains were fast acidifiers with a change in pH of two units after six h and 12 h. At 0.3% and 1.0% bile salts concentration, *L. fermentum* W10 and *L. fermentum* OB9 had the highest values of 84.1±0.58% and 74.3±0.57% respectively. *Lactobacillus fermentum* WO1 displayed the highest value of 4.11±0.89 log CFU mL^{-1} while *L. fermentum* W10 had the smallest value of 2.94 log CFU mL^{-1} tolerance to gastric acidity after 180 min. This was comparable to reference strain *L. acidophilus* CNRZ 1923. *Lactobacillus plantarum* 2GM2 displayed the highest value of 76.30±0.92% and *L. fermentum* OB13 the least of 49.95±6.94% to human fibronectin. *L. plantarum* 2GM2 exhibited the highest value of 76.25±0.81% and the smallest value of 49.40±5.63% to human collagen Type IV while *L. plantarum* CG4 exhibited the highest value of 57.30±6.9% and the least value of 10.95±1.24% to human plasma fibrinogen. All the strains were negative to hemolysis and gelatinase activities.

Significance: In vitro determination of probiotic potentials has shown strain specific variance among *Lactobacillus* strains isolated from traditional fermented foods. These strains are interesting candidates for selection as functional starter cultures.

P1-245 Prevalence of Extended Spectrum β-Lactamase *Escherichia coli*, *Enterococcus* spp. and *Salmonella* in Soil and Water after Hurricane Florence Flooding in North Carolina

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Introduction: Hurricane Florence made landfall near Wrightsville Beach, North Carolina on September 14, 2018, causing significant economic loss to life, property and food animal production in the Carolinas. It is estimated that 3.4 million poultry, 5,500 hogs, and 37 people died due to Florence flooding. In addition, floodwater affected the landscape resulting in an overflow of sewage, both animal and human, across cities resulting in contamination of soil and water bodies. This is a serious public health concern as animal and human wastewaters contain diverse populations of bacteria, which can potentially infect humans and animals.

Purpose: To determine the prevalence of *E. coli*, including ESBL *E. coli*, *Salmonella* and *Enterococcus* spp. in soil and water from flood-affected regions.

Methods: Soil (10 g) and water (10 ml) were collected at six discrete sites monthly from November 2018 to January 2019 along the Neuse river basin from Kinston to New Bern cities. A total of 210 soil (n=60 per visit) and water (n=10 per visit) samples were collected. All samples were tested for isolation and identification of *E. coli*, ESBL *E.coli*, *Salmonella* and *Enterococcus* using standard microbiological culture methods. The ESBL *E. coli* were isolated using chromagar with cefotaxime four μg/ml.

Results: From soil samples (n=180), the prevalence of *Enterococcus* spp., at 174 (96.6%) of 180, was higher followed by *E. coli* at 120 (66.6%) of 180; *Salmonella* at eight (4.4%) of 180; and ESBL *E. coli* at six (3.3%) of 180. All water (n=30) samples were positive for *E. coli* (30 of 30) and *Enterococcus* spp. (30 of 30). The prevalence of ESBL *E. coli* and *Salmonella* in water was 23.3 and 10% respectively.

Significance: This initial baseline serves to reinforce the assumption that the environmental microbiota 'resets' following catastrophic events such as flooding. This study will continue over time to study the changes that occur following such events and should provide insight into the dissemination of bacterial populations into human and animal communities.

Poster

P1-246 Microbial Quality and Safety of Pesto, Salsa, Guacamole and Tapenades at Retail Markets

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Introduction: Due to attention to a more sustainable diet with foods of non-animal origin, a wide variety of vegetarian dips are being introduced on the retail market. Few food-borne outbreaks have been associated with pesto, salsa or guacamole or tapenades.

Purpose: To collect information on the scope of these vegetarian dips available on the Flemish retail market and to evaluate microbiological quality and safety of these types of products. Vegetarian spreads with major component plant-based proteins (e.g., chickpeas) were excluded from this study.

Methods: Local supermarkets were visited to establish the type of vegetarian dips available at retail and information on storage conditions and ingredients were collected. Ca. 40 refrigerated and eight ambient-stable products were subjected to microbial analyses both at time of purchase and (enforced) end of shelf life for both overall quality and hygiene indicators and *Listeria monocytogenes*. Also pH and a_w was measured.

Results: In total 172 different food items were found, mainly pestos (31%) and often tomato based (31%). In total 75% of these product items contained an organic acid preservative. The pH varied between 3.6 and 5.2, the a_w varied between 0.91 and 0.99. No *Listeria monocytogenes* counts of >10 CFU/g were noted. In the refrigerated products *Bacillus cereus,* sulphite reducing *Clostridia, E. coli* and coliforms were occasionally found but never exceeded 3.65 log CFU/g, aerobic psychotropic count and psychotropic lactic acid bacteria ranged between less than two to 9.44 log CFU/g and yeast and fungi ranged between less than one to 6.67 log CFU/g. The commercial stability was confirmed for the ambient-stable products.

Significance: Large differences were found between the overall microbial quality parameters, indicating variation in production conditions. Most products were at end of shelf life still of very good microbial quality.

P1-247 Thermal Reduction of *Salmonella* Inoculated Gelatin in Marshmallow

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Introduction: Marshmallow and its microbiologically sensitive ingredients (gelatin and/or eggs) have been linked to *Salmonella* outbreaks. As consumers can ingest marshmallows without further processing, it is important that the food industry properly controls the risk of *Salmonella* in the product. Possible contamination vectors as well as understanding the worst case conditions are important factors in validating a biological control step to manage the risk of *Salmonella* in marshmallow.

Purpose: *Salmonella* inoculated marshmallow was thermally treated at various temperatures and lengths of time to determine the effectiveness of the marshmallow cook step.

Methods: Several strains of *Salmonella* were freeze-dried on gelatin to mimic the potential mode of contamination and incorporated into a marshmallow. The inoculated marshmallow was then portioned into 20-g samples which were rolled into thin layers within Whirlpak bags. The marshmallow samples were treated at various temperatures (68, 71, 74, 77°C) in a hot water bath for up to 15 min. The study was conducted in triplicate. The *Salmonella* were then enumerated on tryptic soy agar with select colonies confirmed on xylose lysine deoxycholate agar. Log reductions and *D*-values were calculated from the data.

Results: The water activity of the marshmallow varied between 0.758 and 0.819 with the pH ranging from 4.49 to 5.84. *Salmonella* reductions in the marshmallow were 1.27±0.16, 2.18±0.24, 2.25±1.07, and 3.05±0.72 log CFU/g at 68, 71, 74, and 77°C, respectively, after 15 minutes of heat treatment. The *D* values were 10.88, 7.43, 6.60, and 5.95 minutes for 68, 71, 74, and 77°C, respectively.

Significance: Overall, freeze-drying of the inoculum on gelatin may have led to more heat resistant *Salmonella* strains, thus indicating a worst case scenario. The data suggest that treatment at 77°C will produce a three-log reduction of *Salmonella* in marshmallow.

P1-248 Lethality of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* during BBQ Sauce Processing

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed foods highlight the need for food industries to validate their thermal process.

Purpose: The purpose of this study was to evaluate the thermal inactivation kinetics of *Salmonella* spp., *Listeria monocytogenes,* and *E. coli* during BBQ sauce processing.

Methods: Two BBQ sauce formulations (A and B) were inoculated with *Salmonella* spp. (five-strain cocktail), *E. coli* (O157, and "big six" non-O157 Shiga toxin-producing serotypes) and *L. monocytogenes* (five-strain cocktail) in separate trials. Samples (25 g) were placed in a water bath set at 165±2°F (73.9°C) (representing conservative worse-case scenario) and allowed to come up to the minimum temperature (163°F) before starting the treatment time. Samples were treated at temperature and pulled at zero, 2.5, 5, 7.5 and 10-minute time points and were immediately placed in an ice bath to stop further heating (n=3). Data was log-transformed and ANOVA (analysis of variance) was performed to determine parameters that deliver a minimum five-log reduction in *Salmonella* spp., *E. coli* and *L. monocytogenes*. Samples inoculated with *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 were enumerated using scientifically valid methods.

Results: Both formulas A and B achieved greater than five-log reduction (P<0.05) of *Salmonella*, *L. monocytogenes* and *E. coli* after a treatment of 2.5 min. Formula A varied from Formula B regarding % titratable acidity, sodium chloride and brix; however, this did not significantly (P>0.05) affect thermal inactivation kinetics of the evaluated pathogens.

Significance: The study findings provide scientific basis that the thermal process (175°F for 25 minutes) employed by Conagra Brands manufacturing facilities achieves an acceptable lethality of vegetative pathogens from a food safety standpoint. This study supports the management of thermal processing as an operational prerequisite program (not a CCP) in a facility's food safety plan.

P1-249 Quantitative Microbial Risk Assessment of *Vibrio cholerae* and *Vibrio vulnificus* by Consumption of Flatfish Sushi and Sashimi

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Introduction: *Vibrio cholerae* and *Vibrio vulnificus* are usually transferred from seawater to seafood, and raw seafood consumption causes the foodborne illness. In some countries, flatfish is consumed as sushi or sashimi.

Purpose: The objective of this study was to estimate the risk of *V. cholerae* and *V. vulnificus* by flatfish sushi and sashimi in Korea.

Methods: Prevalence data of *V. cholerae* and *V. vulnificus* were collected from flatfish sashimi in domestic markets in Korea. Temperature and time data for flatfish distribution were also collected, and appropriate probabilistic distributions were then determined. Predictive models that can describe the fates of *Vibrio* species were cited and the bacterial cell counts were simulated under the distribution condition. Consumption data for flatfish were collected. Dose-response models for *V. cholerae* and *V. vulnificus* were searched. With all collected data, simulation models were prepared, and the risks of *V. cholerae* and *V. vulnificus* were calculated through the simulation model, using @Risk.

Results: Estimated initial contamination level was -3.4 and -2.4 log CFU/g for *V. cholerae* and *V. vulnificus*, respectively. For distribution conditions, pert distribution (zero, five, 48) for time and pert distribution (two, five, 20) for temperature were appropriate. Under the condition, the predictive model showed that the *Vibrio* cell counts increased during distribution. The gamma distribution for consumption data showed that consumption amounts were 71.15 g per day and frequency was 0.91%. Beta-Poisson models (Risk=1-(1+Dose/β)$^{-\alpha}$: *V. cholera*: α=0.495, β=3,364; *V. vulnificus*: α=9.3×10^{-6}, β=1.1×10^{5}) were appropriate for dose-response of *V. cholerae* and *V. vulnificus*. The simulation with all data showed that the probabilities of *V. cholerae* and *V. vulnificus* foodborne illness by raw flatfish consumption were 6.01×10^{-8} and 2.22×10^{-13}, respectively.

S**ignificance:** This result suggests that the risks of *V. cholerae* and *V. vulnificus* in flatfish sashimi are low in Korea.

P1-250 Enhancement of Thermal Inactivation of Foodborne Pathogenic Bacteria at Mild Heating Temperatures with Inclusion of Parabens

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Introduction: While high temperature heat treatments can efficiently reduce pathogen levels, they also affect the quality and nutritional profile of foods, as well as increasing the cost of processing. Approved preservatives (parabens, as processing aid agents) were investigated for their bactericidal and fungicidal properties, including the synergistic effects of these compounds with mild heating.

Purpose: This study examined the potential enhancement of inactivation of four foodborne pathogens, *Cronobacter sakazakii* 607, *Salmonella* Typhimurium, attenuated *Escherichia coli* O157:H7 and *Listeria monocytogenes,* during mild heating by inclusion of parabens in a model matrix.

Methods: The target microorganisms were grown to early stationery phase, concentrated by centrifugation, and transferred into fresh brain heart infusion broth with selected concentrations of butyl paraben (<250 ppm) just prior to thermal treatment (54 to 58°C) for up to 15 minutes. Heating was conducted with a submerged coil apparatus (Sherwood Technologies), with samples collected at designated intervals. Samples were plated on trypticase soy agar for recovery and enumeration. Survivor curves were plotted, and *D*-values were calculated and compared using ANOVA.

Results: Low concentrations of butyl paraben combined with temperatures < 60 °C achieved five to six-log reductions in less than 15 minutes (*D*-values below 100 seconds) with all tested microorganisms, whereas reductions without butyl paraben were only one to two log (*D*-values above 500 seconds). *Listeria monocytogenes* was the most resistant strain to butyl paraben. Microbial levels in room temperature controls with and without addition of butyl paraben remained unchanged during treatment, demonstrating butyl paraben and mild heating were acting synergistically.

Significance: Addition of paraben can significantly enhance the rate of thermal inactivation of foodborne pathogens, which may enable mild heating temperatures or shorter processing time. This study provides a proof of concept that identification of synergistic processing aid compounds could be a means of enhancing the lower temperature thermal processing.

P1-251 Validation of a Drum Roaster for Peanut Roasting in a Jhagadia, Gujarat (India) Peanut Butter Facility

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Introduction: *Salmonella* is a hazard associated with raw peanuts. Validation of the roasting process is necessary to determine its elimination from raw peanuts. Owing to differences in equipment design, validation protocols for typically used, belt-type roasters cannot be applied to drum roasters. A different validation strategy was needed for a drum roaster used in a peanut butter facility in India.

Purpose: To establish a validation strategy and determine processing parameters to achieve > 5 log reduction of *Enterococcus faecium* (surrogate for *Salmonella*) during the drum roasting process.

Methods: The validation study was performed in a commercial facility hence, pathogens were not used. Peanut inoculation/heat resistance of surrogate organisms was performed using ABC protocol. Aluminum mesh packets containing 50 grams of 'Bold' variety peanuts inoculated with *E. faecium* NRRL B-2354, were interspersed with 299.8 kg uninoculated raw peanuts in the roaster. To ensure uniform heat transfer, mesh sizes for packets were evaluated in advance by comparing L-values (color reading) of peanuts inside the mesh and the bed peanuts after roasting. The smallest mesh size gave the most comparable results. The validation consisted of three replicate trials of three run times of 11, 13, and 15 minutes *after* 100°C (calibrated thermometer installed in the roaster) was reached in the rotating peanuts. Following roasting, samples were retrieved, cooled, and enumerated for *E. faecium* using trypticase soy agar with KF agar overlay.

Results: A 6-log reduction (P < 0.05) of *E. faecium* was achieved regardless of the roasting time after 100°C was reached.

Significance: Acceptable lethality of *E. faecium* on peanuts is achieved during drum roasting. This study highlights consideration of other process parameters (incoming peanut temperature/moisture variation, sample containment material) that play a key role in validation of drum roasting process and provides scientific basis for the facility's food safety plan.

P1-252 Validation of Baking as a Kill-Step for Controlling *Salmonella* in Fruit Filled Pastry

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Introduction: Foodborne pathogens, such as *Salmonella*, can survive in dry ingredients such as flour for long periods, resulting in contaminated batter or dough. Inactivation of these pathogens during the baking step is vital to ensure the safety of finished bakery products such as pastries.

Purpose: To validate baking as a kill-step to control *Salmonella* in fruit-filled pastries and determine *D*- and *z*-values of a seven-serovar *Salmonella* cocktail in pastry dough.

Methods: Two independent experiments (designed as completely randomized blocks with three replications) were conducted to validate a general fruit-filled pastry baking process and to determinate *D*-value during dough heating. The pastry dough was prepared using inoculated flour (~seven log CFU/g). Pastries were baked at 375°F (190.6°C) oven temperature for 15 min followed by 30 min of ambient air cooling (B+C). A pastry was removed and analyzed

Poster

to determine *Salmonella* reductions at defined intervals. *D*-values in pastry dough were determined at 55, 58 and 61°C using thermal-death-time disks and hot-water baths. In both studies, samples were enumerated using an injury-recovery plating protocol.

Results: The mean internal temperature of pastries increased from 36.7°C to ~101°C at the end of 15 min baking. The *Salmonella* population in pastries decreased by greater than five log CFU/g by nine min of baking and was completely eliminated at B+C (as determined by enrichment). The pH and water activity of pastry dough at B+C were 5.33 and 0.677, respectively. *D*-values of the *Salmonella* cocktail at 55, 58 and 61°C were 32.8, 15.5 and 5.3 min, respectively, with a *z*-value of 7.7°C for the *Salmonella* cocktail determined.

Significance: Baking fruit-filled pastries at 375°F (190.6°C) oven temperature for at least nine minutes ensures greater than five log CFU/g reduction in *Salmonella* population. The *D*- and *z*-values determined in this study will help food manufacturers in process lethality determinations.

P1-253 Isolation and Characterization of a Novel *Salmonella* Bacteriophage from Livestock Farms in Ohio

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Introduction: *Salmonella* spp. are considered some of the most important foodborne pathogens. Use of bacteriophages as a biocontrol agent has gained worldwide attention in recent years in terms of its safety and efficacy to control foodborne pathogens. Unlike chemical agents, some commercial phage products show no or only subtle adverse impacts on food texture, taste and aroma. Additionally, the use of phage reduces the possibility of emergence of antibiotic resistant bacteria.

Purpose: Develop an effective anti-*Salmonella* biocontrol method by screening and isolating lytic phages from livestock farms around Ohio, followed by selecting and characterizing the most promising phage isolates to control multiple *Salmonella* strains.

Methods: Phage candidates were screened after inoculating environmental samples with a multi-strain *Salmonella* culture (*Salmonella* Typhimurium, Enteritidis and Heidelberg). The most promising isolates were selected based on host-range determination on 37 different *Salmonella* strains. Furthermore, growth curve, storage stability and multiplicity of infection-dependent inhibition of a selected isolate, A-Organ-ST, were measured.

Results: A total of 32 phages were isolated from livestock farms in Ohio including five poultry farms, two cattle farms, a sheep farm and a swine farm. Among these, seven isolates yielded strong lytic activities against multiple *Salmonella* serotypes. In particular, A-Organ-ST isolate could infected 28 *Salmonella* strains plus two *Escherichia coli* strains. A-Organ-ST presented two distinct phases in one-step growth curve with a burst size of 116 PFU/cell. Reduction in *Salmonella* Typhimurium population was observed at all MOI performed in this research. In particular, at MOI of 1,000 and 10,000, the *Salmonella* population decreased from 4.5 log CFU to 2.4 log CFU and 1.7 log CFU, respectively, within 15 h.

Significance: These results suggest the abundance of bacteriophage in farm environments. Specifically, phage isolate A-Organ-ST is a promising candidate for controlling a wide range of *Salmonella* serovars in future food applications.

P1-254 Stress Response and Survival of *Salmonella* Enteritidis in Single and Dual Species Biofilms with *Pseudomonas fluorescens* Following Repeated Exposure to Quaternary Ammonium Compounds

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Introduction: As an important foodborne pathogen, *Salmonella* could develop adaptation when being exposed to low doses of disinfectant due to underdosing or insufficient. However, little is known about the stress response of *Salmonella* in mixed species biofilms during exposure to quaternary ammonium compounds (QAC).

Purpose: The objective of this study was to evaluate how *S.* Enteritidis biofilm cells in single species and dual species with *P. fluorescens* respond to continuous exposure to sublethal dose of QAC.

Methods: The 48 h-biofilms of *S.* Enteritidis and *P. fluorescens* formed on stainless steel coupons were exposed to 20 ppm QAC for 5 days. The attached cells as well as detached cells were subject to QAC treatment at 200 ppm and 100 ppm, respectively. Biofilm cells as well as EPS were observed by confocal laser scanning microscopy.

Results: The 15 min-QAC treatment led to log reduction (>5.6 log CFU/cm^2) of *S.* Enteritidis in QAC-exposed and non-exposed biofilms below detection limit. However, less than 2 log reductions were obtained in dual species biofilms. There were lower reductions (2.0-3.4 log CFU/cm^2) of *S.* Enteritidis detached from dual species biofilms than those (2.3-5.6 log CFU/cm^2) from single species biofilms. Both *P. fluorescens* and *S.* Enteritidis single species biofilms displayed flat and thin structure, in contrast to the big microcolonies protruding from the bottom layers in dual species biofilms. QAC-exposure led to increased biovolume of membrane damaged cells and reduced biovolume of live cells. In addition, 19.2 % of the total biovolume was occupied by polysaccharides in dual species biofilms, which is 10-fold higher than the percentage of polysaccharides (2%) in *S.* Enteritidis biofilms.

Significance: These results indicate that *P. fluorescens* could enhance survival of *S.* Enteritidis biofilm cells under sublethal QAC stress and QAC treatment. Thus, the protective effect of *Pseudomonas* on *Salmonella* biofilm cells poses a challenge for sanitation efforts, suggesting better sanitation strategy to control *Salmonella* in food processing environment.

P1-255 Biofilm Formation of O157 and Non-O157 Wild-Type and Pressure-stressed Shiga Toxin-producing *Escherichia coli* at 7°C and 25°C and Their Sensitivity to Quaternary Ammonium Compound-based Sanitizer

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❖ ***Developing Scientist Entrant***

Introduction: Biofilms of microbial pathogens are a major concern in manufacturing and healthcare settings and estimated to be the etiological agent of >80% of bacterial infections.

Purpose: Current study evaluates the sensitivity of a quaternary ammonium compound-based sanitizer against two phenotypes wild-type (WT) and pressure-stressed (PS) of O157, O26, O45, O103, O111, O121, and O145 Shiga toxin-producing *Escherichia coli* at 7 and 25°C.

Methods: A six-strain mixture of WT and PS *Escherichia coli* O157 and non-O157 *Escherichia coli* (CDC's "big six") were utilized for biofilm formation for up to two weeks on the surface of stainless steel (finish 2b) coupons at 7 and 25°C. After removal of loosely attached cells, samples (six replicates per sample per treatment) were neutralized using D/E neutralizing broth and removed from coupons using sonication, prior to culture dependent analyses. The PS phenotypes were prepared by exposing the isolates to the sublethal elevated hydrostatic pressure of 15,000 PSI (c.100 MPa) for 15 minutes. The experiment was analyzed statistically by SAS, using a Tukey-adjusted mean separation.

Results: Counts of WT and PS *Escherichia coli* O157 at 7°C were 2.04±0.7 and 3.29±0.2 prior to treatment, respectively. These counts on day zero were reduced (P<0.05) to 0.63±0.2 and 0.98±0.3, respectively, after the treatment. In contrast, counts of two-week mature biofilm of WT *Escherichia coli* O157 were 5.22±0.1 and 4.56±1.0 before and after treatment, respectively, exhibiting low efficacy (P≥0.05) of the sanitizer against two-week mature biofilm.

Significance: The tested serogroups and phenotypes of pathogen exhibited similar biofilm formation capability and sensitivity to QAC. The sanitizer tested at the highest concentration recommended by the manufacturer appears to be efficacious only against planktonic cells while exhibiting inability for the complete removal of one- and two-week mature biofilms.

P1-256 Phenotypic Characterization of Biofilm-Forming *Bacillus* spp. Identified in the Irish Artisan Bakery Environment

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial biofilms on food processing surfaces play a key role in food (re)contamination. Approximately 40% of the outbreaks in Europe from 2007 to 2012 were due to biofilm formation in diverse low-moisture food industries.

Purpose: The purpose of this study was to assess selected *Bacillus* isolates from the Irish artisan bakery environment for biofilm-forming ability and their potential to survive on food processing surfaces.

Methods: Phenotypic characterization of five selected isolates and *Bacillus subtilis* NCTC3610 as a control was performed using Congo Red-Calcofluor assay for the co-expression of extracellular matrix components (namely curli fimbriae and cellulose), crystal violet biomass assay, and pellicle formation at the air–broth interface at 30, 37 and 45°C from 18 to 96 h.

Results: All the isolates had the ability to form weak, moderate and strong biofilms at different test time and temperature. The isolates exhibited a red, dry and rough (RDAR) morphotype on Congo Red–Calcofluor agar after 72 h at 30, 37 and 45°C, indicating the presence of curli fimbriae, while two isolates showed very RDAR (VRDAR) morphotypes at 45°C. No fluorescence was detected in the isolates suggesting no or low cellulose production. Biomass production during static growth in M9 medium assessed using crystal violet decreased over time at 30 and 37°C with weak to moderate attachment in all but one isolate which consistently formed strong films at both the temperatures. At 45°C, the biomass production appeared to decrease from 18 to 24 h and then increased for three out of five isolates, however attachment to the surface was strong for all the isolates. Fragile pellicles at the air-broth interface at 37 and 45°C suggested biofilm forming ability despite low cellulose production.

Significance: The increased understanding of the nature of *Bacillus* biofilms will contribute significantly to the development of eradication and control strategies in the relevant food processing industries.

P1-257 Antimicrobial Resistance of Enterococci in Surface and Reclaimed Water in the Mid-Atlantic Region

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Introduction: Increasing demands for agricultural water require identification and safety assessment of alternative water sources. Some species of *Enterococcus*, an opportunistic bacterial pathogen and water quality indicator, exhibit antimicrobial resistance (AMR) and can transfer resistance traits to other bacterial taxa, including human pathogens.

Purpose: To evaluate the distribution and AMR of two *Enterococcus* species of most concern to human health, *E. faecalis* and *E. faecium,* in surface and reclaimed water in the Mid-Atlantic region of the United States.

Methods: A total of 365 isolates from 129 water samples collected from 11 sites (three reclaimed wastewater treatment plants, four non-tidal and two tidal rivers, two ponds) between October 2016 and October 2017 were identified as *E. faecalis* (40.5%), *E. faecium* (7.7%) or other *Enterococcus* species (51.8%). Antimicrobial susceptibility testing was conducted on 95 *E. faecalis* (n=75) and *E. faecium* (n=20) isolates using microbroth dilution and the standard gram positive NARMS GPN3F plates. Chi-squared and Fisher's exact tests were used to assess significant relationships between categorical variables and differences in proportions. Logistic regression was used to measure association of season, water type, temperature, pH and salinity with species and AMR.

Results: Season (P<0.01), water type (P<0.01), temperature (P<0.01) and salinity (P=0.023) were significantly associated with species probability, and season with antimicrobial resistance of *E. faecalis*. Pan-susceptibility to antimicrobials tested was detected in 1.3% of *E. faecalis* and five percent of *E. faecium*, but all isolates (n=95) were susceptible to ampicillin, vancomycin, daptomycin and linezolid. Sixteen percent of *E. faecalis* and 70% of *E. faecium* isolates were multidrug resistant. In *E. faecalis*, season was a factor in AMR to erythromycin (P<0.01), streptomycin (P=0.043), tetracycline (P<0.01) and in multidrug resistance (P<0.01).

Significance: While *Enterococcus* was ubiquitous in water tested and exhibited resistance to multiple antimicrobials, resistance to antimicrobials of last resort for enterococcal infections was non-existent.

P1-258 Distribution of Pathogenic *E. coli* in Surface and Reclaimed Water: A Conserve Study

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Introduction: *Escherichia coli* is a widely used bacterial water quality indicator that can carry pathogenic traits causing gastric and urinary tract infections. Fresh crop surfaces can recruit *E. coli* via irrigation water. The characterization of *E. coli* recovered from irrigation water is an important step in ensuring food safety.

Purpose: Assess the prevalence and diversity of *E. coli* collected from surface and reclaimed irrigation water in the Mid-Atlantic region of the United States.

Methods: Water samples (n=166) were collected from 12 sites (two ponds, two tidal rivers, four non-tidal rivers/creeks, three reclaimed wastewater treatment plants and one vegetable processing water) over a 12-month period. *E. coli* were isolated by standard membrane filtration (EPA Method 1604) on MI agar. Isolates (n=308) were PCR-confirmed with *uidA* gene amplification. Phylotyping (Groups A, B1, B2 and D) was done by amplification of three virulence genes: heme transport gene (*chuA*), a stress-related gene (*yjaA*) and lipase/esterase (TspE4.C2).

Results: *E. coli* was isolated from 149 (87.6%) water samples at 0.3 to 4.1 log CFU/100 ml. Non-tidal water samples harbored significantly higher levels of *E. coli* compared to the tidal, pond and reclaimed water ($P<0.05$). Phylogenetic analysis revealed that the largest proportion of isolates in all water types belonged to the B1 group- 170 (55.2%) of 308. Group B2, which includes enterohemorrhagic, enteropathogenic and Shiga toxin-producing *E. coli*, was the second most predominant group. Phylotype distribution was significantly different across water types ($\chi^2=45.39$, $P<0.0001$). Group B2 was highest in tidal brackish water and Group D in non-tidal and tidal water. These groups were absent in vegetable processing water, which harbored groups B1 (88.9%) and A (11.1%). A seasonal effect on phylotype distribution was not detected.

Significance: These findings indicate that non-tidal river water contained the highest levels of *E. coli* and tidal brackish water the highest percentage of potentially pathogenic strains.

P1-259 Prevalence of Methicillin-resistant *Staphylococcus aureus* in the Isidro Ayora General Hospital in the City of Loja, Ecuador

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) causes various infections of both community and hospital origin. The interest in this study is due to insufficient information about this pathogen in Ecuador.

Purpose: To determine the prevalence of MRSA in Isidro Ayora Hospital in the city of Loja, Ecuador

Methods: A total of 311 nasal and hand samples and 92 food samples were collected. The food samples were seeded in Baird Parker agar by inoculating one ml in three boxes. Fifty g of the product was weighed and mixed with 450 ml of Butterfield's phosphate-buffered saline and incubated 45 to 48 h at 35 to 37°C. The identification of *S. aureus* and MRSA in all the samples was carried out by catalase, coagulase and mannitol tests. For the confirmation of MRSA, colonies were inoculated on Mueller Hinton agar with cefoxitin and incubated under aerobic conditions for 18 hours at 37°C. The samples are considered MRSA when the inhibition zone is ≤21 mm.

Results: From 92 food samples, it was determined that 12 (13%) of 92 were positive for *S. aureus,* of which 2 (17%) of 12 were classified as MRSA and sensitive to linezolid. In clinical samples a greater predominance of this microorganism was reported in nurses (50%), doctors (14%) and internal medicine (28%). The results suggest a need for application and implementation of good hygiene and sanitation practices.

Significance: The presence of methicillin-resistant *S. aureus* in prepared foods suggests a potential risk to the health of patients and hospital staff.

P1-260 A Comparison Study of bioMérieux VIDAS SET II and the r-Biopharm Ridascreen SET Total to Detect the Presence of Staphylococcal Enterotoxins Using Matrix Dependent Extractions from a Variety of Foods

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Introduction: The FDA-BAM recommends a secondary confirmation test for *Staphylococcus* enterotoxin-positive samples. This study was conducted with the VIDAS SET2 assay and the Ridascreen SET Total assay for use in detection and confirmation of SETs in food. The VIDAS SET2 assay uses an automated ELFA (Enzyme Linked Fluorescent Assay) based technology and the Ridascreen SET Total is a manual ELISA method. Both kits screen for staphylococcal enterotoxins A, B, C1, C2, C3, D and E but have different extraction protocols based on the food matrix.

Purpose: The purpose of this study was to compare the two assays for toxin detection in a variety of foods and to evaluate the performance of the different extraction methods.

Methods: Twenty-three different food matrices and six proficiency samples were evaluated for a total of 60 VIDAS SET2 and 47 Ridascreen SET analysis. Each matrix was spiked with SET toxins A or B at one ng/g of food. Once detected via VIDAS SET2, the same extraction material was used for confirmation with the Ridascreen SET Total assay.

Results: Both test kits correctly reported all the spiked samples as positive and the control samples as negative. The VIDAS SET2 was able to correctly detect the toxin, when present, following five different matrices' unique extraction protocols. The Ridascreen also correctly detected toxin presence/absence in all 25 spiked samples and six negative controls that were extracted following the Ridascreen simplified extraction methods and 16 additional spiked extraction samples from the alternate test kit method.

Significance: We concluded that not only could both assays be used to reliably detect the presence of SETs using the recommended sample extraction methods but that both the initial and the secondary testing could be carried out using the same extraction material. Lessons learned and challenges with the different extraction methods will be shared.

P1-261 Hurdle Enhancement of Antimicrobial Efficacy of Acidic Electrolyzed Water on *Bacillus cereus* Spores Using Ultrasonication

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Developing Scientist Entrant

Introduction: *Bacillus cereus* spores have tough and metabolically inert structures whose formation is a strategy for this bacterium to survive in unfavorable environments. It is of significant concern to the food industry because it produces two thermostable toxins and causes massive foodborne disease. Thus, it is critical to develop effective technology for inactivation of bacterial spores.

Purpose: This study evaluated the inactivation effects of ultrasonic treatment combined with acidic electrolyzed water (AEW) on *B. cereus* spores.

Methods: *B. cereus* spores were treated with ultrasound and AEW separately and combined, followed by investigation of the antimicrobial effects. Flow cytometry and electron microscopy were used to investigate changes in the physiological status and ultrastructure of spores. The trials were replicated 3 times in each experiment.

Results: AEW treatment induced 1.05 to 1.37-log CFU/ml reduction of *B. cereus* spores while the sporicidal effect of ultrasound was minor. In comparison, simultaneous ultrasonic and AEW treatment for 30 min produced a 2.29-log reduction of spores and was determined to have a synergistic effect. Moreover, simultaneous antimicrobial treatment was more effective to inactivate spores than successive antimicrobial treatments. Flow cytometry combined with SYTO 16/PI staining analysis revealed that ultrasound hydrolyzed the cortex and AEW partially damaged the integrity of the inner membrane of spores. We also identified that ultrasound promoted the detachment of exosporium and destroyed spore structure (e.g., cortex) that subsequently decreased the resistance of spores. Electron density of spores appeared to be heterogeneous after AEW treatment.

Significance: Combining ultrasound with AEW had a significant sporicidal effect on *B. cereus* spores and was validated as a promising sterilization technology for use in the food industry.

P1-262 Isolation and Characterization of *Vibrio parahaemolyticus* Protected from Laboratory Validation from Natural Seafood Sources

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Undergraduate Student Award Entrant

Introduction: The viable but non-culturable (VBNC) state of foodborne bacteria is a contributor to false-negative validation by the standard examination/evaluation protocol and causes a high risk to seafood safety. Some strains of *Vibrio parahaemolyticus* behave as such and are more likely to contribute to contamination found in seafood products.

Purpose: Our research was to explore a novel reviving method that can vitally improve the detection method devised today in evaluating seafood samples for foodborne *Vibrio* varieties.

Methods: An improved preenrichment method was devised for investigative isolation of VBNC *V. parahaemolyticus* from various sources. Buffered indicator plates containing thiosulfate-citrate-bile salts-sucrose media (TCBS) were subsequently utilized for selective culturing and isolation of *V. parahaemolyticus*. Gene-specific PCR, urease and Kanagawa hemolytic tests were followed for virulence characterization of the isolates. Significant differential revivability was determined for 15 biological and three technical tests using Student's *t*-test ($P<0.05$). Extracted DNA using a bead collision method was analyzed using 16S rDNA sequencing with an ABI 3730XL sequencer.

Results: TCBS screening for preenriched or preenriched and heated (80°C) shellfish samples exhibited 15 green or dark green colonies, a representative color of *V. parahaemolyticus*; however, a small subset of the isolates (five of 15) were acquired following a prolonged heat treatment (20 min) of preenriched samples, suggesting the availability of a novel group of *V. parahaemolyticus*, in which their revival from VBNC state depends on preenrichment followed by heat treatment. Positive urease production and negative hemolysis formation were noted. The DNA analysis of two dark green isolates by virulence genes PCR and 16S rDNA sequencing confirmed that these isolates were TLH-positive *V. parahaemolyticus*.

Significance: Food outbreaks driven by false negatives may involve novel bacterial properties that may contribute to the lower detection rate of *V. parahaemolyticus* compared to the hospitalization rate of patients. Our findings suggest a method using combined conditions for detection of this group of *V. parahaemolyticus*.

P1-263 The Migration of Phthalate Esters from Packaging Materials to Olive Oil under High Temperate Storage

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Introduction: Phthalate esters (PAEs) are popular for use in plastic bottles and for food packaging materials. The goal was to establish a fast and simple method of high-performance liquid chromatography-photo diode array detector (HPLC-PDAD) that can analyze PAEs.

Purpose: Through measured temperature snack bars, we simulated a high temperature environment of 50±2℃ for the storage test. The oil samples were analyzed to examine the migration of plasticized content in different packaging materials under high temperatures at 50°C for 30 and 60 d. This data was used to establish risk analysis for PAEs released into olive oil.

Methods: The standards included DMP (dimethyl phthalate),DEP (diethyl phthalate), DBP (di-n-butyl phthalate), BBP (benzylbutyl phthalate), DEHP (di-(2-ethylhexyl) phthalate) and DNOP (di-n-octyl phthalate) were analyzed. The packaging materials included PET, LDPE and HDPE. The optimized conditions were a column that was Luna C18 (2) (250 by 4.6 mm, five µm), flow rate 0.5 ml/min, mobile phase is acetonitrile : H_2O=80 : 20 (v:v).

Results: All PAEs were with well linear ranges, which were 0.033 to 16.66 µg/ml, $R^2>0.995$, and the limit of detection and limit of quantitation were 0.01 to 0.13 µg/ml and 0.03 to 0.26 µg/ml, respectively. The precision test finished such that the lowest range of DEHP can reach to five percent (relative standard deviation, RSD %).The DMP and DEP migrated out of containers, including PET, HDPE and LDPE. Sealed and unsealed were within 4.77 to 6.84 µg/ml and 0.97 to 9.18 µg/ml in the olive oil samples for 60 days. Other PAEs were not detected. DMP migration levels at 60 days increased 6.5-fold over that at 30 days for sealed PET bottles.

Significance: The highest released level for DMP (PET bottle) was 16.84±2.66 (µg/ml), and the predicted consumption levels were 0.0092 and 0.0089 mg/kg body weight/day for male (64 kg body weight/35 g fat/day) and female (52 kg body weight/27.5 g fat/day) adults, respectively. The levels are well below 0.05 mg/kg body weight/day as recommended by the Scientific Committee on Food of the European Commission (EC-SCF).

P1-264 WITHDRAWN

P1-265 Insight into Bacterial Communities Present in Commercial Chopped Romaine Lettuce Processed in Early and Late Seasons

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Developing Scientist Entrant

Introduction: Due to recent outbreaks associated with chopped romaine lettuce, the abundance and diversity of bacterial communities present in these products needs to be better evaluated.

Purpose: The aim of this study was to evaluate the changes of bacterial communities present in three brands of chopped romaine lettuce products harvested and processed in early and late seasons on their "use by" dates.

Methods: Commercial romaine lettuce products (a total of 72 bags) were purchased from the local grocery market in early (September to October) and late seasons (March to April) of 2018. Three brands of commercial products were chosen with two brands from California and one brand from Florida. DNA was extracted from these samples on their "use by" dates and the dynamic changes of microbial communities were evaluated by plating salad homogenate onto plate count agar (APC) and anaerobic agar (AnPC) and by conducting 16S rRNA sequencing.

Results: The APC and AnPC of all three brands on their "use by" dates were 6.78±0.65 log CFU/g and 5.65±1.81 log CFU/g respectively. No significant difference was observed among the three brands. Products of the late season had significantly higher APC (average 0.55 log CFU/g higher) and AnPC (average 1.0 log CFU/g higher) than that of the early season. *Proteobacteria, Firmicutes, Bacteroidetes*, and *Actinobacteria* were top four phyla present in three brands with

Poster

Proteobacteria (with relative abundance from 15.09 to 99.46%) and *Firmicutes* (with relative abundance from zero to 84.48%) dominating across all samples. Early season samples had significantly higher *Firmicutes* than late season samples ($P<0.05$). At the genus level, *Pseudomonas*, *Leuconostoc*, and *Rahnella* were top three genera in all samples.

Significance: This study provides important insight into bacterial communities present in commercial romaine lettuce products. Results will directly benefit the risk assessment of products when they approach "use by" dates.

P1-266 Analyzing Microbial Community Change of Turkey Litter Compost Due to Heat Exposure Using 16S High Throughput Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Turkey litter compost is commonly used as a biological soil amendment. However, there is a lack of knowledge on microbial community changes after heat processing of compost.

Purpose: The objective of this study was to evaluate the microbial profiling in the turkey litter compost before and after heat treatment using 16S high throughput sequencing, and the phylogenetic diversity of the microbial community categorized by analyzing operational taxonomic units (OTUs).

Methods: Turkey litter compost samples (six) before and after dryer were sourced from a commercial plant. According to the manufacturer's instruction, 0.25 g of each sample was used for DNA extraction. Before genomic DNA extraction, DNA from the dead cells was removed by propidium monoazide (PMA). The purified DNA samples were sequenced, and high-quality sequenced reads were analyzed using a custom modified QIIME analysis pipeline. Alpha diversity including Shanno, Simpson index, and species richness estimator of Chao I was calculated. Weighted UniFrac was calculated using QIIME, which was used to measure phylogenic beta diversity.

Results: The bacterial community structure in the compost following thermal process was reviewed by PCoA analysis and UPGMA clustering. The principal coordinates one, two, and three explained 97.41%, 1.79%, and 0.45% of the total structure variations, respectively. The clustering analysis showed that the turkey litter compost before the heat treatment was well-clustered together and separated from the heat-treated compost. Taxonomically, the phyla *Proteobacteria, Actinobacteria*, and *Bacteroidetes* were the most abundant in both before and after heat treatment, whereas *Firmicutes* and *Tenericutes* were predominant in the compost before and after heat treatment, respectively.

Significance: The microbial community analysis method was optimized for turkey litter compost, and the effectiveness of PMA treatment for compost on removing DNA from dead cells was confirmed. Further, the identification of indigenous bacteria in poultry litter surviving the physical heat treatment may lead to future studies on biological control of pathogens in soil amendment.

P1-267 Development of an Olfactory Test as a Tool for Food Safety

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Introduction: Olfactory disorder compromises safety from food poisoning and toxic agents. It influences food selection by things such as inability to recognize burnt cooking and accidental ingestion of spoiled food. There are olfactory test tools widely used in the United States and Europe but they are limited in Asian countries such as Taiwan. Odors reflect marked geographic variation. Choosing highly recognized odors is the elementary step in developing a good olfactory test tool.

Purpose: The purpose of this study is to provide odor information for further development of an olfactory test in Taiwan, potentially in Asian or Chinese populated countries.

Methods: Six hundred people were interviewed about familiar odors from their daily life. A total of 2,259 odors were obtained. One-hundred forty out of 2,259 odors were selected based on high frequency. Among 140 odors, we included 82 odors that can be obtained. After pretests and professionals meeting, 40 odors were selected, presented in liquid form with a standard concentration. Eighty participants with normal smell functions were invited to name the odors and rank the degree of odor intensity, familiarity, irritation, pleasantness, and coolness via VAS scale.

Results: Among 40 odors, the most intense is garlic, the most familiar is almond, the most irritating is garlic, and the most pleasant is grapefruit. The coolest odor is chrysanthemum. The warmest is garlic. These data proved a strong statistical relationship between the degree of discrimination and the odor's intensity (OR=1.15, P=0.000), familiarity (OR=1.40, P=0.000), irritation (OR=0.95, P=0.002), pleasantness (OR=1.36, P=0.000), coolness (OR=1.06, P=0.001). This study also affirmed the capability of odor identification intensity (OR=1.13, P=0.000), familiarity (OR=1.29, P=0.000), and pleasantness (OR=1.25, P=0.000).

Significance: These data provide 40 odors for developing a Taiwan/Asian olfactory test tool to recognize olfactory disorder for food safety.

P1-268 Growth Profile of Bacteria, Molds and Yeasts in Sliced Mozzarella Cheese Stored under Refrigeration

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Introduction: The handling of sausages and/or cheeses when cutting and stored can undergo change in the level of contamination, making the product unfit for consumption even before shelf life expires. The reason may be a lack of sanitation of slicers and/or inadequate storage of semi-cut pieces.

Purpose: The aim of this study was to analyze the growth of bacteria, molds and yeasts in sliced mozzarella cheese, packaged in styrofoam trays and stored under refrigeration (4°C) as stipulated by the supplier and current legislation for 96 h.

Methods: Three different samples, 25 g each, were analyzed (every 24 h up to 96 h), named week one (opened piece just before the first slicing), week two and week three (parts that were already sliced, stored under refrigeration in the sales packages). The samples were homogenized, diluted, inoculated in 3M Petrifilm plates and incubated, in duplicate. Growth curves were plotted in Excel and compared using ANOVA. The mathematical model was elaborated in Microfit.

Results: There were no statistically significant differences (P>0.05) in the counts of lactic acid bacteria during the 96 h. In other cases, both week two and three had counts higher than week one. The total coliform contamination of all samples was higher than that established by Brazilian legislation ($<5\times10^3$ CFU/g), with a mean value of 3.1×10^4 CFU/g. For *Staphylococcus aureus*, the limit is 10^3 CFU/g; only week one was within the standards (2.8×10^2 CFU/g) and both week two and three averaged 9.4×10^3 CFU/g. The adequate mathematical model was the logistic for total coliforms, lactic acid bacteria, molds and yeasts; for the others (*S. aureus* and aerobic bacteria) a model was not applied.

Significance: These data suggest that samples were probably badly stored and improperly handled before being sold, reducing the product's shelf life and possibly affecting consumer health.

P1-269 Acidified Sushi Rice Safety

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❖ ***Developing Scientist Entrant***

Introduction: Sushi rice held at room temperature in food service before preparation of rice rolls may support the growth of pathogenic microorganisms (e.g., *Bacillus cereus* and *Clostridium perfringens*) if not adequately acidified. Hence, it is critical to investigate the efficacy of sushi rice acidification on the survival and growth of these microorganisms.

Purpose: The purpose of this study was to investigate the survival and growth of *B. cereus* and *C. perfringens* inoculated in acidified sushi white rice.

Methods: Rice acidification methods performed by a retail sushi supplier were investigated. Briefly, the white sushi rice was cooked in a rice cooker and the pH was adjusted with rice vinegar to 4.2 or lower. The rice was inoculated with two strains of *C. perfringens* (ATCC 13124 strain s107; ATCC 3624 strain 26), and one *B. cereus* strain (ATCC 11778) to achieve a final count of 10^4 CFU/g. Three samples were taken at zero, 12, and 24 h postinoculation and plated on TSC and MYP media to quantify each pathogen. Three replicates of the experiment were carried out.

Results: The mean and SD of *B. cereus* and *C. perfringens* at time zero were 3.4±0.2 and 3.0±0.2 log CFU/g, respectively. No recovery of *C. perfringens* was found at 12 or 24 h.

Significance: This study found that adjusting white sushi to pH of 4.2 or less inhibits the growth *C. perfringens* and *B. cereus* at room temperature for up to 24 h.

P1-270 Assessment of Mercury Contamination in Sardine and Swordfish Using Inductively Coupled Plasma Atomic Emission Spectroscopy

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Introduction: Consuming fish is highly recommended by nutritionists due their content of high-quality proteins, omega-3, and vitamins, components that contribute to a healthy diet However, fishe are also known to bioaccumulate toxic heavy metals which contaminate seas and oceans, especially mercury, which is known to be a neurotoxin and a serious threat to the health.

Purpose: Objectives of this study were to estimate the rates of mercury (Hg) contamination in sardines (*Sardina pilchardus)* and swordfish (*Xiphias gladius)* and compare them with the regulatory threshold values

Methods: Hg contamination was investigated by sampling sardines (n=70) and swordfish (n=30). Determination of mercury concentration was done with inductively coupled plasma atomic emission spectrometer after mineralization. Quality control of the analysis was performed to verify the analytical performance by processing certified reference materials.

To compare the difference in Hg content in the two fish species, statistical analysis was performed with the Mann–Whitney test, and the threshold P value was set to 5%.

Results: The mean Hg concentration in sardines was 0.62 mg/kg wet weight, while in swordfish it was 0.56 mg/kg wet weight. The statistical analysis showed a significant difference in the concentrations of Hg between the two species (P<0.05).

The concentration in sardines exceeded Algerian and European legislation recommended threshold values (0.56 mg/kg wet weight), whereas in swordfish it was close to the threshold values.

Significance: The study demonstrated that consuming these two fish species could represent a hazard for consumers in Algeria, especially sardine which is the most consumed fish. Systematic and periodic controls of heavy metals in fish in general are recommended, and risk assessment is needed to protect the consumer.

P1-271 Development of a New and Natural Food Colorant Type Time-Temperature Indicator

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Introduction: A time-temperature indicator (TTI) is a simple, inexpensive device that can attach to a package surface and integrate the cumulative time-temperature history of foodstuffs fully or partially. TTIs are generally categorized as physical, chemical and biological (microbial) systems. The mechanisms of those TTIs are based on irreversible changes and reactions; most of the changes are expressed as a color change.

Purpose: The purpose of this study was to develop a prototype of a novel, chemical TTI, which is low cost, non-toxic, and user-friendly for cold food management. The principle of the novel TTI is an anthocyanin natural colorant (purple sweet potato, PSP) in alkaline solution that changes from deep blue to yellow.

Methods: In this study, seven natural colorants were evaluated as TTIs as determined by color change at different pH and temperatures. Among them, PSP colorant is the most suitable colorant for a TTI. Therefore, the color change of the PSP colorant in different alkaline solutions exposed to temperatures from 4 to 35ºC was determined by a colorimeter.

Results: The color of PSP colorant in alkaline solution changes from deep blue to greenish blue, brown, yellow-brown, and finally yellow at time progesses. The rate of color change of this TTI is affected by temperature, while L*a*b* and ΔE values of TTI increased as temperature and storage time increased. Based on a b* value =-10 and ΔE value=75 as the warning color change value, the warning times of this TTI at temperatures of 4, 10, 15, 20, 25, 30, and 35ºC are 102, 72, 48, 34, 18, 14, and 12 hours, respectively.

Significance: As a result, this new and non-toxic TTI made by natural purple sweet potato colorant is low cost and is suitable for commercial use. It has potential as an indicator to alarm temperature abuse in a cold food chain.

P1-272 Food Contamination Incidence by Foreign Materials Reported in Japan, 2014 to 2016

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Introduction: Complaints on contamination by foreign materials in food are reported to the health centers in Japan. Each health center has archived data for the complaints, however these are not shared with others nor aggregated, due to differences in reporting formats between municipalities. Nationally aggregated data are necessary to guide food business operators in introducing control measures against food contamination by foreign materials.

Purpose: To analyze the situation and the risk factors for contamination by foreign materials in food in Japan.

Methods: Reported complaints on contamination by foreign materials in food were collected for a period from April 2014 to November 2016 from 127 municipalities out of 147 (89%) in Japan by a questionnaire. We compiled the data by foreign materials, food items, business types, production/handling procedures related to the contamination, occurrence of injury, physical size of the contaminants, and hard object contaminants.

Results: There were 14,379 complaints reported on contamination by foreign materials during the data collection period (approximately three years). Most frequently reported foreign materials were bugs followed by hairs, metals, and plastics. Prepared dishes, confectionery and farm products were the most reported food items. 31.4% of the contamination events occurred in food production/processing/cooking business sectors with 34% of the contaminants being hard objects. Prepared dishes, confectionery and farm products were the most reported food items for hard object contamination events occurring in the business sectors, and the most reported hard objects were metals followed by plastics, animal derived materials, plant derived materials, and glasses. Approximately 90% of the injury related to the contamination events in the food business sectors were caused by the hard object contaminants.

Significance: These data enable us to recognize the situation of contamination by foreign materials in food in Japan, and to guide food business operators in preventing food contamination by foreign materials.

P1-273 Estimating the Burden of Foodborne Illness for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan from 2006 to 2016

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Introduction: In Japan, the numbers of food poisoning incidence and cases are reported mandatory; however, these do not exactly reflect the real burden of foodborne illnesses due to the passive surveillance nature. We have been estimating the real burden of foodborne diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan since 2006.

Purpose: Estimating the burden of foodborne illnesses associated with three pathogens in Japan from laboratory-confirmed numbers of infections.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories that test stool samples submitted from all over Japan or from Miyagi Prefecture, from January 2006 to December 2016. The physician consultation rate and the stool submission rate were estimated from telephone population surveys conducted for the whole of Japan and for Miyagi prefecture. We merged the telephone survey data conducted in 2016 with previous data. Each estimate was introduced into the Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *V. parahaemolyticus* in the whole of Japan were 5.5 to 13.6 million, 1.2 to 2.8 million, and 49 to 438 thousand from 2006 to 2016, respectively. Those estimated for the whole of Japan from data on Miyagi prefecture were 0.64 to 1.6 million, 78 to 190 thousand, and 7 to 63 thousand from 2006 to 2016, respectively. The numbers of reported foodborne illnesses per year in Japan from 2006 to 2016, for *Campylobacter*, *Salmonella* and *V. parahaemolyticus*, were 1,600 to 3,100, 440 to 3,600 and 50 to 1,300, respectively.

Significance: These data reveal a significant difference in numbers and trends between our estimates of the burden of foodborne illnesses and the reported foodborne disease cases associated with three pathogens. Need for continuing active surveillance system to complement the present passive surveillance is strongly suggested, in order to identify and prioritize food safety measures more precisely and to monitor the effectiveness of risk management options.

P1-274 Occupational and Food Safety Risks among Slaughterhouse Workers in Ilorin, North Central Nigeria

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Introduction: Basic food safety and occupational health perceptions are poor among slaughterhouse workers in Nigeria, and these are public health concerns.

Purpose: This study analyzed work-associated injuries and their association with food safety risks among slaughterhouse workers.

Methods: A cross-sectional survey involving random sampling of 203 workers from five slaughterhouses were assessed using a pretested structured questionnaire. Information on food safety risks was obtained using a numeric scoring system. Data were analyzed with descriptive statistics, and univariate and multivariate logistic regression at $P<0.05$.

Results: Respondents (age: 36.7±9.3 years) were mostly meat traders (106, 52.2%) and butchers (56, 27.6%) with 178 (87.7%) reporting work-associated-injuries affecting mainly workers' hands (65, 32%), though 34 (16.7%) reported injuries on more than three parts of their bodies. The majority of respondents reported injuries caused by hand equipment (145, 71.4%) rather than animals (57, 28.1%), floor (18, 8.8%), fire (10, 4.9%), vehicles (nine, 4.4%) and big machines (three, 1.5%). About one-fourth of respondents have poor knowledge of zoonosis and spread of meat-borne infections. No worker reported access to occupational health services, and training on hazards and safety at work. Scores on food safety risk ranged from zero to 10 with 177 (87.2%) of respondents receiving unsatisfactory scores (scores<mean+standard deviation). Use of personal protective equipment (PPE) ($P=0.00$) and sex ($P=0.013$) of workers were associated with food safety risks. Workers using PPE (OR=9.0; 95% CI: 3.5 to 22.9; $P<0.001$) were more likely to have better practices limiting food safety risks.

Significance: Current occupational health status and food safety risk perceptions are unsatisfactory among slaughterhouse workers. These findings provide empirical basis for the development of suitable policies and interventions to mitigate food safety risks in Nigerian slaughterhouses.

P1-275 Evaluation of HACCP Implementation in Food Manufacturing Companies in the Emirates of Dubai

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Introduction: The way that food is produced and distributed has undergone fundamental changes in recent decades particularly in Dubai and Middle Eastern region. The food safety area has become more complex, driven by widespread changes in methods of food production and processing, coupled with rapid increases in global food trade and increased tourism. Consumers today are demanding more meaningful information about food safety and quality. To meet this demand, some companies are engaging third-party audit bodies to provide greater assurance that their products meet quality and safety requirements.

Purpose: The purpose of the study was to evaluate the level of implementation and operation of hazard analysis critical control points (HACCP) and PRPs (Prerequisite Programme) as per the codex alementarious commission protocol of 12 logical steps and codex GHP(Good Hygiene Practices)

Methods: Both qualitative and quantitative analysis techniques of in-depth interviews, observations and review of documents were used in this study to complement each other The triangulation method used in this research was to look at the problems from different angles, Five cluster random samples were collected from the sampling frame of 112 food manufacturing companies of DM FCD(Dubai Municipality Food Control Department) list.

Results: Research identified lower compliance rates of Good Hygiene practices (PRPs) which compromise 37.4% for the sampled factories and 31.8% compliance rate for HACCP protocol logical step. Research also identified 50 manufacturing units out of 112 units are implemented and certified with HACCP system which compromise 44.6% also indicate lower plementation level.

Significance: Findings from this study is aimed to provide insights into a fairly new but evolving research area of HACCP Implementation in the food manufacturing sector in the Emirates. The outcomes of this study are expected to have national implications for the enhancement of food safety management system implementation through effective training and enforcement.

P1-276 Effects of Food Safety Training on Achieving Food Safety Knowledge and Practices in Restaurants in the Emirates of Dubai

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Introduction: Food safety, specifically in restaurants, is becoming a key public health priority because of the increased number of meals eaten outside the home. Foodborne illness prevention thus is a significant concern and a public health priority in the United Arab Emirates, particularly Dubai, because of the extensive tourism industry.

Purpose: The purpose of the study was to evaluate the effectiveness of using demonstrations in training sessions to improve food safety knowledge and practices amongst food handlers.

Methods: The population for this study was food handlers in commercial independent and chain restaurants licensed to sell food in Deira and Bur Dubai area. A control group and an intervention group were used to test the internal validity of the training effect. Six independent restaurants were invited to participate voluntarily as the control group. Which did not receive food safety training. Eight other independent restaurants also were invited as the intervention group and also did not attend any food safety training.

Results: On comparison of the pre-test scores between the intervention and the control group, the t-test analysis showed significant difference in the level of food safety knowledge between the two groups. Pretest score for the control group was 78.94 and post test score was 104.67. In the case of the intervention group, pretest score was 91.37 and post test score was 135.11. The scores of food handlers' food safety practice for control group, pretreatment score was 470 and post treatment score was 646. For intervention group pretest score was 723 and pot test score was 1056. The study concluded that training with demonstration techniques is an effective way of improving compliance with food safety guidelines

Significance: Findings from this study aim to provide insights into a fairly new but evolving research area in the retail foodservice sector. The outcomes of this study are expected to have national and international implications for the enhancement.

P1-277 Occurrence of *Campylobacter jejuni and Campylobacter coli* in Chilled Poultry Carcasses in Algeria

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Introduction: *Campylobacter* is considered worldwide as a major cause of gastroenteritis in humans. Among foodstuffs, poultry meat, notably broiler chickens and turkey, are considered to be the main vehicles of transmission of thermophilic *Campylobacter* to humans.

Purpose: This study aimed to detect thermophilic *Campylobacter* species in chilled poultry carcasses.

Methods: During 2015, 115 samples of poultry neck skin were collected from five slaughterhouses located in the area of Algiers (Algeria). Detection and identification of thermophilic *Campylobacter* were done according to the OIE:2005 and ISO 10272:2006 protocols. To detect *Campylobacter* species, a multiplex PCR assay was performed using specific primers.

Results: Of the 115 tested samples, 17 thermophilic *Campylobacter* strains were isolated, which represents an overall prevalence of 14.78%. *C. coli* comprised 70.59% of isolates and *C. jejuni*, 29.41%.

Significance: The poultry industry could be the cause of a major public health problem in Algeria through the spread of pathogenic strains of *Campylobacter*.

P1-278 Transcriptomic Analysis of Botulinum Neurotoxin Expression in *Clostridium botulinum* Strain 62A in Culture Media Using RNA Sequencing

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Introduction: *Clostridium botulinum* produces the potent toxin botulinum neurotoxin (BoNT). Despite the extreme potency of BoNTs the mechanisms underlying their genetic regulation are unknown.

Purpose: The objective of this study was to perform a transcriptomic analysis of strain 62A grown in two media, toxin production medium (TPM) and trypticase-peptone-glucose-yeast extract broth (TPGY) using RNA sequencing to elucidate genes involved in BoNT gene expression.

Methods: *C. botulinum* strain 62A was inoculated separately into 1.5 L of TPGY and TPM, pH 7.0 and incubated anaerobically at 37°C. RNA was extracted from samples removed during the log, late log/early stationary, stationary and lytic growth phases, sequencing libraries were prepared using the TruSeq

mRNA stranded RNA sequencing library kit and sequenced on an Illumina MiSeq. Samples removed at 24, 48, 72 and 96 hours of growth were analyzed for toxin production using DIG-ELISA and Western blot analysis.

Results: Although higher cell density trends were observed when 62A was grown in TPGY (peak absorbance at OD_{600nm} of 4.672±0.93, 11 h) than in TPM (peak absorbance at OD_{600nm} of 3.54±0.59 at 17 h), Western blot and DIG-ELISA analysis showed higher quantities of BoNT/A1 produced in TPM. The transcriptional activator gene, *botR,* was not differentially expressed (log fold change (logFC) = -3.96 to 0.86; false discovery rate (FDR>1.0) throughout growth in two experiments. In one experiment *bont/A1* was not differentially expressed, however in a second experiment the toxin gene was down regulated in the log (logFC =-0.51; FDR <0.05) and early stationary phase (logFC=-1.01; FDR = <0.05).

Significance: Although BoNT/A1 production was greater when *C. botulinum* strain 62A was grown in TPM, *botR*, *bont/A1* and genes within the toxin gene cluster were not upregulated during growth. Understanding the regulation of BoNTs with respect to nutrient availability is paramount to their control in foods.

P1-279 Fate of *Listeria monocytogenes* in Frozen Strawberries

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Introduction: Recently, there have been multiple outbreaks of *Listeria monocytogenes* in frozen produce. These outbreaks are shining the light on a potential major concern to human health. Many consumers use frozen produce as a ready-to-eat food to make smoothies. We therefore must determine the fate of *L. monocytogenes* in frozen products.

Purpose: The purpose of this study was to determine the fate of *L. monocytogenes* in frozen strawberries and to evaluate the best enumeration method for this matrix and environmental condition combination.

Methods: Samples of fresh, whole, hulled strawberries were contaminated with different levels of *L. monocytogenes* and then aged for 48 to 72 hours at 4°C. Half of the samples were placed in -30°C for four to six days after aging, while the remaining were analyzed via direct plating and MPN. The frozen samples were then thawed and analyzed in the same manner as the fresh samples.

Results: Overall, there was a significant reduction in *L. monocytogenes* concentration after aging freezing, but some growth was still present. The enumeration method that showed to be more sensitive in this scenario was MPN.

Significance: Our study provides evidence that we must be concerned about *L. monocytogenes* in not only fresh but also frozen produce.

P1-280 Effect of Growth Conditions on Desiccation Tolerance in *Salmonella enterica, Escherichia coli*, and *Listeria monocytogenes*

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Introduction: Growth on solid media as sessile cells has been shown to increase the desiccation tolerance of *Salmonella*. However, the same effect has not been explored for *Escherichia coli* or *Listeria monocytogenes*.

Purpose: The purpose of this project was to determine the effect of sessile versus planktonic growth on the desiccation resistance of *Salmonella*, *E. coli*, and *L. monocytogenes*.

Methods: *Salmonella enterica* (*Salmonella* Agona, Orienburg, and Tennessee), *Escherichia coli* (0157 and 0121), and *L. monocytogenes* (Scott A and 004) were cultured in trypticase soy broth with 0.6% yeast extract (TSBYE), with and without shaking to obtain different levels of oxygenation, and on TSBYE with agar to provide the same media as solid form. Cultures were harvested after incubation at 37°C at the stationary growth phase. A 100-µL aliquot was pipetted onto sterile cellulose filter disks, dried in a biosafety cabinet, and stored in a chamber at 25°C and 30% relative humidity. Disks were enumerated at 24 and 48 h on trypticase soy agar with 0.6% yeast extract. Plates were incubated at 37°C for 24 h for *Salmonella* and *E. coli* samples or 48 h for *Listeria* samples.

Results: The agar grown cultures of the three *Salmonella* serotypes and *E. coli* 0157 were found to be significantly more desiccation resistant (P<0.05) than both liquid grown cultures. Agar grown cells of *E. coli* 0121 were observed to be significantly more resistant (P<0.05) than anaerobic liquid grown cells, while the aerobically grown cells were not significantly different (P>0.05) from either agar or anaerobically grown cells. For *Listeria* serotypes tested, no significant differences (P>0.05) were found between the three culturing methods.

Significance: The results suggest that cell culturing method can significantly affect survival during desiccation, particularly for *E. coli* and *Salmonella*. Growth method should be considered when testing desiccation resistance or survival in food systems.

P1-281 Applications of DNA Sequencing in Food Microbiology: Looking Beyond Outbreak Investigations

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Introduction: Whole genome DNA sequencing is a rapidly developing technology in the field of food microbiology. DNA sequencing can quickly identify all organisms in a sample in one assay, and potentially give a rough estimate of the relative quantity of organisms present. While most are aware of how DNA sequencing can be used to track the origins of an outbreak, DNA sequencing has multiple other beneficial applications.

Purpose: We demonstrate how DNA sequencing can be an important tool in quality control, presumptive confirmations, and environmental monitoring.

Methods: We sequenced four samples, including isolated colonies and probiotic preparations, using Oxford Nanopore's MinION device and the Rapid Sequencing Kit. Data analysis was performed using the EPI2ME (Metrichor) Fastq What's In My Pot (WIMP) workflow, which uses the NCBI database, to identify organisms present in each sample. Samples were sequenced between one and six hours, with live basecalling performed by the MinKNOW.

Results: We confirmed the presence of *Salmonella* at 1,000 CFU/ml in about one h, and could clearly identify 10 of 14 species claimed in a probiotic mix. The remaining four species were present at very low levels (<10/418,875 reads). Furthermore, four species not listed as an ingredient in the probiotic mix were also identified. DNA sequencing can also be used to track recurring contamination in a food processing plant, as organism recovery can be identified as a new or previously observed organism. Importantly, species or strain identification is only as good as the database used, as we were unable to reliably distinguish between *Bacillus cereus* and *B. thuringiensis*, which are commonly misclassified.

Significance: DNA sequencing is an important tool to identify all organisms in a sample in one assay. Sequencing is especially useful when strain-level identification is important, such as in quality control of probiotic products, environmental contamination tracking, or pathogen confirmation.

P2-01 Polyphenolic Compounds Kill *Escherichia coli* or Affect Growth, Swarming Motility and Virulence Gene Expression at Sublethal Concentrations

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Introduction: Some pathotypes of *E. coli* are a common cause of diarrhea. Current trends promote the use of natural compounds to control bacteria; therefore, we studied phenolic compounds as antimicrobials to improve the safety of foods and reduce these diseases.

Purpose: To evaluate the effect of polyphenolic compounds (tannic acid (AT), gallic acid (GA), methyl gallate (MG), and epigallocatechin gallate (EGCG)) on the growth, swarming motility, biofilm formation and expression of virulence genes of three *E. coli* pathotypes (EPEC, EHEC, and ETEC).

Methods: Minimum bactericidal concentrations (MBC) were determined using microtiter plates. Swarming motility was determined in LB agar with sublethal concentrations of compounds. Biofilm formation was determined in microtiter plates stained with crystal violet. The expression of genes involved in biofilm (*flhC*, *fliA*, *fliC* and *csgA*) and those in swarming motility (*csgD* and *cyaA*) were evaluated by qPCR.

Results: All compounds affected bacterial survival of all strains; MBC ranged from 1.78 to 2.49, 2.09 to 2.17, 0.07 to 0.59 and 0.11 to 0.59 mg/ml for TA, GA, MG, and EGCG, respectively. The EPEC strain was the most susceptible, followed by EHEC and ETEC. Also, all compounds reduced swarming motility (14.8 to 100%), TA being the most effective. GA reduced biofilm formation in all the strains analyzed; however, various concentrations of TA, MG and EGCG induced biofilm formation in some strains. Significant differences (P≤0.05) in the expression of genes were observed in all the strains. In general, TA overexpressed *csgA*, *csgD* and *cyaA* genes, while most of the other compounds had no effect or reduced their expression.

Significance: The polyphenolic compounds analyzed show great potential to control these pathogenic *E. coli* strains by killing the bacteria or affecting growth, swarming motility and virulence gene expression at sub-lethal concentrations; however, proper concentrations need to be applied to avoid induction of undesirable virulence factors.

P2-02 Differential Antimicrobial Activity of Thymol and Oregano Oil against *Listeria monocytogenes* Strains

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Introduction: The foodborne pathogen *Listeria monocytogenes* is a challenge due to its extraordinary ability to grow at low temperature and to form biofilms contaminating the environment in food production plants.

Purpose: The main objective of this study was to evaluate the efficacy of thymol and oregano oils as antibiofilm agents on *L. monocytogenes* strains isolated from a variety of foods.

Methods: Biofilm capability of *L. monocytogenes* food isolates were assayed In Vitro by crystal-violet staining. MICs of thymol and oregano oils against planktonic forms and biofilm inhibitory activities were also calculated.

Results: Food isolates of *L. monocytogenes* revealed different strengths in biofilm-forming capability, presenting weak (0.148 to 0.348); moderate (0.370 to 0.542); and strong (0.553 to 0.888) capability expressed as OD values. The thymol showed anti-biofilm activity against 24 h preformed biofilms at the same MIC values as planktonic forms (250 to 400 µg/ml). In contrast, biofilm formation was inhibited at concentrations less than the MIC (200, 100, 75 and 50 µg/ml). The strongest biofilm-producing *L. monocytogenes* strain was grown on a stainless steel surface and biofilm inhibition was observed with thymol at concentrations less than the MIC (100 µg/ml).

Significance: The two different natural compounds act in a different way against *L. monocytogenes* strains. The anti-adhesion property of thymol could play an important role in the food industry sanitation.

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P2-03 Antimicrobial Activity of *Rosemary officinalis* Leaves against Foodborne Pathogens and Application as a Natural Disinfectant on Food Contact Surfaces

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Introduction: Bacterial attachment on food contact surfaces represents a potential source of cross-contamination for food products. Contamination on food contact surfaces can be reduced by cleaning and disinfection. Negative consumer perception of chemical disinfectants has shifted the attention to natural substances, such as plant extracts.

Purpose: The aim of this study was to investigate the possibility of using *Rosemary officinalis* leaves in the removal of attached foodborne pathogens on food contact surfaces.

Methods: Five pathogens were used in the study: *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella* Enteritidis and *Listeria monocytogenes.* Each pathogen was separately inoculated on a cutting board, knife, plastic basket, stainless steel and polypropylene. Contaminated food contact surfaces waere immersed in *R. officinalis* L.-based disinfectant for five min. The viable bacterial counts were examined using 3M Petrifilm aerobic count plate and determined as log CFU/area.

Results: *R. officinalis* L. extracts showed the strongest antibacterial properties, with 0.2 mg/ml against *B. cereus*, followed by *S. aureus* and *E. coli* with 0.3 mg/ml, *Salmonella* Enteritidis with 0.6 mg/ml and *L. monocytogenes* with 1.3 mg/ml. The treatment of *R. officinalis* L.-based disinfectant at 1.25% concentration showed bactericidal efficacy in both Gram-positive and Gram-negative bacteria on the tested food contact surfaces. The *R. officinalis* L.-based disinfectant showed greater than four-log CFU/area reduction against *S. aureus, E. coli, L. monocytogenes* and *Salmonella* Enteritidis within 30 min, and all pathogesn decreased to less than one log CFU/area within two h.

Significance: The findings of this research suggest the potential use of *R. officinalis* L as an alternative sanitizer or in support of the disinfection of contaminated surfaces.

P2-04 Dried Spices and Their Role in Final Product Quality – A Case Study

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Introduction: A food manufacturer experienced severe bloating in their ranch dressing and wanted to identify the contaminant source. Fresh ingredients are a usual suspect with high bacterial levels; however, dried spices are often overlooked due to their low inclusion rate.

Purpose: Enumerate microbial levels in dried spices and dressing, identify spoilage organisms causing bloating, and compare dried spices to irradiated alternatives.

Methods: A 22-g sample of dressing and 12 spices were enumerated, in duplicate, for aerobic plate count (APC), lactic acid bacteria (LAB), yeast/mold (Y/M), and spore-forming bacteria (SFB). The dressing was sampled weekly until gas formation was observed. Irradiated spices were obtained and the new dressing was prepared and analyzed as above. Colonies of gas-producing bacteria were collected and sequenced for 16S PCR identification.

Results: The initial dressing had 2×10^3 CFU/g APC and <100 CFU/g LAB and Y/M. After six weeks, the dressing had the formation of large gas pockets in the LAB agar. Colonies were identified as *Clostridium beijerinckii,* known producers of hydrogen gas and carbon dioxide. Minced onion, onion powder, and garlic powder had the highest microbial levels (>5×10^4 CFU/g APC, >2×10^3 CFU/g LAB, and 1×10^2 to 1×10^4 CFU/g SFB) and similar gas pockets were seen in LAB and SFB agar plates. From each spice, 16 colonies were collected from SFB agar, and *C. beijerinckii* was identified in 43.8% of minced onion and 6.3% of onion powder isolates. Irradiated samples of these three spices had greater than one-log reduction in APC, LAB, and SFB and the dressing produced with irradiated spices had a one-log reduction in APC and LAB, and no gas-producing bacteria were found.

Significance: While dried spices are used at a low rate in prepared foods, the quality of those spices can influence product spoilage, and source tracking bacterial contaminants can help manufacturers increase product integrity.

P2-05 Identification of Nonpathogenic Surrogate Bacteria Applicable for Industrial-Scale Gaseous Chlorine Dioxide Treatment on Baby Carrots

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Introduction: Vegetables are highly associated with outbreaks since they are often consumed raw or minimally processed. Gaseous chlorine dioxide (ClO_2) is an effective method to control foodborne pathogens in fresh produce. Industrial-scale gaseous ClO_2 treatment may help fresh produce industry to decontaminate produce and enhance safety.

Purpose: The purpose of this study was to investigate inactivation kinetics of *Escherichia coli* and potential nonpathogenic surrogate bacteria in order to support future development and validation of commercial gaseous ClO_2 decontamination in carrot manufacturing plants.

Methods: Two separate cocktails of five pathogenic (O157:H7, O26:H11, O45:H2, and O103:H11) and nonpathogenic *E. coli* strains (K-12, nontoxigenic O157:H7, O45:H10 and O6) were inoculated onto 200 grams of baby carrots. Gaseous ClO_2 was generated inside a treatment chamber by mixing equal amounts of sodium chlorite and activating acids in a sachet. Both sample groups were treated simultaneously at different exposure times. The initial treatment doses were 0.01, 0.03 and 0.06 mg ClO_2/g carrots and the total exposure times were 0.5, one, two, and three h. Temperature (25±5°C) and relative humidity (90±8%) were measured inside of the chamber.

Results: After a one-hour treatment, both pathogenic and nonpathogenic *E. coli* had similar reductions of four log, 4.5 log and five log CFU/g at ClO_2 initial doses of 0.01, 0.03 and 0.06 mg ClO_2/g carrots, respectively. Linear trends were observed between initial doses and log reductions. Log reduction was not significantly (P<0.05) different between pathogenic and nonpathogenic strains for all treatments (n=2).

Significance: This is the first study that analyzes the kinetics of gaseous ClO_2 inactivation against both surrogates and pathogens on baby carrots. This study identifies a nonpathogenic surrogate cocktail with equivalent resistance to gaseous ClO_2, suitable for representing pathogenic *E. coli* in future industrial experiments.

P2-06 Phenolic Extracts of Chokeberry Pomace Have Inhibitory Effects on *E. coli* O157:H7 But Not on Probiotic Bacteria and Normal Bacterial Flora

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❖ ***Developing Scientist Entrant***

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is still a major foodborne bacterial infectious agent in the United States and causes both diarrheal disease and hemolytic uremic syndrome (HUS). As antibiotic therapy is not an option, development of appropriate antimicrobial or improvement the host gut health to prevent the colonization of EHEC in the intestine is essential to control the infections with this pathogen, specifically Shiga toxin-producing strains. In this study, we aim to develop an appropriate natural antimicrobial and stimulate host defense to control foodborne illnesses with EHEC.

Purpose: Develop an appropriate natural antimicrobial and stimulate host defense to control foodborne illnesses with EHEC.

Methods: We investigated the role of chokeberry (*Aronia melanocarpa*) pomace (byproducts) extract (CPE) on growth and survival of EHEC EDL-933, probiotic *Lactobacillus*, and common gut bacterial flora, including *Streptococcus thermophilus*, *Enterococcus faecalis* and *Bacillus subtilis*. We also tested the effects of CPE on physicochemical properties, host cell-EHEC interactions, and expression of various virulence genes (*fliC, eaeA, tir, ler, invH, espD*) of EHEC. All the experiments were carried out in replicates.

Results: Antimicrobial activity of CPE against EHEC EDL-933 was evaluated in LB broth culture by the plate count method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CPE were found to be 0.5 mg/ml and 0.6 mg/ml, respectively. The growth of EHEC EDL-933 was reduced by four log (P<0.05) in the presence of 0.5 mg/ml at five h while this concentration promoted the growth of *Lactobacillus* and common gut bacterial flora *S. thermophilus, E. faecalis* and *B. subtilis*. The abilities of EHEC to attach and invade host intestinal epithelial (INT-407) cells were also reduced significantly (P<0.05) which was supported by altered physicochemical properties and virulence genes expression of EHEC.

Significance: These results suggest that CPE has the potential to be an effective therapeutic/preventive for EHEC EDL-933 without affecting the common gut microflora and probiotics.

P2-07 Assessing Bacterial Viability by Monitoring Adenine Nucleotides and Adenylate Charge in Response to Biocide Treatment

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Introduction: Current plate counting and fluorescent based assays detect only viable and proliferating but not viable/nonculturable (VBNC) bacteria. Thus, there is a need for simple and accurate strategies to monitor VBNC, especially when they become antibiotic and biocide tolerant

Purpose: Develop a novel technology to assess the viability of bacteria under a variety of biocide treatments.

Methods: Taking advantage of highly stable and genetically engineered luciferase we embarked on the development of a highly sensitive method to monitor the changes in AMP, ADP, and ATP in bacteria under normal and stressful environmental and biocide treatments. The method monitors the changes in concentrations of ATP using a luciferin/luciferase reaction and AMP and ADP by converting them into ATP and indirectly assesses the concentration of these nucleotides. Bacteria were plated on LB medium and colonies were counted before and after treatment to calculate the log kill effect of the treatment. The ratio of AMP/ADP and ADP/ATP were also calculated and plotted against different concentrations and treatment time with biocides

Results: Adenine nucleotides provide a valuable tool for assessing the effect of biocide treatments such as chlorine (0.5 and 1.5 ppm), chloramine T (two ppm), peroxyacetic acid (one and 2.5 ppm) and glutaraldehyde (0.1%) in a time dependent manner up to 20 min. We also tested these biocides for a period of up to 120 min to determine the log kill for each biocide concentration. These studies were carried out three times for each biocide using gram positive (*B subtilis, Staphylococcus aureus*) and gram negative (*E. coli, Pseudomonas aeruginosa*) bacteria. We observed a significant increase by several-fold in AMP and ADP (up to 20-fold) when compared with ATP which declined significantly upon treatment accompanied by increased log kill. Other methods such as the fluorescent method failed to correlate with viability in some biocide treatments where ambiguous results were observed making it difficult to assess treatment efficacy

Significance: The use of the adenylate ratio (AMP/ATP and ADP/ATP, and ATP) can be used to predict the effect of biocides and stressful conditions on growth even if organisms do not form CFU on plates

P2-08 Fermentation Optimization to Maximize Production of ScarcelY Produced Paenibacillin by *Paenibacillus polymyxa* OSY-EC

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❖ ***Developing Scientist Entrant***

Introduction: Paenibacillin, a novel antimicrobial peptide, has temperature and pH stability as well as strong activity against *Clostridium, Listeria, Staphylococcus, Streptococcus* and others. Further studies on paenibacillin (and similar peptides) are ordinarily limited due to the low productivity of the producing organisms. Food application tests, for example, require large amounts of purified antimicrobial compounds.

Purpose: The objective of this research was to improve paenibacillin yield during fermentation and downstream processes. This was completed through fermentation optimization by response surface methodology (RSM) and by developing a method suitable for large-scale purification of paenibacillin.

Methods: For initial production optimization, a two-level partial factorial screening of selected factors (medium, temperature, air flow, fermentation time and stir speed) was completed. The factors with significant effects were then varied together to follow the path of steepest ascent to maximize paenibacillin yield. Once the local maximum was found, RSM was completed with a central composite design (CCD) to fine-tune the fermentation parameters. The antimicrobial generated was purified by adsorption on XAD resin, separation by fast protein liquid chromatography and precipitation using trichloroacetic acid.

Results: The initial screening determined the temperature and air flow to have significant effects (P<0.05). All other factors were set to the lower value for cost efficiency. After initial screening, paenibacillin yield was 32 µg/ml of fermentate. The step size calculated was 3°C and -1 LPM. The antimicrobial yield was found to be highest within the original screening parameters. Through CCD, the fermentation conditions will be further optimized. The resulting paenibacillin was separated from the coproduced and structurally-similar antimicrobials (e.g., polymyxin) using the new purification method.

Significance: This optimization established a procedure for paenibacillin production and allowed further food application studies. The research proved the feasibility of commercial production of this novel antimicrobial peptide for protection of the food supply against pathogens and spoilage bacteria.

P2-09 Synergistic Antimicrobial Effects of Metal Oxide Nanoparticles and Ajoene against *Campylobacter jejuni*

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Introduction: *Campylobacter* is ubiquitous throughout the poultry production chain, and it is one of the most common causes of human diarrheal disease worldwide. The indiscriminate use of antibiotics for food-producing animals increases the prevalence of multidrug-resistant *Campylobacter* isolates, which becomes an enormous challenge to both the agrifood system and public health. It is, therefore, necessary to develop alternative intervention strategies to reduce foodborne illnesses and prevent the emergence of antibiotic-resistant *Campylobacter*.

Purpose: This study evaluated the synergetic effect of ajoene and metal oxide nanoparticles, namely aluminum oxide (Al_2O_3) and titanium dioxide (TiO_2) nanoparticles, against *Campylobacter jejuni*.

Methods: A cocktail of *C. jejuni* (ATCC 33560, F38011, y110539, z110526) with a concentration of 10^8 CFU/ml was used for antimicrobial tests. The bacterial cocktail was treated at both 22 and 37°C in a microaerobic condition with ajoene at 0.06, 0.125, 0.25, 0.5 and one mM, the nanoparticles at 0.5, one, two, four, eight, and 16 mM, and combinations of 0.06 mM ajoene and nanoparticles at different concentrations. *C. jejuni* cells were separately enumerated at zero, two, four, seven, 10, and 24 h by plating assay.

Results: For individual treatment after 24 h, TiO_2 nanoparticles at 16 mM could achieve eight-log reduction at 22°C; ajoene ≥0.25 mM, TiO_2 nanoparticles greater than or equal to four mM, and Al_2O_3 at 16 mM could completely inactive *C. jejuni* at 37°C. In comparison, the synergistic antimicrobial effect of ajoene and nanoparticles could achieve ≥8-log CFU/ml reduction at both 22 and 37°C after 24 h treatment with the concentration of nanoparticles ≥0.5 mM.

Significance: Using ajoene and metal oxide nanoparticles in a combinatorial manner can effectively inactive *C. jejuni*. This synergistic antimicrobial treatment could potentially serve as a novel intervention strategy to reduce *Campylobacter* in the agro-ecosystem.

P2-10 Evaluation of Bactericidal Effects of Phenyllactic Acid on Shiga Toxin-producing *E. coli* in Beef Products

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Introduction: Shiga toxin-producing *E. coli* and *Salmonella* have emerged in the last 10 years as important foodborne pathogens. New methods are needed for its control in meat products.

Purpose: Bactericidal effects of phenyllactic acid at various concentrations on *E. coli* O157:H7 and *Salmonella* Typhimurium in pure culture, on micro-plates and in the beef meat were studied.

Methods: Two contamination methods, including surface spray and spot inoculation on beef meat, were compared. Surface spray treatment of phenyllactic acid or lactic acid for inactivation of *E. coli* O157:H7 or *Salmonella* Typhimurium on beef meat were conducted.

Results: Results demonstrated that 1.5% phenyllactic acid inactivated all inoculated *E. coli* O157:H7 within one min contact time (greater than six-log CFU/ml reduction) at 21°C; whereas 1.5% lactic acid did not show any reduction. Microbio-plate assay by inoculated ten to 100 CFU/well of *E. coli* O157:H7 indicated that a concentration of 0.25% phenyllactic acid or 0.25% lactic acid inhibited the growth of inoculated *E. coli* O157:H7 after incubated at 37°C for 24 h, whereas the growth of *E. coli* O157:H7 reached >10^8 CFU/well in the negative control. The treatment of 1.5% phenyllactic acid or 1.5% lactic acid on beef meat reduced the counts of *E. coli* O157:H7 for 0.6 and 0.3 log CFU/cm^2 within five min, and reduced *Salmonella* Typhimurium DT 104 count for 0.9 and 0.1 log CFU/cm^2; respectively. The further frozen processing of 1.5% phenyllactic acid treated meat at -20°C could inactivate either *E. coli* O157 or *Salmonella* Typhimurium DT 104 by 1.1 and 1.5 log CFU/cm^2; respectively.

Significance: Results demonstrated that treatment of phenyllactic acid at 1.5% could significantly reduce the population of *E. coli* O157:H7 and *Salmonella* in beef meat.

P2-11 Synergistic Antimicrobial Activities of Gaseous Essential Oils against *Bacillus cereus* Vegetative Cells and Spores on a Laboratory Medium

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❖ ***Developing Scientist Entrant***

Introduction: Gaseous essential oils (EO gases) have recently received attention as natural antimicrobial agents, but the antimicrobial activities of individual EO gas and their combinations against *Bacillus cereus* (vegetative cells and spores) have not been intensively studied.

Purpose: This study was done to measure the minimum inhibitory concentrations (MICs) of EO gases and to determine the combinations of EO gases causing synergistic antimicrobial activities against *B. cereus* on a laboratory medium.

Methods: Among 97 commercially available EO gases, EO gases with relatively strong inhibitory activities against *B. cereus* cells were screened by vapor diffusion assay. Next, the MICs screened EO gases against vegetative cells and spores of *B. cereus* on nutrient agar were measured. Finally, the combinations of two EO gases showing synergistic effects against *B. cereus* cells were determined using a modified checkerboard assay.

Results: Five types of EO gases (citronella, lemongrass, may chang, oregano, and thyme thymol) showed relatively strong antimicrobial activities against *B. cereus* cells. The MICs of screened EO gases against *B. cereus* were 0.0391 to 0.0781 µl/ml. The combination of oregano and thyme thymol EO gases showed synergistic antimicrobial effects (fractional inhibitory concentration (FICI)=0.5000) against *B. cereus* cells. The combinations of citronella and lemongrass, citronella and may chang, lemongrass and oregano, may chang and oregano, may chang and thyme thymol EO gases showed partial synergism (FICI=0.6250) against *B. cereus* spores on nutrient agar.

Significance: This is the first study which measured the MICs of EO gases and determined the combinations of EO gases with synergistic antimicrobial activities against *B. cereus*. These results may provide useful information in developing antimicrobial packaging system using EO gases.

P2-12 WITHDRAWN

P2-13 Assessing the Efficacy of Sodium Bisulfate in Tempering Water to Control Shiga Toxin-producing *Escherichia coli* in Wheat

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❖ ***Developing Scientist Entrant***

Introduction: Wheat as a raw commodity is produced in soil and could be readily contaminated by pathogenic bacteria. Shiga toxin-producing *Escherichia coli* (STEC) strains (O121, O26) associated with recent disease outbreaks in the United States, have been traced back to contaminated wheat. Antimicrobial intervention steps in wheat tempering during flour production could potentially control pathogen contamination.

Purpose: To evaluate the efficacy of sodium bisulfate (SBS) as an antimicrobial agent in tempering water to control STEC in wheat.

Methods: The minimum inhibitory concentration (MIC) of SBS required to inhibit the growth of *Escherichia coli* ATCC 1427 was determined using tryptic soy broth micro-dilution method as follows: *E. coli* were treated with various concentrations of SBS ranging from 0.009% to 20% w/v. The lowest concentration inhibiting visible growth of bacteria after 24 h of incubation at 37°C was considered as the MIC. Wheat inoculated with *E. coli* (~10^6) was tempered to 16% moisture. Tempering water treatments included 0.125, 0.25, 0.5, 1, 2, 5, 10, 15 or 20% SBS, 200 ppm chlorine, a positive and a negative control. The *E. coli* counts were enumerated using tryptic soy agar after incubation of the treated wheat for one h at 37°C. Logarithmic reductions of the bacterial counts were calculated and the treatment means were compared using Tukey's test.

Results: The MIC of SBS was determined to be 0.25%. Wheat tempered with 20% SBS solution (final concentration 0.62% w/v) resulted in the highest reduction (P<0.05) of 2.8 log CFU/gram in *E. coli* counts, while 200 ppm chlorine resulted in reduction of 0.13 log CFU/g.

Significance: The data suggests that SBS solution at concentrations greater than 15% may be used as an antimicrobial agent in tempering of wheat to control STEC, with an advantage over the commonly used chlorinated water.

Poster

P2-14 Screening Cultures for Nitrate Reduction and Their Use in the Fermentation of Vegetable Extracts to Generate 'Natural Nitrite', a Clean Label Ingredient

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❖ ***Developing Scientist Entrant***

Introduction: Sodium nitrite is a regulated food additive that is added as 'cure' in many processed meats and must be listed as an ingredient. Commercial interest in nitrites was piqued by a USDA-FSIS position whereby vegetable-based nitrites (vegetable nitrate fermented to nitrite) were considered 'natural nitrite' and not subject to being listed as a preservative. As such, a 'no preservatives added' declaration is allowed, but the product cannot be called 'cured'.

Purpose: The purpose of our study was to screen cultures in our collections, or those occurring naturally, that can reduce vegetable nitrate to nitrite efficiently.

Methods: The nitrate reduction assay using nitrate broth was used as a qualitative method for detection of bacterial reduction of nitrate. For lactic acid bacteria, M17 broth containing 0.1% potassium nitrate (KNO_3) was used as a screening medium for culture isolates. Reagent A (sulfanilic acid) and Reagent B (alpha-napthylamine) were used for red color confirmation of nitrite while zinc dust was used to detect nitrate (red with zinc) if nitrite was negative; absence of color by either method indicates nitrate was reduced beyond nitrite.

Results: Out of 500 cultures screened for nitrate reduction, seven isolates were found to reduce nitrate to nitrite. *Propionibacterium jensenii P-42, Propionibacterium acidopropionici P-5, Bifidobacterium breve T11, Lactobacillus salivarius PIG 3-1, Lactobacillus salivarius PIG1-2, Lactobacillus salivarus PIG1-3, Lactobacillus acidophilus ML811* were able to produce nitrate reductase enzyme which reduces nitrate into nitrite. Multiple bacterial isolates were able to ferment vegetable extracts from celery, cabbage, and lettuce to nitrite reduction using above test as confirmation.

Significance: Nitrite is a crucial agent in the preservation of processed meat products. Vegetable sourced nitrite is considered 'natural nitrite' and may provide a 'green/clean label' tag in processed meats. New sources of nitrate reducing organisms may provide more efficient methods of generating nitrite.

P2-15 *Listeria monocytogenes* Control in Cold Smoked Salmon Using Natural and Sodium-free Preservatives

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Introduction: In response to WHO recommendations salt is being reduced in food products, which results in a challenge to the control of *L. monocytogenes* growth in cold smoked salmon (CSS). Acetic acid/acetates are well known for their inhibitory activity against *Listeria*. Natural alternatives based on vinegar have been developed in order to have a natural ingredient to increase the safety of low-sodium CSS.

Purpose: To compare the inhibitory properties of a chemical and a natural sodium-free preservative on the growth of *Listeria* in CSS.

Methods: A Danish CSS processor in collaboration with DTU Food used a combination of salting with injection brining followed by treatments on the surface of filets in order to have an optimal distribution of additives. The main product characteristics of CSS were three percent water-phase NaCl and pH of 6.1 to 6.3 (control). Other treatments included 60% solution of lactate/diacetate (2.5%); 0.25% solid acetate/diacetate Provian K (0.5% and 0.75%); and solid vinegar Provian NDV (0.3%, 0.6%, 0.9%). For each treatment, sliced products were inoculated with approximately two log CFU/g of an *L. monocytogenes* cocktail. Inoculated slices were vacuum packaged and stored for 50 days at 4°C and 7°C. At each time of sampling during storage *L. monocytogenes* were enumerated in triplicate by surface plating on PALCAM agar.

Results: The growth of *L. monocytogenes* to seven to eight log CFU/g was observed during 50 days on the CSS control treatment at both 4°C and 7°C, with an increase of one log CFU/g reached after one week for this treatment. All other treatments showed reduced growth of *L. monocytogenes.* Provian K (0.75% w/w) and Provian NDV (0.9% w/w) both reduced growth to less than a two-log CFU/g increase during 50 days at 4°C and 7°C.

Significance: This research demonstrated the possibility to increase the safety of cold smoked salmon using preservatives that meet current food trends, like sodium reduction and natural origin.

P2-16 Antimicrobial Activity of Different Cabbages Based on In Vitro and In Silico Methods

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Introduction: *Brassica* vegetables are rich in phenolic compounds that have a wide range of biological functions, including antioxidant and antimicrobial activities.

Purpose: The objective of this study was to evaluate the possibility of using polyphenolic extract from the stem and leaves of different cabbages: *Brassica oleracea* var. *capitata* f, *rubra,* commonly known as red cabbage (RC), and *Brassica oleracea* var. *capitata* f, *albal,* commonly known as white cabbage (WC), as natural antimicrobial agents in meat products.

Methods: In Vitro antimicrobial potential of the chloroform, dichloromethane, ethyl ether, toluene, methanol, ethanol and aqueous extracts of RC and WC were evaluated by using seven pathogenic bacteria and four fungi of importance in human health and vegetable spoilage. The antimicrobial activities of WC and RC were evaluated using different solvents by the disk diffusion method. In addition, WC and RC were evaluated with GC-MS and molecular docking methods to confirm the compound responsible for antimicrobial activity of the extracts. Minimum inhibitory concentration and minimum bactericidal concentration were determined against foodborne pathogens using the disk diffusion method and growth inhibition assay.

Results: All extracts were found to significantly inhibit (P<0.05) the activity against all microorganisms examined. However, chloroform extract shows the highest activity among all extracts with the concentration of 33 mg.mL^{-1}. Qualitative investigation on structure elucidation of bioactive compounds using GC-MS analysis revealed the presence of eight compounds responsible for the antimicrobial activity of the cabbage. WC and RC were found to be potential candidate for the development of novel antimicrobial agents with low cost and fewer side effects. In addition, antimicrobial components in crude extracts were thermoresistant and the highest activity was observed in chloroform extract of WC.

Significance: Our results showed that WC and RC extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning and may find a place as antimicrobial agents applied in the food industry as constituents of chemical preservatives.

Poster

P2-17 Physiological Damages Caused to Cells of *Salmonella* Enteritidis PT4 by Continuous Exposure to Mint (*Mentha piperita L.*) Essential Oil

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Introduction: The *Mentha piperita* L. essential oil (MPEO) is a "green" antimicrobial. The activity of MPEO against *Salmonella* has been primarily associated with changes to cell membrane permeability, which may cause leakage of vital intracellular components. Little is known about the effects of continuous exposure of *Salmonella* cells to MPEO.

Purpose: This study evaluates the effects of continuous exposure to MPEO on the physiology of a strain of *Salmonella* Enteritidis PT4 *isolated* from chicken meat associated with foodborne disease outbreaks.

Methods: *Salmonella* Enteritidis PT4 cells (10^8 log CFU/ml) were exposed to 2.5 µl/ml of MPEO in brain heart infusion (BHI) broth over 252 h in 14 cycles of 18 h exposure. Cells were harvested by centrifugation (4500*g*×10 min, 4°C), washed twice and resuspended in PBS after every two cycles of 18 h exposure. Cells were labeled with propidium iodide for membrane integrity; bis-1,3-dibutyl barbituric acid for membrane potential and ethidium bromide for efflux activity all measured by flow cytometry. One hundred µl aliquots were also serially diluted and viable cell counts enumerated on BHI agar. The same procedures were performed with cells not exposed to MPEO.

Results: After 72 h, ~95% of *Salmonella* Enteritidis PT4 cells exposed to MPEO presented depolarized membranes, 41% showed compromised efflux activity and 28% had damaged membranes. Cells not exposed to MPEO remained largely polarized with normal efflux pump activity and intact membranes. A decrease of approximately 0.5 log CFU/ml was observed over 252 h. Increasing exposure time reduced the population of cells with a compromised membrane or efflux activity but did not change the population of viable cells.

***S*ignificance:** These results show that MPEO exerts inhibitory effects through a multi-target mechanism in *Salmonella* Enteritidis PT4 cells, but continuous exposure appears to create a subpopulation of cells able to repair the injuries.

P2-18 Cell Damage Caused by Mandarin Essential Oil to Autochthonous Spoilage Lactic Acid Bacteria in Orange Juice

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Introduction: Lactic acid bacteria comprise the largest group of raw fruit microbiota and can easily be transferred to juices causing deterioration through fermentative metabolism. The combined use of *Citrus reticulata* essential oil (CREO) with mild heat treatment or other technologies has been suggested for orange juice preservation. However, the effects of CREO on the physiological functions in spoilage bacteria remain unknown.

Purpose: This study determines cell damages caused by CREO in *Lactobacillus brevis* and *Leuconostoc mesenteroides,* well-known juice spoilage bacteria of orange juices.

Methods: The test strains (10^8 log CFU/ml) were exposed (12 min) to 0.5 µl/ml of CREO in orange juice. Cells were harvested by centrifugation (4500×*g,* 10 min, 4°C), washed twice and resuspended in PBS and immediately labeled with the fluorochromes: propidium iodide for membrane integrity; bis-1,3-dibutylbarbituric acid for membrane potential, ethidium bromide for efflux activity and 5-cyano-2,3-ditolyl tetrazolium chloride for respiratory activity. Flow cytometry for data acquisition was set on 10,000 for FSC and cells were gated per FSC/SSC. In each acquisition, 10,000 events were analyzed. Density plots were obtained along measurements and dot plot analysis of FL1 vs. FL3 was used to establish fluorescence of each population. Ctograms were analyzed using BD Accuri C6 Software.

Results: After exposure to CREO, approximately 93% of *Leuconostoc mesenteroides* cells presented membrane damage: 96% depolarized membranes, 91% compromised efflux activity and 81% without respiratory activity. The percentages of injuries in *Lactobacillus brevis* after exposure to CREO were 66% of cells with membrane damage, 86% with the depolarized membrane, 88% with compromised efflux activity and 78% without respiratory activity. Cells not exposed to CREO remained largely polarized, presented intact membranes and normal efflux and respiratory activity.

Significance: Results show that CREO exerts its activity acting in distinct membrane cell targets; however, the extent of the effects varies with the intrinsic resistance of spoilage bacteria.

P2-19 Antimicrobial Resistance of *Salmonella* Recovered from Environmental Samples on Three North Carolina Tomato Farms

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Introduction: Tomatoes have been repeatedly implicated in *Salmonella* outbreaks.

Purpose: To evaluate the antimicrobial susceptibilities of *Salmonella* isolates recovered from tomato farms.

Methods: Environmental samples of farm soil, irrigation water, irrigation filtration unit, pond water, stream sediment, stream water, and tomatoes were collected from three North Carolina tomato research farms (two mountain, one Piedmont regions) in 2012, 2013 and 2014 and tested for *Salmonella* using cultural methods. All presumptive positive *Salmonella* isolates (*n*=169) were tested for antimicrobial susceptibility using the Sensititre microbroth dilution system and the National Antimicrobial Resistance Monitoring System (NARMS) custom plate CMV3AGNF. Breakpoints were used to determine minimum inhibitory concentrations (MIC), which were recorded as the lowest concentration of an antimicrobial that completely inhibited bacterial growth. Multidrug resistance (MDR) was defined as resistance to two or more classes of antibiotics.

Results: Out of the 169 total isolates tested, (82.2%) (139) were pan-susceptible to the 14 antibiotics tested. Two stream water isolates (1.2%) showed intermediate resistance against chloramphenicol. Twenty-eight isolates (16.6%) demonstrated resistance to one antibiotic (streptomycin or ampicillin): twenty-seven (16%) were resistant to streptomycin (pond water, stream sediment, and stream water) and one isolate (0.6%) was resistant to ampicillin (irrigation water). Twenty-six (15.4%) of the antimicrobial resistant isolates were found at Farm Site B. A single MDR sample (cefoxitin, amoxicillin/clavulanic, and ampicillin) was obtained from a stream sediment sample isolated from Farm Site B. Twenty-two isolates (13%) from stream water were found resistant to streptomycin. The majority of resistant isolates (11.8%) were identified as serotype IV.

Significance: The presence of antimicrobials in the environment has led to antimicrobial resistance (especially streptomycin) in water and sediment samples from 3 tomato farms in North Carolina.

P2-20 Assessment of Antibiotic Usage and Oxytetracycline Residues in Eggs from Commercial Poultry Farms in Ilorin, Nigeria

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Introduction: The misuse of veterinary drugs is one of the causes of drug residues in animal products.

Purpose: A cross-sectional study was carried out to investigate the antibiotic usage patterns in commercial poultry farms and the presence of antibiotic residues in eggs in Ilorin, Nigeria.

Methods: Poultry farmers (*n*=200) were interviewed using a pretested questionnaire on their patterns of antibiotic usage and knowledge of occurrence and risks of antimicrobial residues in eggs. Egg samples (*n*=200) were obtained from randomly selected commercial poultry farms (*n*=20) across the Ilorin metropolis. Pooled egg samples (*n*=10) from each farm were analyzed with high-performance liquid chromatography (HPLC) for oxytetracycline residue levels.

Results: A significant percentage of the respondents (61.5%) frequently administered drugs without a veterinary prescription and 73.3% admitted to non-observance of the withdrawal periods. Tetracycline was the most widely used antibiotic (26.0%) according to the survey with almost half of the farmers purchasing drugs over-the-counter. Many farmers (63.5%) were unaware of the residual effects of antibiotics. Out of a total of 20 homogenized egg samples, 15 were found to contain oxytetracycline residues with a total prevalence of 70% and mean concentration of 398.302±186.73 µg/kg (oxytetracycline standard: y=4.4954x+3.3266; r^2=0.9808). Five samples had mean detectable concentrations higher than recommended WHO/FAO maximum residue limit.

Significance: This study highlighted a lack of implementation of the recommended drug withdrawal period possibly due to lack of awareness of the poultry farmers and the absence of government regulations. Compliance with the observance of the withdrawal period of drugs used for treatment in laying birds is emphasized.

P2-21 Effect of Nutrient Enrichment on Antimicrobial-resistance Dynamics of Native Soil Bacteria

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Introduction: Speculation has been growing that antimicrobial use in food animal production settings is having a large effect on the increase in antimicrobial resistance (AMR) observed in human clinical cases.

Purpose: To observe the dynamics of antimicrobial resistance when native soil bacteria are enriched In Situ to levels observed in livestock production environments.

Methods: Field plots were created in an area with little to no impact by antimicrobials or fecal material derived from humans, livestock, or companion animals for at least 20 years. Four plots were developed and within each plot three enrichment sites were designated. Sites received sterilized, liquid bacteriological media three times per week (Phase I – 21 weeks, Phase II – four weeks) and samples were collected once per week. Soil samples (one sample per site per week; *n*= 4 per treatment per week) were analyzed using microbial culture, qPCR, and metagenomics.

Results: The addition of nutrients in the form of sterile bacteriological media led to changes in concentrations from < 1-log CFU/g of soil of tetracycline-resistant (TET^r) *Enterococcus* spp. and erythromycin-resistant (ERY^r) *Enterococcus* spp. to five-log and four-log CFU/g soil, respectively. The levels of these resistant bacterial populations are equivalent to those typically found in conventionally-reared cattle feedlots. When nutrient addition was discontinued, TET^r and ERY^r *Enterococcus* spp. populations rapidly decreased to undetectable levels. In addition, both qPCR and metagenomic analyses showed that nutrient addition increased the levels of several genes (qPCR targets included: bla_{CMY2}, $bla_{CTX\text{-}M}$, *erm*B, *tet*M, *tet*A, *tet*B, and *aad*1) of multiple antibiotic resistance classes (aminoglycosides, beta-lactams, macrolides, and tetracyclines) to levels observed in cattle feedlot environments. Principal coordinate plots calculated using a Bray-Curtiss dissimilarity matrix shows clustering of enriched samples with samples from cattle feedlot pen surface material.

Significance: Currently, associations are made between antimicrobial use in livestock populations and AMR bacterial populations in the environment without accounting for the ecological dynamics such as outgrowth of native bacterial populations through nutrient supply in the form of fecal deposition. This project demonstrates that AMR can flourish in environments in the absence of human-applied antimicrobial selective pressure.

P2-22 Influence of pH on the Effectiveness of a Natural Antimicrobial to Control *Listeria monocytogenes* on Ready-to-Eat, Clean Label, Smoked Pork Sausage during Extended Storage at 4° and 10°C

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Introduction: Alkaline phosphate is used in traditional cooked sausage products, in part to modify pH. Potassium carbonate may be used as a processing aid, also to modify or control pH. The efficacy of natural antimicrobials may be affected by pH.

Purpose: Evaluate the effect of pH on the efficacy of buffered vinegar to control *Listeria monocytogenes* on an RTE pork sausage during refrigerated storage.

Methods: Commercially-manufactured, clean label RTE smoked pork sausage was formulated with or without 0.5% sodium tripolyphosphate (STPP) or 0.07, 0.12, or 0.17% of potassium carbonate (K_2CO_3) as pH modifiers, or with 1.7% of liquid buffered vinegar (LBV) as a natural antimicrobial. Sausage (one link per package; ca. 60 gram) were surface inoculated with 500 µl of a five-strain rifampicin-resistant (100 mg/ml) cocktail of *L. monocytogenes* (ca. four log CFU/package), and then packages were vacuum-sealed and stored at four or 10°C for up to 135 days

Results: Inclusion of STPP or K_2CO_3 in the formulation increased pH of sausage by 0.2 to 0.6 pH units, whereas inclusion of BV, alone or in combination with STPP or K_2CO_3 decreased pH by ca. 0.2 pH units when compared with sausage formulated without pH modifiers. At 4°C, in sausage formulated with or without K_2CO_3 or K_2CO_3 plus BV, *L. monocytogenes* numbers remained unchanged or increased by ca.1.4 to 5.8 log/package, whereas inclusion of STPP plus BV or BV alone, decreased pathogen numbers by ca. 1.2 log CFU/package after 135 days of storage. At 10°C, regardless of the pH modifier added to the formulation, pathogen numbers increased by 2.5 to 5.9 log CFU/package after 135 days of storage.

Significance: When used alone or in combination with 0.07% K_2CO_3, as a pH modifier for natural, RTE pork sausage, BV provides inhibition of *L. monocytogenes* throughout extended shelf life.

Poster

P2-23 Investigation into the Detection of Semicarbzide, a Nitrofurazone Indicator, in Chicken

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Introduction: The Food Safety and Inspection Service (FSIS) is the public health agency in the United States Department of Agriculture responsible for ensuring that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged. Under the United States National Residue Program (NRP), FSIS routinely monitors these products for the presence of chemical residues from veterinary drugs, pesticides and environmental contaminants based on tolerances set by regulatory partners. Nitrofuran antibiotics have been banned from use in food-producing animals by many food regulatory agencies, including the European Food Safety Authority in 1993, the Food and Drug Administration in 2002, the Thailand Ministry of Health in 2003, and the New Zealand Food Safety Authority in 2003. Nitrofurazone is a nitrofuran antibiotic, and its metabolism results in tissue-bound metabolites, including semicarbazide (SEM). SEM usefulness as an indicator of nitrofurazone usage has been called into question as research has identified alternative sources of SEM, such as environmental contaminants or reactive by-products formed during food processing. Even though nitrofurazone has been unauthorized in the United States, there is documented evidence that SEM residues have been found in poultry products.

Purpose: FSIS conducted a study to determine whether SEM findings were the result of illegal drug use or generated as a by-product of food processing.

Methods: Fresh and frozen chicken samples were collected and analyzed for the presence of SEM, at various time points in poultry production.

Results: No samples collected prior to chemical interventions tested positive for SEM, whereas several post-intervention samples tested positive.

Significance: While the detection of SEM in samples prior to intervention could have been indicative of nitrofurazone use, its absence in these samples suggests that the detection of SEM exclusively after the application of chemical interventions may be a result of by-products formed during poultry processing.

P2-24 Survival and Inactivation of *Listeria monocytogenes* from Common Specialty Crop Food Contact and Non-Food Contact Surfaces Using Different Antimicrobials

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Introduction: Biofilm formation may be the underlying element for the persistence of *Listeria* in fresh produce operations, even when physical access for cleaning and sanitizing is not limiting.

Purpose: To evaluate the efficacy of different antimicrobials on *Listeria* following biofilm formation on common food and non-food contact surfaces.

Methods: Under In Vitro conditions, four by four cm pieces of materials recovered from packing operations were sterilized and biofilms using three strong biofilm-producing *Listeria monocytogenes* strains from these operations were established. Following 15, 21 and 30 days post-inoculation (DPI), materials were air-dried. Eight commercial antimicrobials (chlorine, peroxyacetic acid; PAA, Safe-Zone, RelyOn, Steroklor, Chico Wash and Decon7) were applied following manufacturer recommendations.

In three In Situ trials, efficacy was assessed at locations repeatedly positive for *Listeria* spp. An area one by one m^2 was swabbed in long sweeping motions. Three sequential swabs were conducted including pre-cleaning, post-cleaning, and post-treatment. Five antimicrobials (PAA, Safe Zone, RelyOn, Decon7) were assessed.

L. monocytogenes quantitative and/or qualitative analysis was performed, including culture confirmation and serogrouping.

Results: In vitro trials showed greater log reduction when applying antimicrobials after 15 DPI, with PAA, Safe Zone and RelyOn. However, three to 4.5-log reduction was only observed on some materials. Although RelyOn, Decon7 and Steroklor showed significant log reductions at 30 DPI, high rates of *Listeria* survival were observed after 21 DPI. In trials conducted In Situ, both *Listeria* spp. and *L. monocytogenes* were confirmed pre-cleaning. Interestingly, different *L. monocytogenes* serogroups were recovered before treatment and only two (IIb and IVa) were recoverable after antimicrobial application. With revised cleaning procedures at different sites, all antimicrobials resulted in non-detection of *Listeria*.

Significance: Outcomes provide additional evidence that prevention of establishment of environmental *Listeria*, whether due to failures in robust cleaning and sanitizing programs or failures to minimize establishment in harborage sites, is necessary to realize the efficacy of available antimicrobials.

P2-25 Susceptibility of *Listeria monocytogenes* Isolates from Food, Environmental, and Clinical Origin in South Africa against a Commercial Bacteriophage

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❖ ***Developing Scientist Entrant***

Introduction: Listeriosis is a serious infection in humans caused by the bacterium *Listeria monocytogenes.* In South Africa, a national *Listeria* outbreak declared in December 2017 was believed to be the largest-ever global outbreak of listeriosis. To date, there has been 1064 laboratory confirmed cases with 216 deaths. Prior to December 2017, listeriosis was not a notifiable disease, and published data relating to food borne listeriosis is lacking in South Africa. The application of bacteriophages in food processing facilities is still relatively new, thus not much is yet known about possible occurrence of resistant organisms in the processing environment.

Purpose: The aim of this study was to test the effectiveness of a commercial bacteriophage against *L. monocytogenes* isolates from clinical and food origin (acquired 2018 – 2019) and to determine whether strains of varying origin show differences in susceptibility to the phage.

Methods: *Listeria monocytogenes* colonies were inoculated from fresh tryptic soy agar plates into brain heart infusion broth and grown for three h at 37°C, to obtain log phase growth. Plates were flooded with 100 µl of this culture, spread and left to dry for 30 min. Subsequently, 10 µl of 1×10^9 PFU/ml phage suspension (diluted with 0.85% saline solution) was dropped on the plate and left to dry completely at room temperature. Plates were then incubated at 30°C for 18 to 20 h. After incubation, plates were inspected to see whether or not a plaque has formed.

Results: Plaque formation was detected in 124 (63.6%) of 199 samples, 13 (65.0%) of 20 clinical, 24 (70.6%) of 34 food processing environment, nine (26.5%) of 34 raw food, 15 (42.9%) of 35 ready-to-eat, and 62 (87.5%) of 72 unknown.

Significance: Although the commercial bacteriophage is designed to be effective as an antilisterial agent in the food processing environment, it was interesting to note that a large percentage of isolates showed no susceptibility to the phage. Upon further study, it can be established whether these isolates also show the same resistance towards antibiotics currently used as treatment for listeriosis.

Poster

P2-26 Antimicrobial Effect of Citral-based Emulsions against *Escherichia coli* (MTCC 443) on Fresh-cut Papaya Surface

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Introduction: Application of emulsion-based edible antimicrobial coatings to inhibit the growth of microorganisms on food surfaces to extend shelf life and to enhance the safety of foods has gained popularity recently. Citral, a natural citrus flavor compound has been reported to show antimicrobial activity against different bacteria. Therefore, the potential use of citral as an edible and natural antimicrobial compound to improve the quality and safety of perishable foods warrants investigation.

Purpose: The purpose of our study was to evaluate the impact of citral-based emulsions on the antimicrobial efficacy against *Escherichia coli* MTCC 443 on fresh-cut papaya surface as a model food.

Methods: Citral-based emulsions were stabilized with Tween 80 by ultrasonication and characterized for physical properties and storage stability. The time-kill assay was performed on *E. coli* MTCC 443 spiked fresh-cut papaya surfaces for eight days at 4°C to study the antibacterial effect of emulsion-based formulations as compared to non-emulsion (citral-only) during prolonged refrigerated storage. All experiments were performed in triplicates.

Results: The emulsions demonstrated greater stability than citral-only formulation with mean particle diameter (245±10 nm), polydispersity index (0.27±0.01) and zeta-potential (-24±2 mV). Data obtained from time-kill assay on cut papaya revealed that the citral-emulsion coating reduced the *E. coli* MTCC 443 count by 4.01 (day three) and 2.62 (day eight) log CFU/g when compared with control (no citral coating). The bacterial inhibition by emulsion formulations was found to be significantly (P<0.05) higher than citral-only formulation at the same time-points, which resulted in 1.11-(day three) and 0.716- (day eight) log CFU/g reduction in bacterial counts, respectively.

Significance: The oil-in-water type formulation of citral-emulsion could significantly reduce viable *E. coli* MTCC 443 counts on fresh-cut papaya surface during prolonged refrigerated storage. The study showed the benefit of using an oil-in-water emulsion for extending the antimicrobial activity of citral.

P2-27 The Effect of Cranberry Pomace Ethanol Extract on the Growth of Meat Starter Cultures, *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Cranberry pomace ethanol extract (CE) is an 80% ethanol extract of a crude cranberry processing byproduct that has gained considerable interest. Several studies have demonstrated its excellent antimicrobial activities on foodborne pathogens, but there are only limited studies investigating its effect on the growth of beneficial bacteria, especially meat fermentation starter cultures.

Purpose: Examine the effect of CE on the growth of commonly used meat fermentation starter cultures, and selected foodborne pathogens.

Methods: Ten meat starter culture strains belonging to genus *Lactobacillus*, *Pediococcus*, and *Staphylococcus*, and three pathogens (*Escherichia coli* O157:H7*, Salmonella* Enteritidis*,* and *Listeria monocytogenes*) were grown in dextrose-free De Man, Rogosa and Sharpe broth, or dextrose-free tryptic soy broth, respectively, supplemented with different concentrations of micro-filtered CE prepared in 80% methanol. Changes in bacterial growth at 37°C were monitored for 48 h by measuring OD_{600} using Bioscreen C.

Results: Concentration-effect was observed for all starter cultures studied. *Lactobacillus* spp. and *Pediococcus* spp. showed maximum OD_{600} in the presence of 0.50 to 0.75% wt/v CE; and could possibly be attributed to the carbohydrates in CE. *Staphylococcus* spp. showed a higher sensitivity towards CE than other starter cultures and no growth was observed starting from 0.50% and higher wt/v CE. Reduced growth at higher CE levels could be related to the increased amount of CE phenolic compounds and inherited acidity. All pathogens studied showed a higher sensitivity towards CE than the starter cultures. Gram-positive pathogens were found to be more susceptible to CE than Gram-negative pathogens.

Significance: To the best of our knowledge, this is the first study reporting the effect of berry extracts on the growth of common meat starter cultures. Findings suggest that CE can potentially be used as a natural antimicrobial against foodborne pathogens and growth promoter for certain meat starter cultures.

P2-28 Evaluation of Maqui (*Aristotelia chilensis)* extract and Copper against Biofilm Production in *Listeria monocytogenes.*

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Introduction: *Listeria monocytogenes* (*Lm*) is a foodborne pathogen responsible for causing listeriosis.This widespread bacterium can survive and grow in a wide range of environments. Biofilm formation is the main mechanism that promotes *Lm* survival, persistence, and dissemination in the food industry. Therefore, biofilm control is imperative to assure a safe food supply.

Purpose: This study aimed to investigate the effect of two compounds over the biofilm formation in *Lm* isolated from different food sources.

Methods: First, we evaluated the biofilm formation ability of 43 *Lm*isolates at 37ºC. Then, we selected 8 representative strains to evaluate their response to non-toxic copper concentrations (0.5 and 1mM) and non-toxic concentrations of maqui (*Aristotelia chilensis*) extract (0.25 and 0.5 mgEAG/mL). Strains were inoculated (10^5CFU/mL) into 96 well polystyrene plates, and biofilm formation was measured with the dye crystal violet method after 7-days incubation at 37ºC.

Results: Almost every strain decreased their ability to form biofilm when exposed to both compounds, and the higher concentration used, the stronger reduction in biofilm formation was achieved. However, one strain showed a different behavior; it increased its biofilm production in the presence of both concentrations of maqui extract.

Significance: These results suggest that natural antimicrobial compounds, such as copper and maqui extract, could be useful to control *Lm* biofilm production.

Poster

P2-29 Antimicrobial and Physical Properties of Chitosan/Acetylated Starch Edible Films Containing Cinnamon and Clove Essential Oils

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Introduction: Edible food packaging can slow down or prevent the degradation of foods due to environmental factors. Novel approaches to improving the safety and quality of food products are very important to the food industry and public health. Clove and cinnamon essential oils (EOs) have been discovered to be effective natural antimicrobial agents, especially for food packaging applications.

Purpose: The objective of this study was to study the effect of cinnamon and clove EOs on the antimicrobial and physical properties of acetylated starch/chitosan film for food packaging applications.

Methods: The acetylated starch/chitosan film films were prepared in a one-to-one ratio with different concentrations of clove and cinnamon EOs in a one to three ratio using a solution casting method. The antimicrobial properties were examined by exposing a cocktail (≈10^5 CFU/g of bacteria) of five strains of *Escherichia coli* O157:H7 to the films (1.5 cm by 3 cm) in duplicates and replicated twice. The tensile strength (TS) and elongation at break (%E) of the films (1 cm by 5 cm) were determined using a texture analyzer. The color variations of the films were measured using a colorimeter in duplicates. The moisture content and solubility of the films (2.5 cm by 2.5 cm) were measured in triplicates.

Results: The acetylated starch/chitosan films were successfully optimized and developed with different concentrations of EOs. In the antimicrobial test, the growth of *E. coli* O157:H7 was completely eliminated using films containing 2.00% EOs after 24 h of incubation. The TS and %E of the films was reduced by 25.6% and 40.4%, respectively, upon the incorporation of two percent EOs. As the concentration of EOs increased, the films were darker, redder and yellower. With a higher concentration of EOs, the moisture content and solubility showed a decreasing trend.

Significance: These data suggest that incorporation of EOs may improve the antimicrobial activity of edible packaging films.

P2-30 Evaluation of Two Antimicrobial Treatments, Chlorine and Peroxyacetic Acid, to Effectively Control *Listeria monocytogenes, Salmonella* spp., and *Escherichia coli* O157:H7 on Celery Stalks

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Introduction: Celery, a raw agricultural product is considered an RTE item with the potential to contain food pathogens such as *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 pre and post-harvest, a significant food safety and public health threat.

Purpose: The objective of this study was to identify an antimicrobial concentration and method of application that would effectively provide a twi-log or greater reduction of the food pathogens *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7 inoculated onto celery stalks.

Methods: This study used *n*=300 inoculated celery stalks to evaluate two antimicrobial intervention chemicals, chlorine (sodium hypochlorite) and peroxyacetic acid used at two concentrations, applied as a spray and by immersion, to reduce *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 inoculated onto celery stalks. Inoculated stalks were exposed to chlorine at five and 50 ppm, peroxyacetic acid at 80 and 120 ppm. Treated stalks were then quantitatively evaluated for viable *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 using selective and differential microbiological media. Inoculated untreated celery stalks were used as controls to calculate the log reduction.

Results: A two-way ANOVA analysis adjusted for bias using Dunnett's posthoc analysis showed the concentration of the antimicrobial was more significant (P<0.05) than the antimicrobial or the method of application at reducing *L. monocytogenes*. The same analysis showed the antimicrobial and concentration being more significant than the method of application at reducing *Salmonella* and *E. coli* O157:H7.

Significance: Consumer food safety is realized when antimicrobial interventions to reduce food pathogens are applied effectively at a functional concentration onto RTE Items pre and post-harvest.

P2-31 Influence of Pre-Adaptation to Sub-lethal Concentrations of a Sanitizer on the Susceptibility of Fecal Coliforms to Antibiotics

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Introduction: Adaptive exposure to sublethal concentrations of sanitizers was previously reported to lead to the development of cross-resistance in bacteria to antibiotics.

Purpose: This study were undertaken to compare the susceptibility of sanitizer-adapted and non-adapted fecal coliform cells to antibiotics commonly used in human medicine.

Methods: To accomplish this, the antibiotic resistance profiles of selected fecal coliform isolates (*n*=55) was determined using the standard disk diffusion assay. Selected fecal coliforms were then adapted in one-half or one-fourth of manufacturer-recommended concentration (5 ppm) of liquid chlorine dioxide. The susceptibility of adapted and non-adapted cells to 15 different antibiotics, commonly used in treating human gastrointestinal infections, were determined by observing the changes in their minimal inhibitory concentrations (MIC).

Results: Results showed that the fecal coliforms were resistant to at least one antibiotic. None of the fecal coliforms were resistant to kanamycin, trimethoprim, tetracycline, gentamicin, chloramphenicol and doxycycline. Of the 55 isolates, 53 (96.3%) were resistant to ampicillin, 44 (80%) to nitrofurantoin, 43 (78.1%) to sulfisoxazole, 23 (41.8%) to amoxicillin-clavulanic acid, 21 (38.1%) to cephalothin and 19 (34.5%) to cefazolin. Additionally, 15 (27.2%) isolates were resistant to cefazolin, two (3.63%) to nalidixic acid and ciprofloxacin and one (1.81%) to trimethoprim. Pre-adaptation to sublethal concentrations of chlorine dioxide, in general, did not enhance the resistance of fecal coliforms to the 15 antibiotics, with only two exceptions: the pre-adaptation increased the MIC of kanamycin against two of the fecal coliform isolates from eight to 16 µg/ml and 16 to 32 µg/ml, respectively and of nalidixic acid against one of the two fecal coliform isolates from 16 to 32 µg/ml.

Significance: This study suggests that pre-adaptation of fecal coliforms to sub-lethal concentrations of chlorine dioxide did not appear to induce cross-resistance of selected fecal coliforms to most of the antibiotics used in the study.

P2-32 Bacterial Contamination of Touch Screens in Restaurants and Grocery Stores

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Introduction: Touch screen electronic devices have become increasingly common in public facilities. They have been placed into grocery stores for customer use in checkout lines. Restaurants have also placed units onto tables for diners to order and pay for meals, as well as for entertainment purposes. In hospital and clinical settings, their involvement in the transmission of pathogenic bacteria has been demonstrated.

Purpose: To determine the types and concentrations of bacteria on touch screens used at tables in restaurants, and in grocery stores at checkout counters.

Methods: Sponge sticks (3M Corporation, Minneapolis, MN) were used to sample touch screens at tables in full-service restaurants (*n*=40) and at checkout counters in grocery stores (*n*=20) per standard methods. An area of approximately 100 cm^2 was sampled on each screen. Samples in the restaurants were collected immediately following lunchtime and dinnertime service hours. Samples in grocery stores were collected randomly during the daytime. Each sample was assayed for levels of total culturable bacteria, coliforms, and *Escherichia coli*.

Results: Bacterial numbers per screen in restaurants averaged 3.54 log CFU, and 4.12 log CFU on checkout counter screens. Coliform bacteria and *E. coli* were detected on 50% and 10% of the checkout counter devices, respectively. The higher numbers of bacteria on checkout counter devices may reflect their greater use by the public.

Significance: Touch screens used by the public may serve to transfer potentially pathogenic microorganisms from one person's hands to another, and thereby increase infection risk. The levels of bacteria on these devices are greater than we have reported previously for touch screen devices used in healthcare environments (3.35 log CFU).

P2-33 Potential Application of the Photosensitizer Curcumin in Inactivating Foodborne Pathogens on Chicken

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Introduction: The European Union has prohibited the import of poultry exposed to chlorine since 1997. Alternatives to traditional chemical interventions are needed. A photosensitizer was found to inactivate a variety of microorganisms. However, studies on its food applications and its impact on food quality are still limited.

Purpose: The goal of this research is to investigate the antimicrobial efficacy of the water-soluble photosensitizer curcumin (PSC) and the impact of PSC on the appearance of chicken skin/meat.

Methods: The absorption spectrum of PSC was determined by UV-Vis spectroscopy. A light-emitting diode (430 nm, 107 W/m^2) was chosen to activate PSC. The antimicrobial effects of PSC on *Listeria monocytogenes* (three isolates), *Escherichia coli* O157:H7 (two isolates), and *Salmonella* (eight isolates) were evaluated on both media and chicken skin. The effects of interaction time before illumination and illumination time were studied. Skin color (L*, a*, and b*) was measured to calculate total color difference and whiteness index. Results were compared using ANOVA; P<0.05 was considered statistically significant.

Results: Both the light source and photosensitizer had to be present to induce antimicrobial effects under the conditions examined. MIC and MBC of PSC on *L. monocytogenes* were 0.001 and 0.002%, respectively. Treatment with 0.02% PSC resulted in maximum 5.1- and 3.6-log reduction of *E. coli* O157:H7 and *Salmonella* on media, respectively. The population of *L. monocytogenes* was reduced from five to ~three log CFU/cm^2 on chicken skin, after five-minute dipping with 0.03% PSC followed by five-minute illumination. There were no significant differences in antimicrobial activities among different interaction (1, 2.5 and 5 min) or illumination time (one, five, and 10 min). Meanwhile, no significant color change was observed on skin exposed to five-minute illumination or more.

Significance: This research suggests that PSC could effectively inactivate pathogens, indicating a potential application as an antimicrobial intervention on poultry without causing changes in appearance.

P2-34 How Water Antimicrobials and Produce Volume Influence Cross-Contamination during Batch Washing in Retail Operations

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Introduction: According to the FDA food code (2017), fresh produce *shall* be thoroughly washed prior to serving to the consumer, but chemicals are not required. Pathogens if present may transfer to water and other uncontaminated products. Typically, a case of lettuce is immersed at the same time but processing high-volume produce per batch might increase the likelihood of cross-contamination due to close contact between commodities.

Purpose: This study aimed to compare the efficacy of tap water, neutralized electrolyzed water, and two commercial acidic sanitizers (lactic acid/phosphoric acid and sodium dodecylbenzenesulfonate/lactic acid) in mitigating cross-contamination. Two settings of produce volume, low (eight heads) and high (24 heads), were also compared.

Methods: Red leaf lettuce were spot-inoculated (~five log CFU/g) with Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes.* In the low-volume scenario, one inoculated lettuce was washed with seven non-inoculated in 76 liters for fie minutes, followed by two washings with eight non-inoculated per batch using the same water. Similarly, three consecutive washings were conducted in the high-volume scenario, but with three inoculated in the first washing.

Results: In the low-volume scenario, two types of antimicrobial could achieve greater than two log reduction of commensal microorganisms (APC), STEC, and *L. monocytogenes*, which was significantly higher than water (P<0.05). However, their efficacy decreased in the high-volume, increasing the risks of cross-contamination. Adding the antimicrobials showed lower levels of APC, STEC, and *L. monocytogenes* in water compared with tap water alone. Except for electrolyzed water, cross-contamination was found in the third washing event, but more positive samples were detected in the high-volume wash.

Significance: Adding an antimicrobial during batch washing can reduce risks of cross-contamination by decreasing and controlling the level of pathogens on contaminated produce and in water. It is recommended that the number of commodities being washed per batch be limited to mitigate cross-contamination.

P2-35 Identifying Nonpathogenic *Salmonella* Surrogates for Industrial Scale Treatment of Almonds Using Gaseous Chlorine Dioxide

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❖ ***Developing Scientist Entrant***

Introduction: In order to conduct successful challenge studies in industrial facilities, non-pathogenic surrogates are used to represent the target pathogens. *Enterococcus faecium* is the most commonly used and approved surrogate for *Salmonella enterica* in almond processing. This study would help fill the research gap of *E. faecium* as a surrogate for gaseous ClO_2 treatment on almonds.

Purpose: The objective of this study was to evaluate the potential of surrogate strain to represent pathogenic *Salmonella* during gaseous chlorine dioxide (ClO_2) treatments.

Poster

Methods: Cocktail of five pathogenic *Salmonella* strains, and *E. faecium* NRRL B-2354 were used separately to inoculate 200 g of almonds. Gaseous ClO_2 was generated on site using dry precursors by mixing sodium chlorite and activating acid. Treatments were conducted at various concentrations (0.3, 0.4, and 0.5 mg of ClO_2/g of almonds) and exposure times (two, three, and four h) in a treatment chamber with attached fans blowing the gas (dry precursor mix method) towards the inoculated almonds.

Results: The log reductions of *Salmonella* demonstrated the same trend as in *E. faecium*, which established its potential as a suitable surrogate for ClO_2 studies. Throughout the entire treatment, held at different concentrations and exposure times, the difference in log reductions between *Salmonella* and *E. faecium* was in the range of 0.01 to 0.71 log CFU/g. These results were duplicates from one repeat and indicated *E. faecium* to be a robust surrogate for validating ClO_2 treatments. Kinetic modeling inferred that the reduction trend was not linear with respect to treatment concentrations.

Significance: *E. faecium* is a suitable surrogate and will be used to scale up the ClO_2 treatment in the almond industry to avoid cross-contamination in the facility.

P2-36 Efficacy of Cinnamon Oil Nanoemulsion in Inhibiting *Salmonella* spp. and *Listeria* spp. on Mung Bean Sprouts

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Introduction: Mung bean sprouts have been associated with deadly outbreaks of foodborne pathogens including *Salmonella* and *Listeria.* Cinnamon oil exhibits antimicrobial properties but is of limited use as food treatment due to its hydrophobicity. Nanoemulsions of cinnamon oil made through sonication offer a stable, homogenous solution that may provide an effective treatment for mung bean sprouts.

Purpose: The purpose of this study was to test the efficacy of cinnamon oil nanoemulsions as an antimicrobial treatment for mung bean sprouts against *Salmonella enterica* and *Listeria monocytogenes*.

Methods: A five percent cinnamon oil nanoemulsion (CONE) was prepared by 20-min sonication of natural 100% cinnamon oil with the surfactant Tween 80 and DI water. Seeds were tested for germination following CONE treatment. To test treatment, the seeds were artificially inoculated with a three-strain cocktail of either *L. monocytogenes* or *S. enterica* and were then soaked in dilute concentrations of the CONE for one minute. The mung bean seeds were watered and allowed to sprout over a 72-h period. Samples of the sprouts were homogenized using a stomacher and bacterial enumeration was performed at zero, 24, and 48 hours after treatment.

Results: The CONE treated seeds showed a reduction in bacteria over the seeds treated with a water control and no negative effect to germination. Following a one percent treatment, the seeds inoculated with *S. enterica* showed the greatest reduction immediately following treatment, at 48 hours, with a 2.64-log reduction (P=0.041). Those inoculated with *L. monocytogenes* showed the greatest reduction after 48 hours, with a 1.07-log reduction (P=0.018). In our future studies we will test increased treatment times and increased concentration of CONE.

Significance: This data suggest that nanoemulsions of cinnamon oil may be effective as an antimicrobial treatment for mung bean sprouts.

P2-37 Evaluation of Cranberry Antimicrobial Properties by TLC-Bioautography

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Introduction: Cranberry extracts have antimicrobial activity against foodborne pathogenic and spoilage organisms. However, some applications of high concentrations of cranberry extracts may affect the organoleptic properties of foods. Thus, it is important to identify and isolate the specific compounds in cranberry extract responsible for antimicrobial activity to allow for optimal use.

Purpose: The goals of this study were to separate compounds in cranberry extracts by thin-layer chromatography (TLC) and evaluate the elution bands for antimicrobial activity against *Listeria* using a bioautography assay.

Methods: Silica gel TLC plates were developed using a mixture of hexane: ethyl acetate: formic acid (6:10:1, v/v/v) as a mobile phase for separation of cranberry extracts at room temperature. Developed TLC plates were air-dried to remove solvents and challenged against *L. innocua*, at five to six log CFU/ml by agar overlay bioautography and incubated at 35°C. Inhibition regions were visualized by non-detectable dehydrogenase activity of *Listeria* after thiazolyl blue tetrazolium bromide application.

Results: The TLC system utilized here was able to separate cranberry extract into different elution bands. Bioautograms showed *Listeria* inhibition in some zones and not in others. A benzoate solution at 0.25% was used as a control based on the naturally occurring concentration of benzoic acid in cranberry extract; no *Listeria* inhibitory effect was observed.

Significance: TLC-bioautography demonstrated the antimicrobial activity of specific fractions of cranberry extract against *Listeria*. Furthermore, the antimicrobial activity was not due to the concentration of benzoic acid alone. The method developed here is a rapid technique to assess the antimicrobial activity of cranberry extract fractions that can be purified for identification and further testing.

P2-38 Long-term Survival Phase Cells of *Listeria monocytogenes* Exhibit Increased Tolerance to Cinnamaldehyde in 0.85% Saline and Apple Juice

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Introduction: *Listeria monocytogenes* is an environmental contaminant and can remain viable for months or years in the long-term survival (LTS) phase while showing increased tolerance to different antimicrobial treatments such as heat, high pressure and UV radiation compared to stationary phase cells (STAT).

Purpose: The objective of this study was to compare the tolerance of LTS and STAT cells of *L. monocytogenes* to cinnamaldehyde, an essential oil component in saline (0.85%) and apple juice.

Methods: *L. monocytogenes* Scott A was used to prepare STAT cells by two consecutive 24 h transfers in TSBYE at 35°C. LTS cells were prepared by incubating STAT cells in TSBYE (35°C, 30 d). Saline (0.85% NaCl, 1.0% cinnamaldehyde and 0.5% tween 80 as emulsifier) and apple juice (0.2% cinnamaldehyde and 0.5% tween 80) were prepared. Controls did not contain cinnamaldehyde. LTS and STAT cells (10^8 CFU/ml) of *L. monocytogenes* were exposed to saline and apple juice for five, 10, 15 and 20 minutes. Survivors were enumerated by surface plating 0.1 ml aliquots on tryptic soy agar with yeast extract (TSAYE) and modified oxford agar (MOX) and colonies counted after Incubation (35°C, 48 h) and log reductions calculated.

Results: There was a significant difference (P<0.05) in the tolerance of LTS and STAT cells in both apple juice and saline at 20 min. In saline, STAT cells showed 6.62-log CFU/ml reduction compared to 4.34-log CFU/ml reduction in LTS cells on TSAYE at 20 min. In apple juice (pH 6.3), STAT cells had a 5.71-log CFU/ml reduction compared to LTS cells that showed a reduction of 4.09 log CFU/ml in TSAYE at 15 min. There was a significant difference in recovery (P<0.05) of STAT cells on both TSAYE and MOX media compared to LTS cells.

Significance: LTS cells of *L. monocytogenes* exhibit more tolerance to cinnamaldehyde compared to STAT cells.

P2-39 Comparison of a Novel Lactic Acid-based Antimicrobial Solution (Purac Evolve) to Lactic Acid and Water as a Final Pre-Rigor Beef Carcass Wash to Reduce Shiga Toxin-producing *Escherichia coli* Contamination

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Introduction: Efficacy of antimicrobial carcass washes depends on factors such as concentration, contact time, surface buffering, physical parameters of application, and meat surface characteristics. A novel lactic acid formulation was developed with the goal to enhance Shiga toxin-producing *E. coli* (STEC) lethality during final pre-rigor beef carcass washing by improving solution residence time and contact intimacy on tissue surfaces.

Purpose: Compare the efficacy of heated (54.4°C) solutions of five percent lactic acid (LA), five percent Purac Evolve (LA containing a surfactant and thickener; mLA), and water (control) for reducing STEC populations on pre-rigor beef carcasses.

Methods: Pre-rigor fed beef carcass sides (n=3) were inoculated with a seven-serogroup stationary-phase STEC cocktail (~5.3 log CFU/cm^2). Each side passed through a two-stage CHAD cabinet (Stage 1: high-volume oscillating ambient water wash; Stage 2: specified 54.4 °C treatment spray) prior to being placed in a spray chill cooler. Meat surface excision samples were taken from the bottom, middle, and top external sections of each side at four sampling points: 30 min post-inoculation, post-Stage 1, post-Stage 2, and after 18-h spray chilling. Three experimental replications were conducted.

Results: Stage 1 washing reduced STEC populations by ~0.2 log CFU/cm^2. Stage 2 provided further STEC reductions of 0.4, 1.1, and 2.1 log CFU/cm^2, respectively, for water, LA and mLA sprays (average of reductions from top to bottom). The 18-h water spray chill cycle provided additional STEC reductions of 0.2, 0.5 and 0.6 log CFU/cm^2, respectively, for carcass sides that received the three final wash treatments. The LA and mLA treatments were superior to the 54.4 °C water control (P≤0.05).

Significance: This study validates the effectiveness of five percent LA and five percent mLA against STEC populations on pre-rigor beef carcass tissues. The addition of surfactant/thickening additives in mLA significantly (P≤0.05) enhances the STEC lethality (by ~one log cycle) compared to a standard five percent lactic acid spray.

P2-40 Isolation of Antimicrobial- and Lactase-producing Lactic Acid Bacteria from Farm Animals and Produce

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✦ ***Undergraduate Student Award Entrant***

Introduction: Improving food shelf life, safety and quality (human and animal health-related) by using probiotics are trending applications in the food industry today, but the fact that these generally-recognized-as-safe (GRAS) lactic acid bacteria (LAB) that utilize lactose can improve lactose intolerance in patients is under explored to date.

Purpose: Our purpose was to identify potential antimicrobial peptide- and lactase-producing LAB in farm animals and produce.

Methods: Samples were enriched and screened for antimicrobial LAB against indicator organisms, *Listeria monocytogenes* and *Escherichia coli*, on de Man, Rogosa, and Sharpe (MRS) agar plates using a sandwich overlay technique supplemented with deferred antagonism indicator overlay method. Lactase-producing and sheep blood hemolytic isolates were determined using a modified disk-diffusion technique on agar plates containing X-gal (20 µg/ml) and sheep blood (five percent), respectively. Subsequent 16S rDNA identification of LAB isolates was carried out with an ABI 3730XL sequencer.

Results: Deferred antagonism antimicrobial analyses for 12 samples of farm animals (3) or fresh produce (9) exhibited a total number of 161 LAB with confirmed activities against *E. coli* (34), *L. monocytogenes* (46), both *E. coli* and *L. monocytogenes* (75), and with no activities (6), suggesting that this group of probiotics be utilized for subsequent investigations. Lactase activity analyses for these antimicrobial-positive LAB determined 22 isolates with differential lactase reactivity (dark blue or blue colonies). Subsequent blood agar hemolysis analyses revealed that all these 22 isolates were from alpha-hemolytic group. 16S rDNA analyses for 10 select LAB isolates identified human pathogens (1), human probiotics (1), bacteriocin-producing bacteria (3), starter cultures (4), and an unknown bacterium (1).

Significance: Searching for new GRAS materials for improvement of food safety and quality (shelf-life and health-related) by food safety agencies and industries is an ongoing effort. Our findings suggest a group of probiotics that may serve a greater benefit for both needs.

P2-41 Implementation of Fluorescent Assays to Measure Membrane Damage to *Escherichia coli* O157:H7 after Exposure to Chlorine Dioxide

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Introduction: Aqueous chlorine dioxide (ClO_2) has demonstrated antimicrobial capabilities against foodborne pathogens and may be a valuable alternative to bleach in an industrial setting.

Purpose: This study implements fluorescence-based assays to measure membrane injury to *Escherichia coli* O157:H7 after exposure to aqueous ClO_2.

Methods: *E. coli* O157 was exposed to ClO_2 (2.5, 5, or 10 ppm) for five, 10, or 15 min. For comparison, controls of 0.1% peptone, 70% isopropanol, and 10 ppm NaOCl were applied for 15 min. After treatment, cells were enumerated on selective media and simultaneously analyzed with the following fluorescent probes for cellular damage i) Bis-(1,3-Dibutylbarbituric Acid)trimethine oxonol ($DiBAC_4(3)$) for membrane polarization, ii) SYTO 9/propidium iodide (LIVE/DEAD) for membrane permeability, iii) 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose (2-NBDG) for active glucose uptake, and iv) lipid peroxidation through accumulation of malondialdehyde (MDA). Fluorescent emissions from each probe were expressed as relative fluorescent units (RFU) and compared to controls.

Results: Log reductions after ClO_2 treatment ranged from 0.17 to 5.47. Reductions of 0.01, 7.87, and 0.14 were achieved after treatment with DI water, isopropanol, and NaOCl, respectively. Changes in RFU after the LIVE/DEAD and 2-NBDG assays were not correlated (Pearson's Correlation P<0.05) with reduction as exposure to each concentration increased. Depolarization ($DiBAC_4(3)$) was observed after NaOCl treatment, however, cells treated with ClO_2 showed results like those treated with peptone water (P<0.05). Accumulation of MDA was detected after 10 ppm ClO_2 treatments, indicating that membrane oxidation occurs at higher concentrations, but not at lower ones. This suggests that ClO_2 damage to *E. coli* O157 is significantly different from NaOCl at comparable concentrations, and the damage could be dose-dependent and is not correlated with viability.

Significance: This study demonstrates that oxidative damage caused by ClO_2 is significantly different than the damage caused by NaOCl. Understanding the impact of ClO_2 on microbial physiology is important to determine appropriate post-harvest treatment conditions.

P2-42 The Use of *Bacillus* spp. Isolated from Ready-to-Eat Date Fruits to Control *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes*, an important foodborne pathogen, remains a significant threat to public health as the invasive form of infection can result in high case-fatality rates.

Purpose: To control/inhibit the growth of *L. monocytogenes* using culturable members of the RTE date fruit microbiome.

Methods: RTE date fruits were acquired from five geographic regions: China, Iran, Palestine, Saudi Arabia, and Tunisia. Bacterial isolates were collected by washing the surface of dates with peptone water, then plating the wash on blood agar. Isolated strains were then individually assessed to monitor whether they can prevent growth of *L. monocytogenes* using an agar plate inhibition test (*n*=3). Following, bacterial strains that secreted antimicrobials were then identified using 16S rRNA Sanger sequencing.

Results: A total of 191 isolates belonging to 91 different phenotypes were observed. From this collection, 35 isolates belonging to 21 phenotypes produced a zone of inhibition against *L. monocytogenes*: Zone sizes ranged from 0.5 to 5.7 mm on the agar plate. Sequencing revealed that the inhibitory strains all belonged to the genus *Bacillus,* with different species. Further work was done to identify those *Bacillus* spp. which had no link to clinical illness. Those *Bacillus* spp. that were found to be safe and that produced the largest inhibition zones are being further characterized by whole genome sequencing and probing the genome for potential inhibitors.

Significance: The results from this research could lead to the discovery of either novel antimicrobial metabolites or beneficial *Bacillus* spp. that could be added to foods to inactivate and/or control *L. monocytogenes*. These novel compounds can also be assessed for their potential activity against other foodborne pathogens and could eventually lead to novel probiotics and/or bio-compounds that can help to reduce foodborne illness.

P2-43 Sanitizer Susceptibility of Recurrent and Sporadic *Listeria monocytogenes* from Meat Processing Environments When Grown in Planktonic and Biofilm States

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Introduction: *Listeria monocytogenes* can persist for prolonged times in food processing facilities. Causes behind persistence are multifaceted, often attributed to inadequate cleaning and sanitation, formation of biofilms, increased resistance to sanitizers, variability in biofilm-forming capacity and adaptation to environmental stressors.

Purpose: Investigate sanitizer susceptibility of recurrent and sporadic *L. monocytogenes* from a meat processing facility (MPF) when exposed to sanitizers used in the facility.

Methods: Four *L. monocytogenes* strains from MPF (recurrent (R1, R2) and sporadic (SP1, SP2)) and ATCC 19115 were grown in a liquid suspension (tryptic soy broth, TSB; 35°C, 24 h) or attached (TSB, 23°C, four days) to assess their susceptibility to two commercial sanitizers (quaternary ammonium compound (QAC, E-San; 50 to 5,000 ppm), and hydrogen peroxide and acetic acid (HPAA, Perox-E; 70 to 19,200 ppm)). Recurrent and sporadic strains were isolated at least three times and once, respectively, over 20 months, and fingerprinted with PFGE. Biofilms were grown on stainless steel coupons (12 mm) and plastic surfaces (MBEC device).

Results: All strains exhibited similar susceptibility to tested sanitizers, with higher concentrations required to inactivate biofilms compared to planktonic cells. Concentrations lower than the manufacturer recommended concentrations (MRC; QAC, 200 ppm; HPAA, 1,100 ppm) effectively inactivated planktonic cells. QAC did not inactivate cells on stainless steel and MBEC with concentrations three times and 25 times higher than the MRC, respectively. No viable cells were observed on stainless steel when exposed to HPAA concentrations 1.2 times lower than the MRC, but required four times higher concentration than the MRC to inactivate four-day biofilms on MBEC.

Significance: This study highlights the resilient nature of *L. monocytogenes*, and factors that can influence sanitation efficacy (e.g., sanitizer type, different surfaces, biofilm formation). Additional study of genetic properties of *L. monocytogenes* is warranted to gain insight into their inherent or acquired traits that may be contributing to the adaptation and persistence in food facilities.

P2-44 The Use of Flow Cytometry for the Rapid Detection of Fluorescent-tagged *Salmonella* spp. in Food and Environmental Samples

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Introduction: Green fluorescent protein (GFP)-tagged *Salmonella* strains are often utilized as positive controls for their distinguishability during a cultural confirmation by observing morphological fluorescence on an agar plate using UV light post incubation. Flow cytometry has the ability to distinguish GFP-tagged *Salmonella* without the need of additional labeling or manipulation.

Purpose: The purpose of this study was to demonstrate the ability of flow cytometry to distinguish a GFP-tagged *Salmonella* strain from other *Salmonella* spp.

Methods: GFP-tagged *Salmonella* Typhimurium Sal54 was propagated in buffered peptone water, then transferred and grown up in RV broth. An additional five wild-type *Salmonella* cultures were grown in a similar fashion. The cultures were then diluted and run on a flow cytometer. The instrument setting were adjusted to examine the voltage pulse height of the fluorescent emission through the BL1 530/30 band pass filter when excited by a laser with a wavelength of 488 nm.

Results: The BL1 histogram plot displaying the cellular events produced by the GFP-tagged *Salmonella* show a distinct cellular population emitting a stronger voltage pulse height due to the GFP excitation from the laser. Voltage intensity between 10^3 and 10^4 was observed in the GFP-tagged *Salmonella.* Wild-type *Salmonella* strains did not display the same cellular population and were easily distinguished from the GFP-tagged strain.

Significance: The ability to distinguish GFP-tagged *Salmonella* control strains from wild-type *Salmonella* by flow cytometry allows for result confirmation in real-time. This discernment removes the need to grow cultures overnight to observe fluorescence reducing the time to result.

Poster

P2-45 Tracing Contamination Issues and Challenges with *Listeria* spp. in an Artisan Dairy Plant in British Columbia, Canada Over a Nineteen-Year Period

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Introduction: In British Columbia (BC; Canada) dairy processing facilities (DPFs) are routinely inspected and microbiological testing of ready-to-eat dairy products is required. Here we describe how the provincial dairy inspection system tracked and addressed *Listeria monocytogenes* contamination in one DPF over 19 years.

Purpose: Investigate microbiological contamination issues in an artisan DPF over 19 years.

Methods: Microbiological tests (coliforms, generic *Escherichia coli*, *Listeria* spp., *Salmonella (*food only), and *Staphylococcus aureus (*food only)) were performed on 639 foods (474 industry-submitted, 165 inspector-collected) and 162 environmental swab samples, using standard culture methods (Health Canada, BAM, AOAC). Samples were collected from one DPF from 1998 to 2016. Voluntary recalls were issued and triggered further investigations when >100 CFU/ml or g was observed in foods that support *L. monocytogenes* growth.

Results: Over 19 years, there were intermittent issues in foods and/or food processing environment with coliforms (153 (46%) of 330 food samples and 26 (17%) of 153 environmental samples); generic *E. coli (*20 (nine percent) of 234 food samples and one (two percent) of 50 environmental samples); *Listeria* spp. (20 (five percent) of 406 food samples and 23 (17%) of 137 environmental samples); and *L. monocytogenes* (10 (two percent) of 406 food samples and 10 (seven percent) of 137 environmental samples); *S. aureus* (38 (17%) of 224 food samples) and *Salmonella* spp. (one (one percent) of 91 food samples) were observed. *L. monocytogenes* contaminated cheese led to three voluntary recalls (2006, 2010, 2012), although no outbreaks or illnesses were linked to the DPF. Issues likely to have contributed to microbial contamination were traced to process failure and methods changes (brine and rind washing; spice and herb additions) water for product chilling; UV bulb failure; inadequate worker hygiene and wound policy; mastitis in introduced dairy cows affecting pooled milk and raw cheese; building design; process flow; and inadequate processing environment sanitation.

Significance: This research highlights the importance of risk-based inspection and environmental testing programs in the prevention of foodborne illness outbreaks, and the significance of collaborative approach between the industry and inspection authorities in protecting public health.

P2-46 Efficacy of a Food Acid to Inhibit *Escherichia coli* O157:H7 and Disrupt Its Biofilms on High Density Polyethylene Surface

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✦ ***Undergraduate Student Award Entrant***

Introduction: When addressing *Escherichia coli* O157:H7 contamination, both population reduction and biofilm disruption need to be considered. Sodium acid sulfate (SAS) is a natural food acid that exhibits antimicrobial properties against foodborne pathogens, but its ability to disrupt biofilms is untested.

Purpose: To evaluate the efficacy of a naturally-derived food acid, sodium acid sulfate (SAS), against *E. coli* O157:H7 and its biofilms.

Methods: Sodium acid sulfate (SAS) was evaluated at three concentrations (0.1, 1, and 3%), and compared to water, chlorine (200 ppm), and peracetic-acid (200 ppm). A cocktail of three *E. coli* O157:H7 strains were allowed to form biofilms on high density polyethylene (HDPE) coupons for 2.5 h at 37°C. Coupons were then washed and dried for five h and treated with sterile distilled water, chlorine, peracetic-acid, and SAS for zero and 10 min and eight h. Positive and negative controls were also included. *Escherichia coli* O157:H7 populations were enumerated by plating on tryptic soy and sorbitol McConkey agar. Biofilm disruption was visually analyzed using scanning electron microscopy (SEM). Coupons were prepared for SEM through chemical fixation, dehydration, and a gold/palladium surface-coating. Surfaces were viewed between 1,000 and 40,000x magnification. Data was analyzed using one-way ANOVA (P<0.05).

Results: Compared to the positive control, all treatments except water and 0.1% SAS reduced pathogen populations to undetectable levels immediately. At zero min, the 0.1% SAS treatment reduced the pathogen levels by 3.4 log CFU/cm^2, followed by complete reduction at eight h. The SEM images supported the quantification results and showed significant reduction in viable bacterial cells, in the form of deflated cells. SEM images of HDPE treated with one and three percent SAS also showed visual disruption of biofilms. However, biofilm disruption with chlorine and peracetic acid was not observed.

Significance: Sodium acid sulfate could be used to control *E. coli* O157:H7 biofilms in the food industry.

P2-47 Validation of the USDA Official Method Neutralization Step/Buffer for a Novel Antimicrobial Solution of Five Percent Lactic Acid Plus Surfactants

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Introduction: USDA-FSIS has established methods for isolation and identification of foodborne pathogens, known as the *Microbiology Laboratory Guidebook*. Within methods 5.09 and 5B.05, modified tryptone soy broth (mTSB) is recommended as the primary enrichment buffer for *Escherichia coli* isolation. Furthermore, Appendix 1.09 provides ingredients for a neutralizing buffer. For new antimicrobials, USDA requires validation that there is no carryover effect, or interference, by new processing aids with enrichment buffers.

Purpose: To validate that use of five percent Purac Evolve (five percent lactic acid, surfactant, and gum) on beef tissues during slaughter does not interfere with neutralization or enrichment capacity of USDA-FSIS official method buffers when testing for *E. coli* O157:H7.

Methods: Brisket (fat side) was trimmed into 100 cm^2 sections and inoculated with a Shiga toxin-producing *Escherichia coli* (STEC) surrogate cocktail (ATCC BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) at ca. three log CFU/cm^2. Following 30 min attachment (room temperature), samples were sprayed with 10 ml of five percent treatment or water (control). A core (1.2 cm diameter) was excised from the surface of each sample pre- and post-treatment (10 min treatment), diluted, and enumerated on sorbitol MacConkey agar (SMAC). Additional cores were diluted in one of three buffers (mTSB, USDA-prescribed neutralizing buffer, or buffered peptone water) at a 1:4 ratio, incubated (42°C) for 24 h, streaked for presence/absence, and enumerated on SMAC.

Results: There was no difference (P>0.05) in the results for presence/absence recovery of presumptive *E. coli* following USDA-mandated protocol. In addition, surrogate counts on control and treated beef were not significantly (P>0.05) different.

Significance: Using the novel five percent lactic acid plus surfactants treatment as an antimicrobial intervention/processing aid on beef does not interfere with the neutralization capacity of USDA official method buffers when testing for *E. coli* O157:H7.

Poster

P2-48 Antimicrobial Properties of Ohelo Berry (*Vaccinium reticulatum*) Fractions: Anthocyanins, Non-Anthocyanin Phenolics, and Organic Acids

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❖ ***Developing Scientist Entrant***

Introduction: Ohelo berry, a wild relative of cranberry, is an endemic plant in Hawaii. Our preliminary study suggested ohelo berry extract has strong antimicrobial activity, which might be attributed to various bioactive compounds it contains.

Purpose: The purpose of this study was to determine which constituents of ohelo berry have the most potent antimicrobial effect on pathogenic bacteria.

Methods: The crude extract of ohelo berry was separated using a C-18 Sep-Pak cartridge into sugars plus organic acids (F1), non-anthocyanin phenolics (F2), and anthocyanins (F3). Each fraction was analyzed for pH, °brix/acid ratio, phenolics, and anthocyanin content. The antimicrobial properties of the three fractions at their native pH and neutral pH were evaluated against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Listeria monocytogenes* via the agar well diffusion assay. Moreover, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the fractions were determined against the four bacteria.

Results: F3 showed the highest concentrations of total phenolics and anthocyanins among all three fractions. None of the fractions generated inhibition zone against *E. coli* O157:H7 or *Salmonella* Typhimurium in agar. However, they caused a significant reduction ($P<0.05$) of all tested bacteria in broth in 24 h. F1 at native pH had the same MIC (1.39/0.26 °brix/acid) and MBC (5.55/1.06 °brix/acid) against the four bacteria. After neutralization, F1 lost the antimicrobial activity against any bacteria. The largest inhibition zones against *S. aureus* and *L. monocytogenes* were generated by F3, at 16.5 and 14.25 mm, respectively. The MIC of F3 against *S. aureus* and *L. monocytogenes* were 6.85 and 13.69 cyanidin-3-glucoside equivalent mg/L, respectively, which were not affected by neutralization.

Significance: Both phenolics and organic acids contribute to the antimicrobial properties of ohelo berry. They have the potential to be used as natural antibacterial agents in the food industry.

P2-49 Comparison of the Antimicrobial Activities of *Ohelo* Berry (*Vaccinium reticulatum*) and Cranberry (*Vaccinium macrocarpon*)

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❖ ***Developing Scientist Entrant***

Introduction: Ohelo berry (*Vaccinium reticulatum*) is a Hawaiian wild relative of cranberry (*Vaccinium macrocarpon*). Previous research indicates that ohelo berry is a rich source of phenolic compounds, which may hold antimicrobial potential.

Purpose: This study aimed to determine and compare the phenolic contents and antimicrobial activities of ohelo berry and cranberry.

Methods: The concentrations of phenolic compounds and anthocyanins in the crude extracts of ohelo berry and cranberry were determined by the Folin Ciocalteu method and the pH differential method, respectively. Both extracts were evaluated against four pathogenic and two probiotic bacteria via the agar well diffusion assay. Moreover, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined against the six bacteria. Transmission electron microscopy (TEM) was used to assess the damages of *Escherichia coli* O157:H7 and *Listeria monocytogenes* cell structures caused by ohelo berry extract.

Results: Ohelo berry extract had significantly higher total phenolic content (21.15 vs 6.81 gallic acid equivalent mg/ml) and lower anthocyanins (477.42 vs 996.59 cyanidin-3-glucoside equivalent mg/l) than cranberry extract. In the agar well diffusion assay, the inhibition zones generated by ohelo berry and cranberry extracts against the same bacteria were not significantly different. However, *E. coli* O157:H7 was more sensitive to cranberry extract than ohelo berry extract, as indicated by MBC of 6.25% vs 12.5%. Lower MIC and MBC were shown with ohelo berry extract against *Staphylococcus aureus* and *L. monocytogenes* than cranberry extract. Moreover, probiotic *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* were most resistant to the extracts. TEM disclosed that *ohelo* berry extract caused significant damages, including irregular shapes and localized disintegration of outer membranes, on *E. coli* O157:H7 and *L. monocytogenes* cells.

Significance: Ohelo berry has antimicrobial activity comparable to cranberry. It might be used as a natural preservative and a functional food.

P2-50 Effects of Sodium Lactate on the Growth of *Bacillus cereus* in a Rice-based Model Food

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Introduction: Sweet and salty rice-based food products are a popular food worldwide, and the main pathogenic microorganism of concern for these products is *Bacillus cereus*. Its spores can survive cooking temperatures and grow at temperatures commonly used for storage of rice-based products.

Purpose: The objective of this study was to determine the effect of sodium lactate as an antimicrobial on the growth of *B. cereus* in rice-based food products.

Methods: Starch mixtures of rice flour and water (4:6), two percent NaCl, and one, two, and three percent sodium lactate were inoculated with *B. cereus* spores to 10^2 to 10^3 CFU/g, and heated at 80°C for 15 min to cook the starch and activate the spores. Samples were stored at 16, 22, and 30°C and the populations of *B. cereus* during storage were determined. The growth curves were used to estimate the lag phase duration (LPD), growth rate (GR), and maximum population density (MPD).

Results: In samples containing one to three percent lactate stored at 16, 22, and 30°C, the LPD of *B. cereus* were 10 to 25, 5 to 15, and two to four h, respectively, and the GR were 0.11 to 0.10, 0.20 to 0.13, and 0.57 to 0.30 log CFU/h, respectively. In samples containing three percent lactate, the MPD of *B. cereus* reached 4.6 and 4.8 log CFU/g stored at 16 and 22°C, respectively, whereas the MPD reached greater than six log CFU/g in other samples. Results showed that the increase of lactate concentration significantly extended the LPD of *B. cereus* at 16 and 22°C and reduced the GR at 22 and 30°C, and three percent lactate reduced the MPD at 22 and 16°C.

Significance: This study identified the levels of sodium lactate that have significant effect in reducing the growth of *B. cereus* in rice-based food products. The information could help the producers to improve product safety by using lactate levels that are applicable to their products' storage temperatures.

Poster

P2-51 Discovery of Novel Small Molecules, Metabolites and Probiotic Strains from Plant Ecosystems to Control Foodborne Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: The seed microbial community consists of both beneficial bacteria and fungi. Seed endophytes can assist plant cells by providing beneficial compounds and protecting the host organism from plant pathogens, by producing antimicrobial compounds. These biological compounds could be used in medicine, agriculture and even in the food industry. *Listeria monocytogenes* and *Cronobacter sakazakii* are both important foodborne pathogens. They can survive for long periods of time in low-moisture foods and can potentially grow in products such as reconstituted powdered infant formula and a wide variety of other RTE foods. The discovery of novel compounds from bacterial endophytes can also be very useful with regards to food applications such as packaging or coating treatment.

Purpose: The objective of this study is to isolate bacterial endophytes from fruit seeds to see whether they are capable of producing antimicrobial compounds that can decrease or inhibit the growth of *L. monocytogenes* and/or *C. sakazakii*.

Methods: Tropical fruits, collected from retail stores in Canada, were processed under sterile conditions. Seeds were collected and washed with peptone water. Consequently, bacterial endophyte libraries were constructed from these tropical fruit seeds and identified by using 16S rRNA sequencing. In addition, isolated bacterial endophytes were screened for their inhibitory activity against *L. monocytogenes* and *C. sakazakii* by using the growth inhibition test.

Results: The number of different phenotypes of bacterial endophytes found inside papayas, sugar apples, dragon fruits and guavas were 28, 13, 59, 13, respectively. The strains identified as *Bacillus* spp. had antagonistic activity against *L. monocytogenes* and *C. sakazakii.* Whole genome sequencing is being conducted to probe the genome of these endophytes looking for the potential antimicrobial compounds.

Significance: These data above demonstrate that we can develop bio-protective compounds from bacterial endophytes to control foodborne pathogens such as *L. monocytogenes* and *C. sakazakii*.

P2-52 Effect of Gallic Acid and Protocatechuic Acid on *Salmonella* Typhimurium

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❖ ***Developing Scientist Entrant***

Introduction: An increase in the incidence of antibiotic resistant *Salmonella* Typhimurium has led to the search for bioactive phytochemicals that can serve as alternative antimicrobials. Phenolic compounds are a diverse group of molecules that have been found to have antimicrobial and anti-inflammatory properties and are abundant in various fruits and vegetables. However, their inhibitory mechanisms of action against the bacterial pathogens are not fully understood yet.

Purpose: To elucidate the antimicrobial effectiveness of gallic acid (GA) and protocatechuic acid (PA), two prevalent phenolic compounds, against the survival ability and expression of virulence genes of *Salmonella* Typhimurium.

Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both gallic and protocatechuic acids were evaluated In Vitro by separately treating *Salmonella* Typhimurium with increasing concentrations of the compounds (0.5 mg/ml to four mg/ml) at 37°C. The relative expression of genes which are crucial for the survival and pathogenesis of *Salmonella* Typhimurium (*hilA*, *fliC*, *invH*, *sipA* and *prgK*) was also evaluated in the presence of sublethal concentrations of both compounds. For statistical analysis, Student's *t* test was used.

Results: *Salmonella* Typhimurium treated with both GA and PA demonstrated a significant ($P<0.05$) growth reduction (ranges from 0.27 log CFU/ml and 0.14 log CFU/ml to no visible growth, respectively) in a concentration dependent manner. MBC and MIC of GA were four mg/m and 3.5 mg/m, respectively. For PA, MBC and MIC were 2.5 mg/ml and two mg/ml, respectively. Relative gene expression of *Salmonella* Typhimurium was significantly reduced ($P<0.05$) when the *Salmonella* Typhimurium cells were pretreated with GA (three mg/ml), showing a log-fold reduction of 3.04, 3.71, 2.27, 2.52 and 2.50 for the genes *hilA*, *fliC*, *invH*, *sipA* and *prgK*, respectively. Gene expression of *Salmonella* Typhimurium cells treated with PA (two mg/ml) was also significantly ($P<0.05$) reduced by 0.89, 1.87, 0.78, 0.08 and 0.12 for *hilA*, *fliC*, *invH*, *sipA* and *prgK*, respectively.

Significance: Our findings suggest that GA and/or PA have the potential to inhibit the growth as well as alter virulence properties of *Salmonella* Typhimurium.

P2-53 Validation of Vinegar Powder to Control *Listeria monocytogenes*, *Salmonella enterica*, Shiga Toxin-producing *Escherichia coli*, and Lactic Acid Bacteria in Fresh Chicken Salad

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Introduction: Ready-to-eat deli salads pose a risk for foodborne illness. The low pH of deli salads typically prevents pathogen growth; however, higher pH products have entered the marketplace. Verdad Powder N6 is a dried vinegar powder used in RTE meat products to enhance food safety and extend shelf-life.

Purpose: To assess the antimicrobial performance of vinegar powder to control pathogenic and spoilage microorganisms in refrigerated chicken salad.

Methods: Chicken salad (65% rotisserie chicken and 35% salad dressing) was prepared with and without 0.50% dried vinegar powder. Independent pathogen cocktails of *Listeria monocytogenes*, *Salmonella enterica*, and Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 were prepared. Ten µl from each was independently inoculated into 25-g chicken salad samples. Samples were stored at 4.4°C (*Listeria*) or 7.2°C (remaining samples) and enumerated on ca. day zero, four, eight, 12, and 22. Pathogen enumeration was performed on MOX agar (*Listeria*), XLT-4 agar (*Salmonella*) and SMAC (STEC), all incubated (35°C) for 48 h. Non-inoculated samples were enumerated on TSAYE agar (35°C for 48 h) and MRS agar (lactic acid bacteria; 35°C for 72 h).

Results: *L. monocytogenes* populations reached one log CFU/g outgrowth by day six in control and by day 15 in treatment. By day four, *Salmonella* counts were significantly ($P\leq0.05$) lower in treatment (2.24 log CFU/g) compared to control (3.24 log CFU/g). On day eight, control STEC counts (7.13 log CFU/g) were significantly ($P\leq0.05$) greater than treatment (2.75 log CFU/g). Control aerobic plate counts were significantly ($P\leq0.05$) higher (6.64 log CFU/g) compared to treatment (3.43 log CFU/g) on day four onward. Control LAB (6.71 log CFU/g) were significantly higher ($P\leq0.05$) than treatment (4.73 log CFU/g) by day 12.

Significance: Application of vinegar powder in chicken salad controls microorganisms and extends shelf life.

Poster

P2-54 Assessment of Five Percent Lactic Acid Plus Surfactants and Ten Percent Lactic Acid Antimicrobial Interventions for Spoilage Microorganism Growth and Survival on Beef Tissues

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Introduction: USDA-FSIS has approved lactic acid as an antimicrobial intervention during beef processing to control pathogens. Processing aids should not alter the growth kinetics of spoilage microflora and should have primary action against pathogens. A novel and 10% lactic acid solution need assessment on their impact on lactic acid bacteria (LAB) growth kinetics.

Purpose: To substantiate that the use of five percent Purac Evolve (five percent lactic acid, surfactant, and gum) or 10% lactic acid on lean and fat beef tissues does result in a residually active antimicrobial effect on spoilage microflora.

Methods: Brisket (lean and fat sides independently sampled) was trimmed into 100 cm^2 sections and inoculated with a *Leuconostoc* spp. spoilage cocktail at three log CFU/cm^2. Following a 20 min attachment period (room temperature), samples were sprayed with 10 ml of novel five percent lactic acid plus surfactants solution, five percent lactic acid solution, 10% lactic acid solution, 600 ppm peroxyacetic acid, or tap water (control). Samples were incubated at 4.4°C in overwrapped film trays and enumerated on ca. day zero, three, 10, and 14. At sampling, three cores (1.2 cm diameter) were excised from the respective sample, diluted, and enumerated on MRS agar following incubation at 30°C for 48 h.

Results: LAB growth behavior over the course of 14 days showed that five percent novel lactic acid treatment solution and 10% lactic acid treatment behaved similarly to control treatment. By day 14, growth kinetics of LAB in all treatments within tissue types were not significantly (P>0.05) different. All treatments showed similar growth curves with noticeable lag and exponential phases.

Significance: Application of five percent novel lactic acid treatment or 10% lactic acid antimicrobial solutions does not impact the growth kinetics of LAB in lean and fat beef. Treatments are not conferring any residual efficacy and are similar to USDA-approved processing treatments and water.

P2-55 Optimization of the Functionality of Sanitizers and Nisin Using Response Surface Methodology: Control of *E. coli* O157:H7 ATCC 43888 and *L. monocytogenes* ATCC 7644 Biofilm

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Introduction: Listeria monocytogenes and *E. coli* can adhere to all the materials commonly used in the food industry. In biofilms *L. monocytogenes* and *E. coli* are significantly more resistant to disinfection than their free-living counterparts. Several novel approaches to disinfection have been proposed, but high costs, practical difficulties or resistance problems limit their practical use. Biofilms resist antimicrobial treatment agents and the most proposed methods when used individually are not effective in exterminating foodborne pathogens and biofilms.

Purpose: To investigate and optimize the efficacy of a composite of sodium dodecyl sulphate (SDS), sodium hychlorite (NaClO) and nisin with the use of response surface methodology.

Methods: A combination of sodium dodecyl sulphate (SDS) (concentration: 200, 400 and 600 ppm), sodium hypochlorite (NaClO) (concentration: 100, 150 and 200 ppm), nisin (concentration: 1000, 5500 and 10000 IU), temperature (25 and 37°C) and time (10, 20 and 30 min) was applied to decontaminate *Escherichia coli* O157:H7 ATCC 43888 and *L. monocytogenes* ATCC 7644 from stainless-steel coupons. Using a Box–Behnken experimental design, predictive quadratic equations were developed for treatment-based population reductions of *E. coli* O157:H7 (R^2=0.92, P<0.001) and *L. monocytogenes* ATCC 7644 (R^2=0.87, P<0.001), and verified using 42 randomly selected treatment conditions.

Results: Among five factors (temperature, time, and concentration of SDS, NaClO, or nisin), temperature had higher significance for inactivation of both pathogenic bacteria. Significant differences (P<0.05) were observed in biofilm quantity between treated and untreated coupons. The optimum treatment conditions were 37°C with 200 ppm NaClO, 400 ppm SDS, and 10000 IU nisin for 30 min for *E. coli* O157:H7 ATCC 43888, and 25°C with 200 ppm NaClO, 150 ppm SDS, and 5500 IU nisin for 30 min for *L. monocytogenes* ATCC 7644. Scanning electron microscopy was used to confirm membrane disruption in the treated microbial cells in each optimal condition. The combined treatment of SDS, NaClO and nisin contributes to the effective inactivation (more than four-log reduction) of both *E. coli* O157:H7 and *L. monocytogenes* on stainless-steel coupons.

Significance: Biofilms resist antimicrobial treatment agents and the most proposed methods when used individually are not effective in exterminating foodborne pathogens and biofilms. Therefore, hurdle technology, a combination of two or more different control techniques can be effective in controlling biofilm.

P2-56 Purification and Structural Elucidation of Paraplantaricin TC318, a Novel Natural Antimicrobial Food Preservative Produced by *Lactobacillus paraplantarum*

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❖ ***Developing Scientist Entrant***

Introduction: Food processors use antimicrobial preservatives to eliminate microorganisms that spoil food or make it unsafe to consume. However, pathogenic and spoilage bacteria are gaining resistance against currently-used preservatives. Therefore, there is a strong research interest in the discovery of new, natural and effective food preservatives.

Purpose: The current study was initiated in search for novel and effective antimicrobials produced by lactic acid bacteria (LAB) to be used as natural food preservatives

Methods: Fermented food products were screened for antimicrobials produced by LAB. A promising bacterium, isolated from a Turkish cheese, produced a stable antimicrobial peptide. The bacterium was identified using mass spectrometry (MS) bio-typing and 16S rRNA gene sequencing. Identity and structure of the antimicrobial peptide were elucidated using MS analysis and bacterial whole genome sequencing. Briefly, the antimicrobial peptide was extracted from the producer's culture using 70% isopropanol and purified using high performance liquid chromatography. The molecular mass, amino acid sequence, and preliminary structure of the antimicrobial peptide were determined using MS analyses and confirmed by bioinformatic analysis of whole genome.

Results: A strain of *Lactobacillus paraplantarum*, isolated from Turkish cheese, produced an antimicrobial peptide that inhibits *Clostridium sporogenes* spores outgrowth, *Bacillus cereus*, *Micrococcus luteus*, *Lactobacillus casei* and *Pediococcus pentosaceus*. The MS and whole genome analyses, taken together, allowed elucidating the structure of a new peptide. The new antimicrobial, designed as Paraplantaricin TC318, has a molecular mass of 2263.900 Da, consists of 22 amino acids and contains four thioether bridges. The amino acid sequence of Paraplantaricin TC318 was determined to be a new member of the epidermin group which belongs to class I antibiotics.

Poster

Significance: Being produced by LAB, and having antimicrobial activity against bacterial spores, Paraplantaricin TC318 is a promising food preservative candidate.

P2-57 Assessment of Probiotic Traits, Antimicrobial Characteristics and Safety of *Enterococcus durans* Osy-Egy Isolated from Artisanal Hard Cheese

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❖ ***Developing Scientist Entrant***

Introduction: Enterococci are lactic acid bacteria found as natural microbiota in fermented food. Some enterococci produce antimicrobial peptides that inhibit foodborne pathogens. A promising *Enterococcus durans* strain, designated OSY-EGY, was isolated from artisanal cheese and showed potent antimicrobial activity. Suitability of this strain to food applications needs to be assessed.

Purpose: Evaluate the probiotic, antimicrobial and safety traits of *E. durans* OSY-EGY based on its genomic and phenotypic characteristics.

Methods: Sequencing of OSY-EGY whole genome was performed to understand the genetic basis of its antimicrobial activity and probiotic characteristics. Furthermore, the genome sequence was screened for genes encoding antimicrobial resistance and virulence factors. To evaluate the probiotic, antimicrobial and safety aspects of OSY-EGY, the following analyses were completed: antimicrobial peptides production, acid and bile salt tolerance, antioxidants activity, cholesterol assimilation, adherence to Caco-2 cells, antibiotic resistance, and hemolysin and gelatinase production.

Results: The mining of OSY-EGY genome revealed genes encoding novel antimicrobial peptides, proteins responsible for cell adhesion, acid resistance, bile tolerance, and antioxidant activity. The genome was void of genes encoding antibiotic resistance or virulence factors. Phenotypically, the OSY-EGY strain was relatively tolerant to acidic pH (3.0), and presence of 0.3% bile salts. The bacterium showed adhesion capability to Caco-2 cells (45% adhesion), cholesterol lowering potential (44.7% reduction) and DPPH scavenging potential (nine percent). The antimicrobial peptides produced by OSY-EGY were effective against *Listeria monocytogenes*, *Staphylococcus aureus*, *E. faecalis*, and *Bacillus cereus*. Additionally, OSY-EGY culture extract inhibits *L. monocytogenes* biofilm formation. More importantly, OSY-EGY is vancomycin sensitive and does not express any hemolytic or gelatinase activity.

Significance: The current work established that *E. durans* OSY-EGY is a safe and beneficial strain that possesses potential probiotic traits and potent antimicrobial activity. These findings support the feasibility of using this strain in food preservation and in promoting human health.

P2-58 WITHDRAWN

P2-59 Interactions of Carvacrol, Caprylic Acid, Habituation, and Mild Heat for Pressure-based Inactivation of O157 and Non-O157 Serogroups of Shiga Toxin-producing *Escherichia coli* in Low-Acid Environments

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Introduction: From 1998 to 2017 at least 599 foodborne outbreaks in the United States were associated with contaminated food products with O157 and non-O157 serogroups of Shiga toxin-producing *Escherichia coli*.

Purpose: The current study investigated synergism of elevated hydrostatic pressure, habituation, mild heat, and antimicrobials for inactivation of O157 and non-O157 serogroups of *Escherichia coli.*

Methods: Various times (zero, one, three, five, and seven minutes) at a pressure intensity level of 450 MPa (65K PSI) were investigated at 4 and 45°C with and without presence of carvacrol and caprylic acid (100 to 500 ppm) before and after seven-day aerobic habituation of O157 and non-O157 serogroups of Shiga toxin-producing *Escherichia coli* inoculated in blueberry juice. Experiments were conducted in three biologically independent repetitions each consist of two replicates. The study was analyzed as a randomized complete block design using GLM procedure of SAS followed by Tukey-adjusted mean separation.

Results: Under the conditions of this experiment, habituation of the pathogen played an influential (P<0.05) role in inactivation rate. As an example, O157 and non-O157 serogroups were reduced (P<0.05) by 1.41 and 1.63 log CFU/ml after a 450 MPa treatment at 4°C, respectively, before habituation. The corresponding log reductions after seven-d aerobic habituation were 2.64, and 3.31, respectively. Carvacrol and caprylic acid both augmented the decontamination efficacy of the treated samples. As an example, *Escherichia coli* O157 were reduced (P<0.05) by 2.64 and 4.17 log CFU/ml after a seven-min treatment at 450 MPa without, and with presence of carvacrol, respectively.

Significance: Results of current study indicate an optimized pressure-based intervention in presence of mild heat and antimicrobial agents could be efficacious for inactivation of >99.9% of microbial pathogens. Current experiment also exhibits the critical role of habituation on increasing the external validity of a microbial challenge study.

P2-60 Synergism of Mild Heat, Nisin, and Elevated Hydrostatic Pressure for Inactivation of *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Epidemiological evidence derived from CDC's active surveillance data indicate >98% of human Listeriosis cases are foodborne in nature with about 94% and 15.9% hospitalization and death rates, respectively.

Purpose: Current study investigated inactivation of *Listeria monocytogenes* using mild heat, hydrostatic pressure, and nisin in buffered environment.

Methods: Four-strain mixture of *Listeria monocytogenes* were exposed to 0, 3, 6, and 9 minutes of six treatments: A) hydrostatic pressure at 4 °C; B) hydrostatic pressure and nisin at 4 °C; C) nisin at 4 °C; D) heat at 40 °C; E) hydrostatic pressure at 40 °C; F) hydrostatic pressure and nisin at 40 °C. Pressure intensity level of 400 MPa (Hub880 Explorer, Pressure BioScience Inc), and nisin concentration of 5000 IU/ml were used for the experiments of inoculated pathogen in phosphate buffered saline. The unit temperature was precisely controlled and monitored by a stainless steel water jacket surrounding the pressure chamber connected to a refrigerated circulating water bath. Analyses of variance were conducted followed by LSD-based mean separation by OpenEpi software.

Results: The six treatments after 9 minutes were all resulted in reductions ($P < 0.05$) of the pathogen. These reductions were 4.5, 4.3, 4.9, 3.1, 5.2, and 4.5 log CFU/ml for treatments of A to F, respectively. Under the condition of this experiment, antimicrobial efficacy of nisin was affected only modestly through synergism with elevated pressure and mild heat. As an example, counts of samples treated for 6 minutes were similarly ($P \geq 0.05$) 2.9 ± 0.4, 3.3 ± 0.7, and 2.9 ± 0.5 for samples treated with nisin alone, nisin and hydrostatic pressure, and nisin and heat, respectively.

Poster

Significance: Results of this study could be incorporated as part of hazard analysis for meeting requirements of FSMA Human Food rule for mitigating the public health burden of foodborne Listeriosis.

P2-61 Reproducible Inactivation of *Staphylococcus aureus* on a Surface Using UV LED

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Introduction: *Staphylococcus aureus* contamination of food processing and contact surfaces is a source for foodborne infection for consumers of meat and meat products

Purpose: In this study surfaces contaminated with high loads of *Staphylococcus aureus* were exposed to 265 nm UV-C LED to assess the effects of multiple doses and irradiances.

Methods: One-inch square stainless steel targets inoculated with *S. aureus* were exposed to a UV-C LED (265 nm) array light source (Phoseon Technology) at 1.3, 1.5, 2.0, 2.5 and 3.0 mW/cm^2 (at the target) from a distance of 15 mm. Doses ranged from 26 mJ/cm^2 through 150 mJ/cm^2 . Surviving bacteria were plated and colony forming units (CFU) assessed. Log reduction was calculated as the difference in the log of geometric means between the unexposed control and the exposed test samples. Each test sample included four independent exposures at each condition.

Results: Irradiances of 1.3 and 1.5 mW/cm^2 resulted in four to five-log reduction of *Staphylococcus aureus* CFU on exposed targets. This corresponded to doses of between 20 and 52 mJ/cm^2. Increasing the irradiance to 2, 2.5, and 3.0 mW/cm^2 to deliver a dose of 150 mJ/cm^2 resulted in a five-log reduction in all cases.

Significance: Short 265 nm UV-C exposures of ≤60 seconds were sufficient to result in a four-log reduction of *Staphylococcus aureus*. Treatment of food products by 265 nm UV-C LEDs represents a viable investigation path for decreasing food-borne *Staphylococcus aureus* infections in consumers.

P2-62 Development a Pilot Plasma Device to Inactivate *Salmonella* spp. on Shell Eggs

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Introduction: Eggs are one of the most consumed food items. Nowadays, disinfection of shell eggs mainly depends on chlorinated water washing, which is effective and affordable but has a negative environmental impact. Plasma is a novel technique without a chemical residual issue. In addition, plasma can be generated under atmospheric pressure at 40-50˚C which is also the commonly used temperature for egg disinfection.

Purpose: We previously demonstrated the high efficacy (greater than five-log CFU/egg reduction) of plasma jets against *Salmonella enterica* serovar Enteritidis on shell eggs on a laboratory scale. Thus, the purpose of this research was to develop a pilot device for commercial use.

Methods: The plasma was generated by argon gas and the eggs were placed on a conveyor belt directly under the plasma jet. To mimic commercial operation, the device was equipped with two jets that operated simultaneously to treat fast moving eggs on the conveyor belt. Two power levels, 300 and 400 W, along with moving speed at 600 or 900 eggs/hr, 30 standard liters per minute (slm) flow rate, and four cm distance between egg and plasma jet were tested. The quality characteristics of eggs, such as egg yolk and albumen index, egg density, and sensory qualities were also determined.

Results: Higher power resulted in a higher reduction. At 400 W, *Salmonella* reduction was achieved at 3.47 and 3.14 CFU/egg at the speeds of 600 and 900 eggs/hr, respectively. In addition, the quality characteristics of plasma-treated eggs were not significantly different from untreated controls.

Significance: This research was the first pilot-scale testing using plasma to inactive shell eggs in Taiwan and results showed plasma could be an effective method for egg sanitation in commercial practice.

P2-63 Heat Resistance of *Clostridium perfringens* Vegetative Cells in *Sous Vide* Processed Ground Beef Supplemented with Grape Seed Extract

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Introduction: *Sous vide* cooked meats are considered as suitable environments for *C. perfringens.* The time and temperature combinations applied during *sous vide* cooking generally are not adequate to destroy *C. perfringens* spores; also, the cooking triggers spore germination. Therefore, the reheating step is a critical step for ensuring the safety of *sous vide* cooked meat in respect to hazards associated with *C. perfringens* vegetative cells.

Purpose: The objective of this study was to quantify the effects of grape seed extract (GSE) on the heat resistance of a three-strain cocktail of *C. perfringens* vegetative cells in *sous vide* processed ground beef.

Methods: Inoculated meat supplemented with zero to three percent GSE in sterile bags was completely immersed in a circulating water bath stabilized at 55, 57.5, 60 or 62.5°C for a predetermined period of time. The surviving cell population was enumerated on tryptose–sulfite–cycloserine agar. Survival curves were obtained by plotting log survivors against time for each heating temperature and the *D*-values were determined from the straight-line portion of the survival curves using Excel Data Tool Pack.

Results: The *D*-values in beef that included no GSE were 67.11±2.62, 17.15±0.00, 4.02±0.00 and 1.62±0.02 min at 57.5, 60, 62.5, and 65°C, respectively. Addition of one percent GSE resulted in concomitant decrease in heat resistance as evidenced by reduced bacterial *D*-values. The *D*-values in beef with added one percent GSE were 62.89±0.40, 13.70±0.48, 3.47±0.17 and 1.46±0.01 min at 57.5, 60, 62.5, and 65°C, respectively. The heat resistance was further decreased when the GSE concentration in beef was increased to two or three percent. The *z*-values in beef with or without GSE were similar, ranging from 4.41 to 4.56°C.

Significance: The results of this study would be beneficial to retail and institutional food service establishments in estimating reheating time and temperature for *sous vide* processed ground beef to ensure safety against *C. perfringens*.

P2-64 Use of Pathogen-specific Bacteriophages to Reduce the Viability of *Escherichia coli* O157:H7 on Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: The increasing incidence of foodborne illness associated with the consumption of raw fruits and vegetables is a growing concern for consumers worldwide. In 2016, the Center for Disease Control and Prevention (CDC) estimated that approximately 48 million new cases of foodborne illness

are being reported every year resulting in 3,000 deaths. Among the diverse array of pathogens that cause foodborne illness, Shiga-toxin producing *E.coli* is one of the top five pathogens that is responsible for over 60 deaths each year in the United States.

Purpose: The objective of the study was to determine the ability of bacteriophages to infect and reduce *E. coli* O157:H7 (EHEC) contamination on fresh produce.

Methods: Lytic bacteriophages isolated from environmental samples were spot tested against EHEC to determine their effectiveness in reducing the pathogen concentration. A microplate growth inhibition assay (n = 36) was used to determine the effect of individual phages and the phage in combination against EHEC. Tomatoes (n = 18) and spinach (n =18), inoculated with the EHEC, were washed in a dunk tank supplemented with a phage cocktail. Wash water and produce samples were tested at 0, 3, 6, 9, and 12 hours to determine the effectiveness of the phage cocktail.

Results: The data, from the microplate, tomato, and spinach study indicates that there was a significant (P<0.05) reduction in the amount of EHEC population compared to the control. The tomato and spinach study indicated a three-log reduction of the pathogen by the end of six hours; however, there was bacterial recovery by the end of 12 hours leading to an overall 2.5-log reduction from the initial inoculum. These results demonstrate that a phage cocktail could potentially act as an antimicrobial to inactivate EHEC and reduce their incidence in produce.

Significance: The results from the studies indicate that the phage cocktail can be commercially used to reduce or eliminate EHEC contamination in fresh produce.

P2-65 Cross-resistance to Phage Infection in *Listeria monocytogenes* Serotype 1/2a

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a foodborne pathogen capable of causing severe illness with a high mortality rate. Bacteriophage products are currently approved for the control of *Listeria* within the food industry. However, bacteriophage resistance is an important consideration regarding their use.

Purpose: The objective of this study was to determine if select *L. monocytogenes* serotype 1/2a strains with known resistance to well characterized phages would possess similar resistance to a diverse collection of *Listeria* phages representing different morphologies and genomic orthoclusters.

Methods: Screening of 120 phages against *L. monocytogenes* 10403S, two 10403S mutant strains whose WTA (wall teichoic acids) lack either rhamnose or GlcNAc (N-acetylglucosamine), and a unique uncharacterized phage-resistant 10403S mutant, was conducted. Each phage was spotted onto duplicate bacterial lawns of each host to determine their efficiency of plaquing or phage activity level. Efficiency of plaquing was determined by observation of visible plaques; phage activity was determined by observation of bacterial growth inhibition as compared to a buffer control. Hierarchical cluster analysis was performed on the averages of three replicates for both plaquing and phage activity.

Results: Only one phage formed plaques on the mutant strain lacking rhamnose in its WTA, and only two other phages showed activity against it. Fourteen phages showed activity or plaquing against the mutant strain lacking GlcNAc in its WTA. The uncharacterized phage-resistant mutant strain had 103 phages that showed activity or plaquing against it. Many of the phages screened showed activity on one or more hosts without formation of visible plaques.

Significance: Mutations in serotype 1/2a strains resulting in loss of rhamnose as a binding receptor leads to resistance that was overcome by only three phages out of 120. Knowledge of phages capable of combating this resistance can aid in the development of more useful bacteriophage products for food safety applications.

P2-66 Antibiotic Resistance Phenotyping and Genotyping of Verocytotoxigenic *Escherichia coli* Isolated from Irrigation Water in British Columbia, Canada and Their Susceptibility to Bacteriophages

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Introduction: The presence of antibiotic-resistant verocytotoxigenic *Escherichia coli* (VTEC) in water used for produce irrigation may be a threat to human health. Bacteriophages have been proposed as a biocontrol method against VTEC.

Purpose: To compare the antibiotic resistance profiles of VTEC isolates from irrigation water in the Lower Mainland of British Columbia, Canada based on phenotyping and DNA sequencing, and to examine their susceptibility to bacteriophages.

Methods: VTEC isolates (n=15) were screened for antibiotic resistance using the broth microdilution method and resistance breakpoints as outlined by the CLSI. VTEC whole genome sequences acquired using the Illumina HiSeq platform were queried against antibiotic resistance gene databases ARG-ANNOT and ResFinder to identify antibiotic resistance genes. Fifteen bacteriophages were isolated from sewage using VTEC as an enrichment host, and their ability to lyse VTEC isolates was tested by spotting five ul of 10^9 pfu/ml of phage lysate on bacterial lawns.

Results: Results showed that 100% (n=15) of VTEC isolates were susceptible to the antibiotics ceftriaxone, chloramphenicol, gentamicin and nalidixic acid. Six isolates (40%) were resistant to one or more of the following antibiotics: ampicillin (n=5), streptomycin (n=4), tetracycline (n=4), and trimethoprim-sulfamethoxazole (n=3), but nine isolates (60%) were susceptible to all eight tested antibiotics. Analysis of the VTEC genomes shows that resistant strains have corresponding known acquired resistance genes to ampicillin (TEM-1), streptomycin (*strA* and *strB*), tetracycline (*tetB*, *tetA* and *tetR*), and trimethoprim-sulfamethoxazole (*sul2* and *dfrA8)*, except for two ampicillin-resistant isolates. Spot tests show that 87% (n=13) of the bacteriophages were capable of lysing 73% (n=11) of the VTEC isolates, including 50% (n=3) of the antibiotic-resistant isolates.

Significance: Antibiotic-resistant VTEC with acquired antibiotic resistance genes may indicate horizontal gene transfer in the environment of British Columbia. Bacteriophages have lysing capabilities against VTEC, indicating their potential for biocontrol.

P2-67 Evaluation of Individual and Cocktails of Bacteriophages against Shiga Toxin-producing *Escherichia coli* and Their Biofilms

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❖ ***Developing Scientist Entrant***

Introduction: Biofilms of Shiga toxin-producing *Escherichia coli* (STEC) pose a significant challenge to the food industry due to their persistence. Conventional control methods reduce the number of pathogens but cannot efficiently disrupt biofilms. Bacteriophages could serve as an effective biofilm control in the food industry.

Purpose: Evaluate the efficacy of STEC-specific bacteriophages, individually and in cocktails, against STEC and their biofilms.

Methods: Bacteriophages (nine log PFU/ml) were evaluated against STEC (O157, O26, O111, O121, O103, O145, O45) and their biofilms, In Vitro and on food-contact surfaces. Phages were used as follows: i) individually against specific serotype; ii) serotype-specific cocktails; iii) 21-phage cocktail against STEC

Poster

Poster

serotypes. STEC biofilms were formed in microtiter-plates (24 h; 37°C) and on stainless-steel and high-density-polyethylene (HDPE) coupons (eight h; 25°C). Plates/coupons were treated with respective phage treatments or control and incubated (37°C). Absorbance (A_{595}) was measured to determine In Vitro biofilm disruption (zero, three, and six h). STEC survival on coupons was measured at zero, three six, and 16 h by plating on CHROM agar. Data were analyzed using one-way ANOVA (P<0.05).

Results: As individual In Vitro treatments, phages effectively disrupted biofilms, reducing absorbance from 2.262 nm (zero h) to 1.179 nm (three h), and 1.537 nm (six h). The O157-specific phages were more effective at six h, while the non-O157-specific phages were at three h. Phage cocktails specific to O111, O121, and O145 serotypes were more effective at three h, while the rest were more effective at six h. The 21-phage cocktail reduced absorbance at three h by 1.45. Greater STEC reduction was observed on HDPE (2.3 to 5.6 log) than on stainless steel (1.9 to 4.1 log), with O121-specific cocktail the most effective. The 21-phage cocktail was only effective at 16 h (stainless steel, 4.1 and HDPE, 4.8-log reduction).

Significance: Bacteriophages can be used individually or as serotype-specific cocktails more efficiently as biocontrol in the food industry.

P2-68 Reduction of *Aeromonas hydrophila* Contamination on Lettuce by Using a Novel *Aeromonas hydrophila*-specific Phage

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❖ ***Developing Scientist Entrant***

Introduction: *Aeromonas hydrophila* has been found in a wide range of foods and fresh produce. Due to the resistance of *A. hydrophila* to antibiotics, *A. hydrophila*-specific phage can be applied to control *A. hydrophila* contamination.

Purpose: The purpose of this study was to investigate the reduction effect of a novel *A. hydrophila*-specific phage (KFS-A9) against *A. hydrophila* contamination on lettuce.

Methods: KFS-A9 has been previously purified from a water sample obtained from a poultry plant. Fifty microlitres of *A. hydrophila* suspension (10^5 CFU/ml) was inoculated on the surface of UV-treated lettuce (two by two cm^2) and placed into a biosafety cabinet for its attachment during one h at 22°C. The same amount of KFS-A9 with MOI of 100 was spread on the *A. hydrophila*-contaminated lettuce and incubated for 30 min at 22°C. Sodium hypochlorite solution and PBS were used as a positive and negative controls, respectively. At every two-h interval, treated lettuce was then placed in a stomacher bag containing 15 ml of PBS for homogenization. Each homogenate was serially diluted for numeration of *A. hydrophila* using an *Aeromonas* selective medium.

Results: The number of *A. hydrophila* treated with KFS-A9 was reduced from initial number of four log CFU/cm^2 to 2.910 log CFU/cm^2 at 30 min and its reduction was sustained up to eight h. However, the number of *A. hydrophila* on the lettuce treated with PBS increased to 7.9 log CFU/cm^2, which was significantly greater than that of KFS-A9 treatment (P<0.05). Although sodium hypochlorite treatment showed bacterial reduction (3.190) at 30 min only, the number of *A. hydrophila* increased significantly after (P<0.05) and the final number of *A. hydrophila* was 7.76003 log CFU/cm^2.

Significance: This study demonstrated the bactericidal effect of KFS-A9 and its potential as a new biocontrol agent.

P2-69 Characterization of a Novel Bacteriophage, EscoHU1, Infecting Both *Escherichia coli* O157:H7 and *Salmonella*

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Introduction: *Escherichia coli* O157:H7 and *Salmonella enterica* are important pathogens worldwide. Bacteriophages are bacterial viruses and promising antimicrobial agents. We previously isolated a novel broad-host-range phage, EscoHU1, which belongs to the family *Siphoviridae*, infecting both *E. coli* O157:H7 and *S. enterica*.

Purpose: The purpose of this study was a characterization of EscoHU1 and examination of antimicrobial potential against *E. coli* O157:H7 and *S. enterica*.

Methods: Adsorption kinetics and one-step growth curves of EscoHU1 were examined using each culture of *E. coli* O157:H7 RIMD0509939 and *S. enterica* serovar Typhimurium IID1000 (OD_{600}=1.0). Also, thermal stability (40 to 70°C) and pH stability (pH 4.0 to 10.0) of EscoHU1 were determined in tryptic soy broth (TSB). Plaque forming units were determined by double agar overlay method. For challenge testing, EscoHU1 (10^8 PFU/ml) and *E. coli* or *S.* Typhimurium were inoculated and incubated in TSB at 30°C. Viable cell counts were determined and compared using Welch's *t*-test.

Results: Adsorption constants of EscoHU1 against *E. coli* O157:H7 and *S.* Typhimurium were 1.68×10^{-10} and 7.00×10^{-11} ml/min. Eclipse period, latent period, and burst size of EscoHU1 against *E. coli* O157:H7 were 10 min, 20 min, and 88±39 PFU/infected cell, respectively, and those against *S.* Typhimurium were 20 min, 20 min, and 100±22 PFU/infected cell, respectively. EscoHU1 was stable for heating at 50°C and incubation at pH 5.0 to 9.0. After EscoHU1 treatment, viable cell counts of *E. coli* O157:H7 and *S.* Typhimurium significantly decreased by 5.4 and 1.1 log CFU/ml compared to control (P<0.05).

Significance: EscoHU1 would be a good antimicrobial agent for the control of foodborne diseases by *E. coli* O157:H7 and *S. enterica*.

P2-70 Genomic Characterization of *Salmonella*-infecting Bacteriophages Isolated from British Columbia, Canada

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❖ ***Developing Scientist Entrant***

Introduction: Bacteriophage therapy is emerging as a novel biocontrol strategy in reducing *Salmonella* contamination of food products. Moreover, they represent an enormous reservoir of genetic materials, however, compared to the amount of publicly available host bacterial genomes, they remain largely underrepresented in the literature.

Purpose: The purpose of this work was to use comparative genomics as a tool to assess the diversity of a collection of 45 *Salmonella* phages isolated from British Columbia, Canada.

Methods: DNA was extracted using a modified phenol-chloroform method and sequenced using the Illumina Miseq platform, with subsequent assembly using SPAdes. Annotation of open reading frames was performed using the RAST pipeline and manual curation with NCBI BLAST. Comparative genomics analyses to assess diversity were conducted using a variety of phylogenetic-based methods.

Results: In Silico genomic analyses revealed an abundance of sequence diversity in our subset of sequenced phages and the elucidation of genes undesirable for biocontrol in foods (e.g., encoding for integrase, virulence factors, etc.). Sequence alignment of the genomes grouped our phages into 12 clusters with four singletons. Phages within certain clusters exhibited extraordinarily high genome homology (>98% nucleotide identity), yet between clusters, genomes exhibited a span of diversity (<50% nucleotide identity). Alignment of the major capsid protein also supported the clustering pattern observed with

alignment of the whole genome. We further observed novel associations between genomic relatedness and the site of isolation, as well as the carriage of interesting genetic elements related to DNA metabolism and virulence.

Significance: The data analyzed in this current work will help support the framework of knowledge we currently possess regarding phage diversity, and will further aid in the development of phage-based applications for food safety.

P2-71 Efficiency of a Phage Intervention against *Salmonella* on Lean Pork, Pork Trim and Bacon

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Introduction: An ongoing baseline sampling program by the Food Safety Inspection Service (FSIS), the Raw Pork Products Exploratory Sampling Program (2015), will provide direction to FSIS to develop a better risk profile and refine the present food safety guidelines for pork products. Thus, new impending regulatory standards will likely prompt establishments to seek interventions that can help in reducing the most probable number of *Salmonella* in their products.

Purpose: To determine the efficacy of a commercially available bacteriophage product, PhageGuard S, against *Salmonella* on several types of pork meat.

Methods: Overnight cultures of *Salmonella* Se13 (streptomycin resistant strain) were inoculated at a concentration of 2×10^4 CFU/cm^2 or CFU/g on lean pork, bacon or pork trims (duplicate samples per treatment). Subsequently, contaminated samples were treated with phage concentrations of 5×10^6, 1×10^7, 2×10^7 or 5×10^7 PFU/cm^2 or PFU/g, or water (negative control). After treatment, samples were stored at 40°F for 18 h before retrieval and enumeration of bacteria on selective agar plates. Reduction values of two individual experiments were used for statistical analysis (ANOVA).

Results: The application of phages at 10^7 and 5×10^7 PFU/cm^2 on lean pork resulted in 1.1 log CFU/cm^2 and 1.6-logCFU/cm^2 (P<0.05) reductions of *Salmonella,* respectively. On bacon, 5×10^7 PFU/cm^2 showed *Salmonella* reduction of 1.3 log CFU/cm^2 and the 5×10^6 PFU/cm^2 resulted in 0.8-log CFU/cm^2 reduction (P<0.05). When applied on pork trim, phage concentrations of 2×10^7 and 5×10^7 PFU/g showed *Salmonella* kill of 1.3 and 1.7 log CFU/g, respectively. Overall, a dose-response was observed where increasing phage concentration resulted in an increasing *Salmonella* kill on different pork meat.

Significance: The above results indicate that the tested phage solution can reduce *Salmonella* contamination on pork by 1.3 to 1.7 log. This shows that bacteriophages are an effective *Salmonella* intervention for processors to reduce risks and allow an increase in consumer safety.

P2-72 Characterization of Selected β-Lactam-resistant *Escherichia coli* Isolates from Food Products

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Introduction: Antimicrobial resistance (AR) is a serious public health threat. Since β-lactam antimicrobials are essential in human and veterinary medicine, the frequent occurrence of β-lactam-resistant *Escherichia coli* isolates from foods, humans and food-producing animals have been a major concern.

Purpose: To characterize β-lactam-resistant *E. coli* isolates from food products to better understand β-lactam-resistant *E. coli* and AR in foods.

Methods: Beta-lactam resistant *Enterobacteriaceae* from five food samples were enriched and isolated using buffered peptone and MacConkey agar, both supplemented with cefotaxime, and HardyCHROM ESBL plates. Isolates were screened for β-lactam AR genes by PCR following DNA sequencing. Three selected isolates were identified by 16S rRNA gene sequence analysis and the expression of β-glucuronidase using the medium from the Colilert system. Antimicrobial susceptibility profiles of the isolates were examined by the disc diffusion method. Class 1 integron was screened and analyzed by PCR following DNA sequencing. Dissemination of the β-lactam-resistance genes was examined by conjugation using *E. coli* Dh5α as the recipient strain.

Results: Three *E. coli* isolates from two food samples were all resistant to cefpodoxime, ampicillin, cefoxitin, cephalothin, and were not susceptible to ceftazidime and cefotaxime. One isolate from a ground chicken sample carrying both bla_{TEM-1} and bla_{CMY-2} genes was also resistant to gentamycin. One isolate from the same sample carrying bla_{CMY-2} gene also carried *aadA1* and in a class 1 integron and *sul1* gene, and was also resistant to gentamycin, chloramphenicol, tetracycline, and streptomycin. One isolate from a raw fresh pork sausage sample carrying both bla_{TEM-1} and bla_{CMY-2} genes was also resistant to tetracycline and streptomycin. The bla_{CMY-2} gene from this isolate could be transferred to *E. coli* Dh5α through conjugation.

Significance: Foods could be reservoirs for β-lactam-resistant and multi-drug resistant bacteria. Antimicrobial resistance genes could potentially be transmitted among organisms through horizontal gene transfer, leading to the distribution of AR.

P2-73 Molecular Epidemiology and Antibiotic Resistance of *Staphylococcus aureus* from Food Animal Carcasses and Carcass Handlers in Nigeria

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❖ ***Developing Scientist Entrant***

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern and a leading cause of mobidity and mortality worldwide, however, in Nigeria, very few molecular studies have been done on *Staphylococcus aureus* in livestock and humans even when there is a close association between the two.

Purpose: We investigated the prevalence and antimicrobial resistance of *Staphylococcus aureus* from chicken and pig carcasses and carcass handlers in Nigeria.

Methods: Samples (1,290) were collected from carcasses and humans and cultured for the isolation of *Staphylococcus aureus*. Isolates were subjected to *spa*, *mecA* and *pvl* PCR typing. *Spa* types were assigned using ridom spa server and were grouped into gene clusters using BURP. Isolates were also tested for susceptibility to 18 antimicrobials using the Vitek2 System. Resistance to ≥3 antimicrobials were regarded as multidrug resistant. Chi-square test was done using GraphPad Prism to evaluate the heterogeneity among variables. Significance was accepted at P<0.05.

Results: The prevalence of *S. aureus* was 4.7, 2.8, 11.1 and 6.7% in chicken, pigs, human poultry and pig processors respectively. Chicken carcasses had significantly higher prevalence of *S. aureus* than others. MRSA was also found to be significantly higher in chicken carcasses. No MRSA was found in humans. Generally, 19 isolates (35.8%) were pvl-positive and 12 (22.6%) carried *mecA* gene. Generally, 66.0% of the isolates and all the MRSA exhibited multidrug resistance. A total of 13 *spa* types and nine singletons including two new *spa* types (18345 and 18346) were detected. Overall, t084 (CC15) and t311 (CC5) were the predominant *spa* types. The *pvl* genes were found more among CC15 while *mecA* was found more among CC1.

Significance: The study suggests that poultry carcasses and their processors harbour more *Staphylococcus aureus* with very high pvl and multidrug resistance, and more MRSA, than pig carcasses and their processors in Nigeria. The CC15 and CC1 are potentially risky clonal complexes for *pvl* and *mecA* genes in Nigeria.

P2-74 The Antimicrobial Activities of Beef Fatty Acids and Their Effects on Virulence Gene Expression in *Listeria monocytogenes* and *Salmonella* Typhimurium

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* and *Salmonella* Typhimurium are significant food-borne pathogens. Bacterial pathogenesis is attributed to the regulation and expression of virulence genes. It has been reported that medium- and long-chain free fatty acids (FAs) may inhibit bacterial growth and decrease the level of virulence gene expression.

Purpose: This study aimed to evaluate the antimicrobial activities and virulence attenuation potential of beef fatty acids on *L. monocytogenes and Salmonella* Typhimurium.

Methods: Free FAs used in this study included commercial FAs (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, conjugated C18:2, C18:3), total beef FAs, and fractions of beef FAs that were rich in monounsaturated fatty acids (UFAs) or poly-UFAs. Minimum inhibitory concentration (MIC) of the FAs on *L. monocytogenes and Salmonella* Typhimurium at pH 7 and pH 5.5 was determined. Meanwhile, both *L. monocytogenes and Salmonella* Typhimurium were treated by FAs listed above, and expression levels of virulence genes (*hlyA*, *actA* and *prfA* for *L. monocytogenes*; *phoP* for *Salmonella* Typhimurium) were quantified by real-time PCR. Assays were completed with three independent experiments..

Results: *L. monocytogenes* was more sensitive to FA treatment compared with *Salmonella* Typhimurium. Generally, FAs showed higher inhibitory activities against *L. monocytogenes* at pH 7 than at pH 5.5. Specifically, MICs of C18:3, C18:2 and C16:1 at pH 7 were 0.25, 0.94 and 0.38 mg/ml, while those at pH 5 were 0.28, greater than six and greater than six mg/ml, respectively. Beef FA fractions, with MICs between 0.06 and 0.33 mg/ml, showed stronger antimicrobial activities compared with commercial FAs. Although fatty acids did not affect the virulence gene expression in *Salmonella* Typhimurium, all the fatty acids except for C16:0 and C18:0 down-regulated the expression level of the virulence genes in *L. monocytogenes* by one to 16-fold.

Significance: Beef FA fractions rich in mono-UFAs or poly-UFAs could be potentially used as natural preservatives against *L. monocytogenes* in the food industry.

P2-75 Antimicrobial Resistance in Surface Water of Two Rivers with Agricultural Use in Chile

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Introduction: Antimicrobial resistance (AMR) is a public health concern with relevance in the food supply as a source of AMR. For produce production, water quality is increasing its relevance, with special significance of agricultural water as a vehicle to disseminate pathogens and AMR. Central Chile is an agricultural location in which fresh produce is produced for local consumption and for international trade. Two rivers, Maipo and Maule, contribute 70% of the irrigation water to the region.

Purpose: The purpose of this study was to identify the presence of antimicrobial resistant bacteria in the Maipo and Maule rivers in central Chile.

Methods: Water samples were collected every three months beginning in August 2017. Samples corresponded to natural zone, agricultural zone, urban zone and livestock/forest area. A total of 20 l of water were ultrafiltrated into 200 ml. Then, one ml of sample was enriched in five ml of buffered peptone water and incubated at 37°C for 24 h; 100 ul of the samples were streaked onto plates of MacConkey supplemented with ciprofloxacin (one mg/l), cefotaxime (one mg/l), and tetracycline (four mg/l) and incubated at 37°C for 24 h.

Results: Among the 48 samples collected during the four samplings, 28 (58.3%) of samples contained gram-negative bacteria that were resistant to at least one of the antibiotics tested. A total of 52, 42, and 25% of the samples contained gram-negative bacteria resistant to cefotaxime, tetracycline and ciprofloxacin, respectively. Gram-negative antibiotic resistance prevalence in sites ranged from zero to 67%. Only one out of the 12 sites tested was negative for antibiotic resistant gram-negative bacteria for all four sampling times.

Significance: Presence of AMR bacteria in water indicates a contaminant of water quality that may impact food safety through foodborne antimicrobial resistance.

P2-76 Prevalence of Extended Spectrum β-Lactamase Encoding Genes: A South African Cucumber Agroecosystem Case Study.

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❖ ***Developing Scientist Entrant***

Introduction: The emergence of extended-spectrum β-lactamase (ESBL) producing bacteria in different environmental compartments has been recognised as a serious threat to human health. This is due to the increase in the number and variety of resistance genes as well as potential horizontal transmission via mobile genetic elements such as plasmids.

Purpose: In this study, the prevalence of relevant ESBL-producing genes in *Enterobacteriaceae* and *Pseudomonas* isolates from the cucumber agroecosystem was investigated.

Methods: A total of 24 samples, including 12 water samples (one liter × 12), and 12 cucumbers (150 g × 12), were collected and processed. Following selective enrichment and streaking onto chromogenic media, 45 presumptive ESBL-producing *Enterobacteriaceae* and *Pseudomonas* spp. isolates were taken and their identities were confirmed using MALDI-TOF MS. Plasmid and chromosomal DNA was extracted and analyzed using PCR targeting seven ESBL genes: bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8/25}$ and $bla_{CTX\text{-}M\text{-}9}$. The PCR reactions were conducted as previously described in the literature. Presumptive positive amplicons were sequenced and then aligned using the BLASTn tool to confirm their identities.

Results: Results when using genomic DNA as reaction template showed that 25 (55.56%) of 45 of the isolates carry the bla_{TEM} gene, 32 (71.11%) of 45 the bla_{SHV}, 24 (53.33%) of 45 the bla_{OXA}, one (2.22%) of 45 the $bla_{CTX\text{-}M\text{-}1}$, zero of 45 the $bla_{CTX\text{-}M\text{-}2}$, zero of 45 the $bla_{CTX\text{-}M\text{-}8/25}$ and seven (15.56%) of 45 the $bla_{CTX\text{-}M\text{-}9}$. Results when using plasmid DNA as reaction template showed that 34 (75.56%) of 45 carry bla_{TEM}, 33 (73.33%) of 45 the bla_{SHV}, 21 (46.67%) of 45 the bla_{OXA}, zero of 45 the $bla_{CTX\text{-}M\text{-}1}$, zero of 45 the $bla_{CTX\text{-}M\text{-}2}$, zero of 45 the $bla_{CTX\text{-}M\text{-}8/25}$ and 26 (57.78%) of 45 the $bla_{CTX\text{-}M\text{-}9}$.

Significance: This study provided valuable data about the prevalence of ESBL genes in the cucumber agroecosystem in South Africa and highlights the need for the development of strategies to contain the further spread of these genes across the different environmental compartments.

Poster

P2-77 Microbial Safety Status of Rape Produced and Sold from Small Scale Farming in South Africa

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❖ ***Developing Scientist Entrant***

Introduction:: *Brassica rapa* (rape) is a favourite African leafy green vegetable often used in traditional meals either raw or cooked. Rape is locally produced by small-scale farmers and sold mainly at informal markets. The food safety status of this crop has not been investigated and given the numerous outbreaks associated with leafy green vegetables it is important to assess the associated hazards and points of potential contamination in the supply chain.

Purpose: Therefore this study assessed the microbial safety of rape in the supply chain.

Methods: Rape samples (*n*=40) were collected from six small scale farms at the stage of harvesting and at the point of sale (*n*=25). Total coliforms, *Escherichia coli* and *Enterobacteriaceae* were enumerated and the prevalence of *Escherichia coli*, *Salmonella* spp., *Listeria* spp. and extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* were assessed.

Results: Coliform counts ranged between 1.40 and 5.35 log CFU/g with 81.25% of rape samples exceeding the previously specified guideline for ready-to-eat foods as defined by the South African Department of Health (2.3 log CFU/g). *Enterobacteriaceae* counts ranged from 1.81 to 5.55 log CFU/g, with 56.25% of samples considered to be of unsatisfactory quality for ready-to-eat foods. *Escherichia coli* was detected from rape samples from three farms (Farm A, B and F) at harvest, as well as from retailer samples (from Farms B, E and F). *Salmonella* spp. were only detected from retailer rape samples originating from Farms C and F. *Listeria monocytogenes* was not detected from any rape samples. Presumptive ESBL-producing *Rhanella aquatilis* was detected from field samples collected from Farms A and E. Presumptive ESBL-producing *Klebsiella* spp. were detected on rape at the point of sale from Farm E and from field samples collected from Farm F. Presumptive ESBL-producing *Serratia* and *Citrobacter* spp. were also detected from Farm F.

Significance: The occurrence of *E. coli, Salmonella* spp. and presumptive ESBL-producing organisms on rape indicates contamination, indicating the necessity to raise awareness of food safety and educate farmers and retailers on good hygiene practices.

P2-78 Beef Contamination with *Salmonella* spp. and Their Resistance to Antibiotics is a Concern and a Threat to Public Health

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❖ ***Developing Scientist Entrant***

Introduction: Beef contamination with *Salmonella spp*. and their resistance to antibiotics is a concern and a threat to public health.

Purpose: This study determined the prevalence of resistant *Salmonella* spp. isolated from beef in Wa Municipality of Ghana.

Methods: One hundred and fifty swab samples were examined. Isolation of *Salmonella* spp. was done according to the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility test was performed using the disc diffusion method and the results interpreted using the CLSI guidelines. Prevalence data were analyzed using SPSS Version 17.0.

Results: Out of the 150 samples examined, 42 (28%) were positive for *Salmonella spp.* The 42 *Salmonella* isolates examined against nine different antibiotics were highly resistant to teicoplanin (97.62%). Resistant to azithromycin and fentamicin were 30.95% and 14.29%, respectively. The *Salmonella* isolates were highly susceptible to chloramphenicol (100%), ciprofloxacin (100%), tetracycline (100%), sulphamethoxazole/trimethoprim (100%) ceftriaxone (95.24%), and amoxycillin/clavulanic acid (90%). Out of the 42 *Salmonella* isolates, 29 were resistant to only one antibiotic, six were resistant to two antibiotics, six were resistant to three antibiotics and one was resistant to four antibiotics. The result revealed that some beef products in Wa municipality are contaminated with *Salmonella* spp. which are resistant to some antibiotics. Therefore, consumers of beef in this municipality are at risk of *Salmonella* infection.

Significance: This study creates the awareness that some meat samples in the Wa Municipality of Ghana are contaminated with *Salmonella* spp., which are resistant to some antibiotics. Therefore, consumers of meat in this municipality are at risk of *Salmonella* infection.

P2-79 Preliminary Investigation of Antimicrobial Resistance Genes in Microbes from Different Types of Retail Food

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Introduction: The increase in microorganisms with antimicrobial resistance (AMR) genes has been recognized as a global problem for public health.

Purpose: To understand the distribution of AMR genes in microorganisms in a broader spectrum of food for effective mitigation, this study investigated the presence of microorganisms containing AMR genes in three main types of retail food in Canada, namely meat, fruits and vegetables.

Methods: Bacteria and possibly fungi in food samples were first enriched (24 h, 37°C) using a quasi-universal media (modified Schaedler medium) followed by genomic DNA extraction and detection of 87 AMR genes relevant to human and agriculture with a commercial AMR gene qPCR array kit (Qiagen).

Results: Among 39 samples tested, AMR genes are present in microorganisms from all food types. The numbers of different AMR gene types detected in individual samples ranged between zero and 27 in 16 meat samples (total of 37 types detected), two and 20 in 16 vegetable samples (total 35 types detected) and zero and five in nine fruit samples (total of eight types detected). Seven AMR genes, particularly class C β-lactamase resistance genes, were detected in three groups, and 18 AMR genes detected in both vegetable and meat groups. Some AMR genes were present in one group of samples only or unique to one source of food, namely, eight and 12 AMR genes detected only in vegetable and meat groups respectively. Ground meat and vegetables in direct contact with soil contained the greatest number of AMR genes indicating the importance of various environments as sources of these genes.

Significance: Overall, the results in this study provide useful baseline data on AMR gene presence in foods in Canada and indicate that different types of food may acquire microorganisms containing certain AMR genes from different sources.

P2-80 Dissemination of Incn Plasmid Carrying *Mpha*, *Oqxab* and $Bla_{CTX\text{-}M\text{-}14}$/$Bla_{CTX\text{-}M\text{-}65}$ in Extensively Drug-resistant *Salmonella* Indiana ST17 Isolated from Humans and Retail Foods in Shanghai, China

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Introduction: *Salmonella* Indiana has become one of the top three serovars in over 70% of reports of *Salmonella* isolation since 2012 in China, and the extensively drug-resistant (XDR) *Salmonella* Indiana have become a serious public health concern.

Poster

Purpose: The aim of study was to determine the prevalence and transmission of azithromycin resistance genes among *Salmonella* isolates from retail foods and humans in China.

Methods: A total of 385 *Salmonella* isolates from foods and humans were screened for azithromycin resistance genes by PCR. Antimicrobial susceptibility to 18 antimicrobials of *mph*A-positive *Salmonella* isolates were determined by agar dilution method. PFGE and MLST were used to determine the genetic correlation among these positive isolates. The transferability of azithromycin resistance genes was tested by conjugation experiments and transformation experiment with *E.coli* C600 as recipient.

Results: The gene *mph*A was identified in 15 *Salmonella* Indiana isolates. These 15 isolates exhibited concurrently high-level resistance to azithromycin, ciprofloxacin and ceftriaxone, which were determined as the same ST (ST17) and exhibited high similar PFGE patterns. Fifteen transformants were obtained by transformation, and exhibited resistance to azithromycin and ceftriaxone. The *mph*A gene was co-transferred with *oqx*AB-$bla_{CTX\text{-}M\text{-}15}$, *oqx*AB-$bla_{CTX\text{-}M\text{-}14}$ and-*oqx*AB-$bla_{CTX\text{-}M\text{-}65}$ in one, five and six isolates, respectively, and was located on an IncN plasmid with approximately size of 220 kb.

Significance: This study revealed that the emergence of concurrent resistance to azithromycin ciprofloxacin, and ceftriaxone in *Salmonella* Indiana isolated from retail foods and humans in China, and this was likely due to spread of *mph*A, *oqx*AB and $bla_{CTX\text{-}M\text{-}65}$, $bla_{CTX\text{-}M\text{-}14}$ carried by epidemic IncN plasmid among these *Salmonella* Indiana isolates.

P2-81 The Cantaloupe Farm Environment Has a Diverse Genetic Pool of Antibiotic-Resistance and Virulence Genes

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❖ ***Developing Scientist Entrant***

Introduction: The actual emergence of multi-resistant bacteria responsible for disease has revitalized interest in understanding their transmission and control. It is well known that foods are important vehicles of infectious bacteria, among which fresh produce has traditionally been associated with high numbers of illnesses. This is due in part to its consumption practices: raw or light-cooked.

Purpose: Since virulence genes of bacteria can act in tandem with antibiotic-resistance and mobile elements, in this study the distribution of antibiotic resistance, virulence and mobile elements genes in the farm environment of cantaloupe was determined.

Methods: A total of 200 samples from cantaloupe melons, farm workers hands, and water (irrigation and source) were collected on three farms in northern México during 2017. Each sample was assayed for presence of resistance to 14 antibiotics, and 15 virulence and five mobile element genes by PCR.

Results: Our results showed that tetracycline and sulfonamide resistance genes were frequent, and mainly found in cantaloupe (18 and 30%, respectively) and farmworker hand rinses (45 and 71%, respectively). Furthermore, colistin resistance genes were detected in 10 of 99 cantaloupe and 15 of 66 farmworker hand rinses. In addition, we observed that the *Salmonella* genes *invA and spiA* were the most abundant (43 and 16%, respectively) among genes of virulence factors, and were homogeneously distributed within all the samples. Although mobile elements genes were widely distributed, the class 1 intregron (*int*1) gene was present in all source water samples.

Significance: From the obtained results, cantaloupe and farmworker hands had, overall, a similar distribution of the studied genes. This suggests that hands could serve as a vehicle to transfer bacteria to produce, which increases the relevance of improving good agricultural practices in this type of environment.

P2-82 A Nationwide Survey of Food Safety Practices on Small Microgreen Farms in the United States

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❖ ***Developing Scientist Entrant***

Introduction: This study represents the first nationwide survey of microgreen farms in the United States.

Purpose: The study objective is to gather operational details and identify food safety practices within microgreen growing operations to inform future research on preventative controls in these systems.

Methods: A seventy-eight question survey was developed using the Qualtrics platform. Questions covered farm characteristics, products, growing systems, agricultural water, hygiene and sanitation, waste disposal, documentation, and training. The summary analysis was performed using Microsoft Excel.

Results: While the survey is ongoing, 77 completed surveys have been summarized. Seventy-eight percent of farms earned less than $10,000 in annual revenue from microgreens and had one to three employees. Eighty-eight percent of farms produced only microgreens or microgreens with other vegetables. Nine farms (12%) raised animals, 69% of which raised chickens. The most common irrigation water sources include municipal (43%) and well water (48%). Fifty percent of all farms test their water for bacteria roughly once per year, but do not test growth media. Seventy-five percent (*n*=71) of farms compost spent growth media. Ninety-one percent (*n*=75) of farms reported soaking seeds in food grade hydrogen peroxide before germination. Post-harvest washing was reported by 36% (*n*=73) of farms. Eighty-six percent of farms harvested microgreens by hand with scissors or a knife. Tools and surfaces are cleaned "daily or more" by 75% of farms, but documentation practices are poor. Seventy-six percent reported at least one type of formal food safety training.

Significance: These findings demonstrate that food safety education for small microgreen farms should focus on documentation, soil testing, and water testing. Future research efforts should explore benefits and risks of composting growth media, pathogen transfer risk from growth media, the effectiveness of the hydrogen peroxide method of seed disinfection, and the effect of post-harvest washing on different microgreen varieties.

P2-83 Bacteria Communities Analysis by 16S Arnr Gene Sequencing in a Melon Producing Agro-Environment

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❖ ***Developing Scientist Entrant***

Introduction: Sequencing technologies in fruits and vegetables have facilitated the study of microbial communities that interact with the product: commensal, food spoilage, and human pathogens.

Purpose: The aim of this study was to analyze the bacterial communities present in two cantaloupe melon-producing farms, and compare the bacterial communities found in melon, hands of farmers and soil.

Methods: A total of 16 samples composed of six DNA samples from rinsates from cantaloupe melons, six DNA samples composed from rinsates from hands of workers and four DNA samples from soil were sequenced using universal 16S primers at 20,000 reads per sample and the sequences were analyzed with QIIME2. Bacteria taxonomy was elucidated using the SILVA 128 16S database.

Results: The α and β diversity indexes of samples indicated that the farms have similar bacterial biodiversity. However, when comparing sample types, the samples of soil were the most diverse, containing over 1000 unique species. Cantaloupe melon and hands contained similar diversity. Most of the species detected were from the dominant phylum *Proteobacteria* (60 to 90%), *Firmicutes* (one to 10%) and *Actinobacteria* (one percent). When the taxonomic analysis was conducted at genus level, enteropathogens related to diseases caused by contaminated melon consumption were detected in some samples. In melon, *Salmonella* was detected in all the samples with a relative abundance of 0.32 to 4.9%, and *Escherichia and Listeria* were not detected. On hands, the relative abundance of *Salmonella* was from non-detectable to 2.33%, and only one sample was positive for *Escherichia-Shigella.* In soils, two of four samples were positive for *Escherichia-Shigella* with a relative abundance of 1.16 to 3.45%, one sample was positive for *Listeria* and no *Salmonella* was detected.

Significance: Farmworkers' hands and cantaloupe melon have similar diversity of microbes with *Salmonella* being present in most samples. The interaction between hands and crops could pose a risk of contamination of cantaloupe melon.

P2-84 Evaluation of Chlorine Dioxide Gas against Four *Salmonella enterica* Serovars Artificially Contaminated on Whole Blueberries

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Introduction: Fresh produce, such as blueberries, continue to be a source of foodborne illness in the United States. Despite new practices and intervention technologies, blueberries and other produce are contaminated with foodborne pathogens, such as *Salmonella* spp.

Purpose: The aim of this study was to evaluate the efficacy of chlorine dioxide gas (CDG) against four *Salmonella enterica* serovars artificially contaminated on whole blueberries.

Methods: CDG was generated with a ClorDiSys Minidox-L system. Blueberries (25 g at a time) were immersed (five min) in 400 ml containing either ca. six or nine log CFU/ml of a *Salmonella* serovar cocktail (*Salmonella* Newport, Stanley, Muenchen, and Anatum) which gave a final cell concentration of up to 3.36±0.26 or 5.72±0.23 log CFU/g berries. Inoculated samples (400 g) were allowed to dry for two or 24 h prior to treatment with up to three mg CDG/liter of air for up to three hours at 22°C and 20 or 90% humidity. Two independent trials of each experiment were performed using four separate sets. Cells were recovered from controls and treated samples by stomaching with 0.1% peptone and enumerated on xylose-lysine-Tergitol-4 agar after CDG challenge. Statistical analysis was completed using JMP Pro 14.0.

Results: Three mg CDG/liter of air for three hours of treatment reduced the *Salmonella* cocktail by up to a 5.62 log CFU/g berries. However, the least efficacious treatment, shorter treatments, were still capable of a 3.86 log CFU/gm reduction. Additionally, all serovars responded similarly to CDG when tested independently (P>0.0861). Finally, relative humidity did not have a significant impact (P=0.5224) on CDG efficacy in this study.

Significance: Our results demonstrated that CDG can be an efficacious treatment option for fresh produce decontamination. Recent advances in CDG technologies makes these tools readily available and economical for fresh produce distributors and should be considered for industrial use.

P2-85 Characteristics of Antimicrobial Resistance of *Salmonella enterica* Isolates from Retail Foods in Shanghai, China

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Introduction: The emergence of antimicrobial-resistant *Salmonella* isolates around the world has raised great concern, and retail foods are important vectors for the transmission of *Salmonella* to humans.

Purpose: The purpose of this study is to characterize the antimicrobial resistance of foodborne *Salmonella* isolates obtained during March 2016 and February 2017 in Shanghai, China.

Methods: A total of 147 *Salmonella* isolates from 1123 food samples were identified using traditional biochemistry tests. Antimicrobial susceptibility was determined by the agar dilution method according to the CLSI guidelines, and the presence of resistance genes was examined in *Salmonella* isolates showing resistance to each category of antimicrobial by PCR assays. MLST and PFGE were used for *Salmonella* isolates subtyping.

Results: The highest antimicrobial resistance to sulfisoxazole was 138 (93.88%) of 147, followed by 90 (61.22%) of 147 resistant to trimethoprim/sulfamethoxazole, and 73 (49.66%) of 147 resistant to nalidixic acid. Antimicrobial resistance genes were detected in *Salmonella* isolates by PCR amplification, including quinolone (*qnr*S, *oqx*AB and *aac(6')-Ib-cr*), β-lactamase (bla_{TEM} and bla_{CMY}), chloramphenicol (*cml*A and *flo*R) and tetracycline (*tet*A, *tet*B and *tet*G). MLST analysis indicated that ST34 and ST11 were predominant clones in *Salmonella* Typhimurium (27 (56.25%) of 48) and *Salmonella* Enteritidis 65 (95.59%) of 68), respectively. Importantly, all of ST34 isolates possessed the ACSSUT resistance pattern (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline). Two pulsotypes contained 16 and eight ST34 isolates from different food sources and areas shared the same PFGE pattern, respectively, suggesting the clonal expansion of ST34 isolates among different foods in Shanghai.

Significance: Our study highlights the wide distribution of antimicrobial-resistant *Salmonella enterica* and clonal expansion of ST34 with ACSSUT resistance patterns in food in Shanghai.

P2-86 Phenotypic and Genotypic Analysis of Antibiotic Resistance of *Listeria monocytogenes* Isolated from Food Products

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Introduction: The gram-positive bacterium *Listeria monocytogenes* is considered as an important foodborne pathogen. Although the antibiotic resistance of *L. monocytogenes* is not as widespread as in some other food-borne pathogens it is important to monitor the antimicrobial susceptibility and presence of resistance genes during surveillance. In addition, little is known about *L. monocytogenes* antimicrobial resistance from food isolates.

Purpose: The purpose of this study was to determine the phenotypic antibiotics resistance pattern of *L. monocytogenes* isolates from various food products and the presence of antibiotic resistance genes by whole genome sequencing.

Methods: A total of 391 *L. monocytogenes* isolates was isolated from from a wide range of food products from 2015 to 2017 using ISO 11290-1. Susceptibility testing was performed with broth microdilution according to ISO 20776-1:2006 in Sensititre panel EUVENC. Whole genome sequencing was performed

Poster

using Nextseq sequencing. Reads were assembled using ABYSS, and Blast was used in combination with the Resfinder database to identify the acquired antimicrobial resistance genes.

Results: Only seven different isolates displayed phenotypic and/or genotypic antibiotic resistance (two percent). In three isolates the displayed phenotypic antibiotic resistance to tetracycline and/or erythromycin matched with the presence of resistance genes *erm*(B), *tet*(L), *tet*(M) revealed by WGS. In two isolates only phenotypic resistance and in three isolates only genotypic resistance was observed. Whole genome sequencing showed to be a useful tool to analyze antibiotic resistance genes in *Listeria monocytogenes*.

Significance: These data show that antibiotic resistance in *L. monocytogenes* is not commonly present. However, regular surveillance should be maintained for detecting any shift in the antimicrobial resistance of *L. monocytogenes* isolated from food.

P2-87 Assessment of Veterinary Drugs Present in Pork Kidney Purchased from Four Retail Stores

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Introduction: Kidney is low-cost, easy to collect in slaughter facilities and is an excellent tissue for assessing drug exposures. Rapid on-site kidney inhibition swab (KIS) tests can screen antibiotic residues efficiently. ELISA can complement KIS screening for non-antibiotic residues or antibiotics that are less sensitive using KIS.

Purpose: To determine the extent of commonly used veterinary drug residues in pork kidney obtained from midwestern United States retail markets as well as their concentrations.

Methods: A total of 1040 pork kidneys, purchased from four retail stores (a local grocery chain, a butcher shop, two ethnic groceries), were screened for antibiotics with the KIS test. Sixty-five samples obtained from each retail location were randomly selected from the respective cohorts of KIS negative samples, as well as 18 KIS positive or "caution" samples, and were analyzed with ELISA to determine the presence of flunixin, ractopamine, sulfamethazine, and/or tetracycline residues. Samples returning positive ELISA results were confirmed using LC-MS/MS.

Results: Six samples (0.6%) tested positive with the KIS. Of the 278 samples evaluated by ELISA, flunixin, ractopamine, sulfamethazine, and tetracycline residues were found to be zero, 22, four, and 10% ELISA positive respectively and had greater than the limit of quantitation concentrations as measured by LC-MS/MS. In the ELISA screening assay, one ethnic shop and the local grocery chain store contained a greater number of ractopamine positive samples in the KIS negative cohort ($P<0.0001$). All residue levels determined by LC-MS/MS were well below United States tolerances. Based on the LC-MS/MS results, the highest concentrations of ractopamine, sulfamethazine, or tetracycline in kidney were all obtained from a local grocery chain store.

Significance: There were chemical residues present in some of the kidney purchased from different sources in a Midwestern town. However, there were no residues in violation in our survey according to United States tolerance standards.

P2-88 WITHDRAWN

P2-89 The Presence of *Cryptosporidium* spp., *Cyclospora cayetanensis, Toxoplasma gondii,* and *Giardia intestinalis* in Potential Alternative Sources of Agricultural Water: A Conserve Study

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❖ ***Developing Scientist Entrant***

Introduction: United States agriculture faces considerable challenges due to climate change. Concerns surrounding freshwater availability and safety have been on the rise, as more outbreaks of foodborne illness have been linked to agricultural water. Protozoan parasites can be isolated from lakes, reservoirs, irrigation water, wastewater, and have a history of association with drinking and recreational water, further substantiating the need to include protozoa in food safety considerations and evaluation of non-traditional water sources.

Purpose: To examine the presence of protozoan parasites in potential alternative sources of agricultural water

Methods: Water samples (n=26) were collected (June to October 2017) from surface water (tidal brackish, pond), and recycled water (vegetable processing, wastewater) sites in the Mid-Atlantic region, and 10 to 20 l were filtered using an Envirochek HV Capsule at a filtration rate of two l/min. Filters were eluted according to the EPA 1623 modified method. The DNeasy Power Water Extraction Kit was used and qPCR was performed with the QuantiNova Probe Assay Kit and confirmed by gel electrophoresis. Data were analyzed using chi-square test.

Results: For *Cryptosporidium parvum,* 65.38% (n=26) of water samples tested positive. By water type, 50.00% (n=10) of recycled water (RW), and 75.00% (n=16) of surface water (SW) tested positive. For *Cyclospora cayetanensis,* 53.84% (n=26) of the water samples tested positive. By water type, 40.00% (n=10) of RW, and 62.50% (n=16) of SW tested positive. For *Toxoplasma gondii,* 7.69% (n=26) of water samples tested positive. By water type, 20.00% (n=10) of RW, and zero (n=16) of SW tested positive. *Giardia intestinalis,* was not detected. There were no statistically significant differences amongst water types for presence of protozoa ($P>0.05$), except for *T. gondii*, which is more likely to be detected in RW ($P=0.0430$).

Significance: Understanding the prevalence of protozoan pathogens in alternative agricultural waters will enable the establishment of interventions and subsequent safe use of these waters in irrigation.

P2-90 Diverse Shiga Toxin-producing *Escherichia coli*-specific Bacteriophages Exist in Goat Feces and the Surrounding Environments on an Organic, Produce-growing Farm in Northern California, USA

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) contamination on produce is primarily associated with ruminant feces, which can result in severe foodborne illness. Although numerous STEC-specific bacteriophages (phages) have been isolated from cattle feces, the presence of STEC-specific phages and any correlation with O157 and the top six non-O157 STECs (the top seven STECs) in goat feces has not been thoroughly investigated.

Purpose: The objective of this research was to investigate the prevalence and diversity of the top seven STECs and STEC-specific phages in ruminant feces and surrounding environments on an organic produce-growing farm.

Methods: One sample each from three sites (goat feces, cattle feces, and soil) was collected monthly for six months (soil was collected for five months; n=17). Individually, samples were tested for STEC using culture and PCR-based methods and STEC-specific phages using enrichment with a cocktail of the top seven STEC strains followed by purification via plaque assay. The isolated phages were subjected to host range tests and morphological observation by transmission electron microscopy.

Results: Ten samples (six goat, three soil, and one cattle) contained various STEC-specific phages belonging to three families (*Myoviridae*, *Siphoviridae*, and *Podoviridae*). The phages isolated from eight samples (five goat, two soil, and one cattle) showed lytic activity against STEC O103, several of which exhibited a wide host range lytic against other top seven STECs. Goat feces contained phages without isolation of STECs throughout the sampling periods. Two STECs, O174 and an O-antigen negative strain, were isolated from one soil and one cattle sample, respectively.

Significance: This study indicates that STEC-specific phages were consistently isolated from goat feces. The prevalence of phages with wide and complimentary host range against the top seven STECs, resulting in zero isolation of the bacterial pathogens, could be indicative of environmental biocontrol of phages in this niche.

P2-91 Crassphage as a Source Tracking Tool to Investigate Human Stool Contamination

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Introduction: CrAssphages are recently-discovered DNA bacteriophages that are prevalent and abundant in human feces and sewage. Because they are practically absent in animal fecal samples, they are considered as an indicator of human fecal pollution.

Purpose: We determined the prevalence and genetic diversity of crAssphages in paired stool and hand rinse samples of 30 symptomatic patients from 12 norovirus outbreaks in long-term care facilities. We also tested human clinical specimens, including vomitus (n=22), saliva (n=43), nasal samples (n=48), and stool samples (n=76) from eight animal species (cow, sheep, horse, pig, monkey, cat, mice).

Methods: CrAssphage DNA was detected by real-time PCR and positive samples were further typed by conventional PCR followed by sequencing of the PCR products.

Results: CrAssphages was detected in 21 (70.0%) of 30 stool samples and 17 (56.7%) of 30 hand rinse samples from norovirus symptomatic patients, but not in vomitus, saliva, nasal or animal stool samples. A total of 30 (78.9%) of 38 crAssphage positive samples were sequenced, including 18 stool samples and 12 hand rinse samples, which could be grouped into two major genetic clusters and further divided into 16 sub-clusters. Identical crAssphage sequences were detected in hand and stool samples from six patients suggesting self-contamination, while different crAssphage sequences were detected on hands from four patients, suggesting cross-contamination with stool from someone else.

Significance: We detected crAssphages in the majority of stool samples from norovirus outbreaks in long-term care facilities and not in other clinical materials or stool samples from animals. Sequence analysis showed that the hands of several patients were likely cross-contaminated with stool samples from other patients. Our data support previous work that crAssphages are uniquely present in human but not animal stool samples making them potentially useful as an indicator of human fecal contamination. Furthermore, we show that crAssphages can be employed to better understand sources of contamination in norovirus outbreaks.

P2-92 Virus Recovery Affected by Contact Surface Physicochemistry of Polymer and Glass

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Introduction: Enteric viruses have been recognized as a major causative agent for foodborne illnesses worldwide. Viruses are transmissible via their adhesion/attachment to contact surfaces of food containers or tools.

Purpose: This research evaluated the recovery or removal of coliphage MS2 as a virus surrogate from abiotic surfaces affected by i) surface chemistry and ii) topographic smoothness (less than one µm RMS), roughness, and porousness.

Methods: Abiotic surfaces of polypropylene (PP), polyvinyl chloride (PVC), polyethylene (low and high densities, LDPE and HDPE), and glass (borosilicate and soda lime) were characterized by atomic force microscopy (AFM), profilometry, tensiometry, and infrared spectroscopy. Observing the MS2 inactivation rate at zero and 0.045 log/day respectively in tryptic soy broth (TSB) and PBS, we prepared MS2 in PBS containing one percent TSB as inocula onto nine surfaces, incubated at 4°C for 24 h, and quantified recoverable viruses by infectivity.

Results: The virus recovery order from four smooth surfaces was PP > HDPE ≥ LDPE ≥ soda lime glass, with strong hydrophobic PP and PE. AFM revealed pinholes (diam. 21±3 nm) on the borosilicate glass. These pinholes (being <28 nm-diam. of MS2) or increased roughness possibly caused virus-trapping, thus decreasing virus recovery. Significantly ($P\leq0.007$) more viruses were recovered from smooth compared to rough surfaces. All nine surface recoveries were distributed into six statistical groups, with the highest and lowest being smooth PP (76±12%) and hole-bearing borosilicate glass (32±6%), respectively. The recoveries of PVC, PP and PE (including smooth and rough surfaces) were classified into five of the six groups.

Significance: The contact surface cleanability could be compromised by scratches or holes occurring during usage or manufacture. Our results illustrated that not all plastic surfaces release attached viruses with equal efficiency; the same was observed for two glass surfaces investigated.

P2-93 The Prevalence of Bacteriophages Lytic against Shiga Toxin-producing *Escherichia coli* (STEC) and Its Correlation with STEC Bacterial Hosts in an Organic Farm

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Introduction: Lytic bacteriophages are increasingly considered as alternative biocontrol agents of bacterial pathogens due to their killing effect. The majority of the phages specific to enteropathogenic bacteria, such as Shiga toxin-producing *E. coli* (STEC), have been frequently isolated from animal-associated environments, such as feedlots. However, the information regarding the prevalence of STEC-specific phages in different types of produce farms is scarce.

Purpose: The purpose of this research was to evaluate the prevalence of lytic bacteriophages specific to STEC strains and its correlation with STEC bacterial hosts in an organic farm.

Methods: Between August 2017 and September 2018, a total of 370 samples, including water, soil, sediment and animal feces, were collected monthly from an organic farm in Northern California containing two separate farming areas: one with and one without animal activity. Cocktails of three non-pathogenic *E. coli* and 14 STEC strains (top six non-O157 and O157 serogroups) were used for phage isolation and host range tests. Culture methods and PCR were used to isolate STEC strains. Information regarding temperature, rain precipitation and solar radiation was also collected.

Results: The results showed that 31 (8.4%) of the samples were positive for lytic bacteriophage active against STEC strains. Most bacteriophage-positive samples (n=26) were collected from the areas with animal activity. Spring season, particularly April, had relatively high prevalence of bacteriophages, which was likely due to high rain precipitation. Additionally, the three most frequently isolated bacteriophages were lytic against STEC O103, O45, and O121. No

Poster

Poster

phages harbored *stx* genes in this study. One STEC O103 was isolated; however, no phages specific to the serogroup were isolated from the particular sample.

Significance: The findings indicate that the prevalence of STEC-specific phages is highly associated with samples collected from the animal-involved areas. Additionally, the presence of these bacteriophages is attributed to the negative correlation with the presence of their STEC hosts.

P2-94 Chemical Inactivation of *Encephalitozoon intestinalis* and *Salmonella* Enteritidis

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❖ ***Developing Scientist Entrant***

Introduction: *Encephalitozoon intestinalis* is a foodborne parasite that causes gastrointestinal illnesses. Currently, there is limited research conducted on the reduction of microbial pathogens via biocides focusing on microsporidia.

Purpose: The chemical inactivation of *E. intestinalis* is explored with *Salmonella* Enteritidis as a bacterial control.

Methods: Six disinfectants and sanitizers (peracetic acid, two peracetic/peroctanoic acids, a fruity peracid blend, a quaternary ammonium compound, and a fatty acid blend) were tested in suspension at 2000 ppm on *E. intestinalis* spores (ATCC #5061) and three strains of *Salmonella* Enteritidis (ME18, H4717, M4639) at 23, 23 and 50°C, respectively. Disinfectant exposure times were five, 15, and 30 minutes. Water was used as the control for *E. intestinalis* and viability was determined using In Vitro cultivation in rabbit kidney cells (RK-13, ATCC CCL-37). Peptone (0.1%) was used as the control for *Salmonella* Enteritidis and cells were recovered in tryptic soy agar (TSA) containing ampicillin. Log reduction (CFU/ml) of viable spores and bacterial cells were calculated and ANOVA was used to analyze the effect of temperature and exposure time.

Results: *E. intestinalis* was completely inactivated by five of the six biocides with no significant differences ($P<0.05$) between exposure times. Spores treated with the quaternary ammonium compound were positive up to three log CFU/ml and confirmed with tissue subculture. *Salmonella* Enteritidis had a log reduction range of 5.91 to 7.71 CFU/ml at 23°C and 6.10 to 7.75 CFU/ml at 50°C for all biocides at all exposure times. There was a significant difference ($P=0.0014$) for temperature only for peracetic acid with higher log reductions observed at 50°C (5.99, 5.91, 6.63 CFU/ml for five, 15, and 30 minutes, respectively) than 23°C (7.45, 7.55, 7.51 CFU/ml for five, 15, and 30 minutes, respectively).

Significance: Complete chemical inactivation of microsporidia was demonstrated and can be used for food safety interventions in the food industry.

P2-95 Optimization and Evaluation of a Viradel Method for Viral Detection in Environmental Source Waters: A Conserve Study

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Introduction: Virus adsorption and elution (VIRADEL) methods originated in the 1970s. The variability of environmental water samples provides challenges in the development of a contemporary standardized method for viral detection.

Purpose: To optimize the recovery and detection of viruses from environmental source waters used for crop irrigation by modifying and evaluating a VIRADEL method.

Methods: Surface (SW) and reclaimed water (RW) samples were filtered in 25 to 40 l volumes and eluted using 300 ml sodium polyphosphate buffer. The eluate was concentrated using four 100 kDa filters prior to nucleic acid extraction. Tulane virus (TV), a surrogate for human norovirus, was used as a process control. Initial water samples ($n=3$), eluates ($n=3$), and concentrates ($n=4$) were inoculated to determine efficiency of filtration, concentration, and extraction, respectively. A one-ml aliquot of TV (7.8 log genomic copies/ml) was used to inoculate each initial water and eluate sample, while 0.025 ml was used in each concentrate. Detection was performed using RT-qPCR and virus quantified using a standard curve generated from TV RNA. Statistical analysis was performed by one-way ANOVA.

Results: TV genomic copies were successfully detected in all inoculated samples ($n=10$). There was no significant difference in TV genomic copies detected from RW or SW samples inoculated prior to filtration($P=0.61$) or eluates ($P=0.19$), the average detected being 5.62±0.25 and 7.26±0.18 log copies/ml; however, detection of TV in SW was significantly lower than in RW when inoculated into the concentrate ($P=0.0024$). Detected copies from RW samples averaged 7.63±0.18 log copies/ml while SW samples averaged 7.05±0.08 log copies/ml. From these data it was determined that 1.19±0.44 log copies/ml out of the total average of 2.19±0.25 log copies/ml lost was due to loss at the initial filtration step.

Significance: These data show the impact that physiological variability of environmental waters has on detection of viruses, contributing to the development of a consistent methodology for detection.

P2-96 Enteric Virus Detection in Leafy Greens

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Introduction: The continued occurrence of viral foodborne outbreaks emphasizes the importance of standardized methods. Currently, FDA's Bacteriological Analytical Manual (BAM) chapter 26B contains a method validated for one matrix: green onion. Creating a leafy green category, exhibited in ISO 16140-2, can broaden the method's application.

Purpose: The objective of this study was to validate the use of FDA BAM chapter 26B with the addition of romaine lettuce and spinach matrices for enteric virus detection including norovirus (NoV) and hepatitis A virus (HAV).

Methods: Following FDA BAM chapter 26B, 50 g of inoculated romaine lettuce and spinach samples were concentrated with ultracentrifugation and RNA extracted with a silica-based column kit. This study included three trials with triplicate samples. Samples were spiked at various inoculum levels of NoV GI (0.3 to 33 genome copies/g), NoV GII (three to 330 genome copies/g), and HAV (0.1 to 10 PFU/g). Murine norovirus (MNV) was used as an extraction control (10^2 PFU/g). RT-qPCR assays on the ABI 7500 Fast were used for detection.

Results: The average internal amplification control Ct in romaine lettuce (26.58±0.27) and spinach (26.36±0.36) matrices demonstrated little to no inhibition for all MNV, HAV, and NoV replicates. HAV and NoV GII were detected in all replicates at all inoculum levels. For NoV GI, the three genome copies/g and 0.3 genome copies/g inoculum detection frequencies in romaine lettuce were 80 and 60%, whereas in spinach they were 47 and 60%.

Significance: The results of this validation study demonstrated that the FDA BAM chapter 26B method can be used to reliably detect NoV and HAV in other leafy green matrices with little to no inhibition. Detection of viral pathogens is essential for food safety. With a leafy green category, surveillance sampling and outbreaks of future implicated matrices that fall into this category can be analyzed with minimal method verification leading to faster analyses and results.

P2-97 Preliminary Study on the Prevalence of Hepatitis A and E Viruses in Feral Fish Obtained from Two Major Lagoons in Lagos, Nigeria

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❖ ***Developing Scientist Entrant***

Introduction: Hepatitis A Virus (HAV) and Hepatitis E Virus (HEV) have been documented as causative agents of foodborne illnesses. These viruses cause acute infections and are responsible for viral enteritis outbreaks in areas of poor sanitation and improper waste disposal into fresh, marine and estuarine waters, thereby exposing aquatic animals to both HAV and HEV. Based on Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) reports in 2000 and 2010 respectively, Africa was classified as an highly endemic region for HAV and HEV.

Purpose: The purpose of this study was to conduct preliminary studies on the prevalence of HAV and HEV in feral fish in two of the major lagoons in Lagos, Nigeria.

Methods: Fifty samples each of feral fish's gills and intestines (making a total of one hundred samples) were pooled separately and tested for both viruses. Samples were homogenized into five each for both gills and intestines respectively. RNA extraction was done using microspin column technology (Jena Bioscience Viral RNA kit). RNA samples were tested for HAV and HEV using Nested Reverse Transcription- Polymerase Chain Reaction (RT-PCR) of the 371 bp region of VP1/P2A junction of HAV genome and 348 bp region of ORF2 region of HEV genome respectively. The resultant nucleotides were visualized after electrophoresis on a 2% agarose gel.

Results: Eighty percent of HEV samples and 100% of HAV samples were positive. The positive RNA samples comprised of three gills and two intestines samples for both HAV and HEV RNA respectively.

Significance: This preliminary study showed contamination of aquatic environment with HAV and HEV. There is ongoing study to determine circulating genotypes in Nigerian aquatic environment in order to protect water bodies, human and seafoods.

P2-98 Adaptation of the Human Intestinal Enteroid Infectivity Assay for Environmental Detection of Noroviruses

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❖ ***Developing Scientist Entrant***

Introduction: Human noroviruses (HuNoVs) are the leading cause of foodborne illness in the US and environmental transmission is important in food handling environments. However, accurate detection of HuNoVs in the environment has been limited as molecular detection cannot differentiate infectious viruses and inhibition from stool matrices poses a significant problem. A HuNoV cell culture model employing human intestinal enteroids (HIEs) has been reported, but environmental applications and effects of purifying viral samples to decrease inhibition have not been examined in detail.

Purpose: The goal was to obtain consistent laboratory growth of infectious HuNoVs in HIE cell culture and to evaluate complex matrices on HuNoV growth in the system.

Methods: The following HIE variables were modified: cell line (J2 from Baylor College of Medicine and 717J from Johns Hopkins Medicine), passage number, and density. Successful HuNoV replication was a fourfold or greater increase in viral RNA between one and 72 h post infection (HPI). HuNoV growth from six viral strains was compared. HuNoV samples were sequentially purified by Vertrel extraction and sucrose cushion and tested.

Results: HuNoV growth was evaluated in 148 cultures with 81 successful replications. An average 16,729 fold increase in viral RNA copies was observed between one and 72 HPI (SD=68,948). Successful growth was observed in two tested HuNoV clinical isolates: a GII.4-16 recombinant and a pediatric GII. The 717J adult jejunum line had a higher growth success rate (59%, $n=137$) than the J2 line (17%, $n=17$). Cell line passage number did not correlate with observed HuNoV growth ($P=0.47$). HIE was challenged with increasingly purified HuNoV samples that showed growth in the system.

Significance: This work expands on the HuNoV cell culture system for environmental applications. Adaptation of HuNoV cell culture to environmental detection and disinfectant testing is crucial for HuNoV detection and control in food environments.

P2-99 Antimicrobial-resistant Bacteria Present in Fresh Produce from the United States and Mexico

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Introduction: Infections caused by antimicrobial resistant (AMR) bacteria are increasing globally, but a potential for exposure to AMR bacteria via fruit and vegetables is unknown.

Purpose: Assess the prevalence of AMR *Enterobacteriaceae*, enterococci and *Staphylococcus aureus* on produce grown in the United States and Mexico.

Methods: United States-grown ($n=60$ of leafy greens, tomato, cucumber, jalapeño peppers, berry varieties) and Mexico-grown ($n=37$ of leafy greens, tomato) produce samples were collected from supermarkets, farmers' markets and farms in Mid-Atlantic United States and Central Mexico regions in fall of 2018. Samples were hand-massaged for two min in buffered peptone water. *Enterococcus* spp. and *S. aureus* was enumerated by direct plating on Enterococcosel and Baird Parker Agar, respectively, then PCR-confirmed. After a three-h incubation at 37°C, extended spectrum β-lactamase (ESBL)- and carbapenemase (CP)-producing *Enterobacteriaceae* were enumerated and isolated on selective ChromAgar ESBL and Chromagar mSuperCarba agars, respectively, then PCR confirmed.

Results: ESBL-producing bacteria (1.0 to 7.5 log CFU/g) were recovered from 56 (93%) of 60 and 33 (89%) of 37 samples in the United States and Mexico, respectively. CP-producing bacteria (1.0 to 7.7 log CFU/g) were recovered from 57 (95%) of 60 in the US and 5 (14%) of 37 samples in Mexico. In the United States, approximately 70% of isolates from these two bacterial groups were PCR-identified as pseudomonads, while in Mexico only eight percent were pseudomonads, *Enterobacteriaceae* (62%) being more abundant. In the United States, *Enterococcus* spp. (0.1 to 3.3 log CFU/g) were recovered from 44 (77%) of 60 samples, of which 11 (11%) of 100 were PCR-confirmed as *E. faecalis* while 17 (17%) of 100 were identified as *E. faecium.* S. *aureus* (0.1 to 4.3 log CFU/g) was recovered from 52 (87%) of 60 United States samples and 27 (73%) of 37 Mexico samples. Sample type was a significant factor for all bacteria groups analyzed ($P<0.01$), with leafy greens supporting the highest populations.

Significance: ESBL- and CP-producing bacteria, opportunistic enterococci and *S. aureus* are prevalent on fruit and vegetables. United States and Mexico-grown produce may be a significant source of human exposure to AMR bacteria.

Poster

P2-100 Optimization of the Human Intestinal Enteroid Model to Study the Efficacy of Sanitizers against Human Norovirus

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Introduction: Successful human norovirus (HNV) cultivation in stem cell-derived human intestinal enteroids (HIE) was recently reported. Its applicability to evaluating disinfection efficacy is of interest but complicated by cytotoxicity, limited assay sensitivity, reagent availability, and cost.

Purpose: To optimize the HIE model to evaluate the efficacy of commercial sanitizers against HNV.

Methods: HIE monolayers were produced as previously described using in-house media and that obtained commercially. One HNV-positive fecal suspension (designated GII.4 Sydney NV14-117) could be propagated on HIE. It was used to screen two sanitizers (Product #1 and #2) by suspension assay (ASTM E1052-11), with virus inactivation evaluated i) directly by RT-qPCR with RNase pre-treatment; and ii) by HIE culture with pre- and post-infection RT-qPCR. Results were presented as log decrease or increase in HNV genome equivalent copies (GEC), respectively. Experiments were done in triplicate.

Results: Commercial media outperformed in-house media, providing higher cell density and faster growth. The Sydney NV14-117 inoculum produced efficient and consistent replication in HIE *(2.6*±0.2 log GEC increase at 72 h post-infection) if first purified by serial filtration. Disinfectant treatment necessitated the use of a spin column to overcome HIE cytotoxicity. Log GEC reductions were 2.8±0.3 and 2.7±0.3 for Products #1 and#2, respectively, when evaluated by RT-qPCR with RNase pre-treatment. No virus replication in HIE was observed after treating Sydney NV14-117 with either product, as compared to the neutralization controls which produced a 2.3±0.02 log GEC increase. Virus adsorption to HIE at one h post-infection was comparatively inefficient, suggesting sanitizer treatment affects capsid binding.

Significance: The HIE model can be used to evaluate HNV disinfection efficacy if appropriately optimized. There remains a need to determine how it can be adapted to produce fully quantitative data on loss of virus infectivity.

P2-101 Efficacy of a Novel Alcohol-based Surface Sanitizer against Human Norovirus

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Introduction: Human Norovirus (HNV) remains the leading cause of foodborne illness and there is a need for more efficacious food-contact surface sanitizing solutions that are practical in terms of contact time and surface compatibility.

Purpose: To evaluate the anti-HNV efficacy of a novel alcohol-based surface sanitizer (ABSS; Purell Multisurface Disinfectant) relative to chlorine solution at concentrations relevant to food service per the FDA Food Code.

Methods: A HNV GII.4 Sydney 20% fecal suspension was used as inoculum. Anti-HNV efficacy was evaluated by suspension assay (ASTM E1052-11) and surface testing (ASTM E1053-11) on stainless steel coupons, with and without additional five percent soil load. Log reduction of HNV genome copy numbers was quantitatively evaluated by RT-qPCR with RNase pretreatment and infectivity loss confirmed using the human intestinal enteroids (HIEs) model.

Results: By suspension assay, the ABSS produced 2.76±0.23 and 2.55±0.08-log reduction in HNV genome copies after a one-min exposure in the absence and presence of soil, respectively. On clean surfaces with one-min contact times the ABSS achieved a 2.04±0.26-log reduction, which was superior to ~0.2 to 0.5-log reduction for 100 ppm bleach (P<0.05) and similar to the ~1.7 to 2.7-log reduction for 500 ppm bleach (P>0.05). HNV exposed to ABSS lost infectivity in its entirety on HIEs after one min in suspension. Experiments were done in triplicate.

Significance: Food-contact surfaces should be cleaned prior to sanitizing to achieve a high level of HNV risk reduction. Mechanism of action, the effect of formulation on ABSS results, contact times and wipe substrates should be further studied.

P2-102 Murine Norovirus Remains Stable at Extreme pH in Association with *Bacillus cereus*

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Introduction: Human noroviruses (hNoVs) are the leading cause of foodborne illnesses in the United States. Human NoV outbreaks have been linked to acidic foods such as orange juice (pH 3.3 to 4.19) and frozen raspberries (pH ~3.20). With respect to persistence in food and resistance to common controls, the relationship between hNoV and bacteria has recently been investigated.

Purpose: This study investigates the stability and binding capacity of murine norovirus (MNV) in suspension with *Bacillus cereus* when exposed to pH extremes at room temperature.

Methods: Equal volumes of *B. cereus* pellet (10^8 CFU/ml) and MNV (10^5 PFU/ml) were mixed in 1X DMEM (adjusted to pH two, three nine, and 10) and incubated at room temperature for 30, 60, and 90 min. First, virus not bound to bacteria was recovered by filtration (0.22-µm pore size). Additional experiments investigated total infectious MNV after exposure to pH extremes by adding imipenem to samples after each time point to inhibit bacterial growth during virus culture. Control samples included MNV alone and 1X DMEM (neutral pH) treatments. MNV log reduction at each treatment was determined by plaque assay. Within each treatment (filtered vs. unfiltered), samples were analyzed in duplicate with only one experimental replicate.

Results: MNV in suspension with and without *B. cereus* was resistant to inactivation at all pH levels. Additionally, MNV fully associated with *B. cereus* at room temperature by 60 min at all pH levels. Mean log reductions were significantly different (P<0.05) for MNV alone and MNV+bacteria by 60 min at pH two, three, and nine and by 30 min at pH 10. Log reductions were minimal (-0.10 to 0.93) but significantly greater for MNV alone.

Significance: Although log reductions of MNV alone and with *B. cereus* were minimal, there were significant differences at all pH extremes while no difference at neutral pH. These data substantiate the need to continue investigating the efficacy of control measures against pathogens within microbial communities.

P2-103 Performance of Traditional and Eco-friendly Sanitizers against *Listeria* spp. at Various Temperatures and Organic Loads

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❖ ***Developing Scientist Entrant***

Introduction: The frequency and variety of recalls linked to *Listeria monocytogenes* highlights ongoing control challenges in food processing environments (FPE). Understanding how factors encountered in FPEs influence sanitizers is critical for designing effective control programs.

Purpose: Evaluate the performance of eco-friendly (EF) and chlorine-based (CB) sanitizers against *Listeria* spp. at different temperatures and organic loads.

Methods: Minimum bactericidal concentrations (MBCs) were determined for four sanitizers based on benzalkonium chloride (BAC), alcohol (AB), citric acid (CAB) and quaternary ammonium compounda (QAC2). MBCs were measured using the microbroth dilution method in tryptic soy broth at four, seven, 15, 23, and 30°C for 24 h for 22 *Listeria* isolates (16 *L. monocytogenes* and two each *L. innocua, L. seeligeri, L. welshimeri*). Eight-mm diameter stainless-steel coupons were soiled with lettuce, cabbage, and beet rinsate by soaking 24 h, rinsing, and air-drying five min. Manufacturer recommendations for one EF (AB) and one chlorine-based sanitizer were tested for their efficacy to inactivate five *L. monocytogenes (*five to six log CFU/ml) in 100 µl adhered to the coupons for five min, 24 h and 48 h.

Results: The MBCs of EF sanitizers (AB, CAB) were significantly impacted (P<0.05, Student's t-test) by treatment temperature, with twofold higher MBCs at 4°C compared to 30°C for AB (20 of 22 isolates) and CAB (18 of 22). Similar results were obtained for QAC2 (11 of 22) and BAC (13 of 22). On stainless steel, sanitizer efficacy was impacted by the time between inoculation and sanitizer treatment. AB and CB eliminated *Listeria* spp. when adherence time was low (five min); however, these sanitizers were less effective with extended times between inoculation and sanitizer treatment (24 and 48 h).

Significance: This study confirmed EF and CB sanitizers are effective against *Listeria* spp. when following manufacturer recommendations. However, their efficacy may be reduced under conditions relevant to FPEs (e.g., temperature, presence of organic material, extended times between sanitation treatments). These findings highlight variables that should be considered when determining suitable sanitizers and sanitation schedules in FPEs.

P2-104 Comparison of Chemical Methods for Removal of *Listeria innocua* Biofilm Attached to a Stainless Steel Surface

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Introduction: Effective antimicrobial treatment of *Listeria innocua* biofilms does not insure removal of inactive biofilms which may serve as a platform for new biofilm growth, making it desirable to not only sanitize food contact surfaces but to also eradicate the biofilm EPS components and attached dead cells.

Purpose: The present study was performed to evaluate different commonly used cleaner/sanitizers for the removal of biofilm components from stainless steel surfaces.

Methods: Eight 2.5 by 7.5 cm stainless steel coupons were immersed 3.5 cm deep into a *L. innocua* inoculum at 25°C for 24 h. Coupons were rinsed and subjected to one of eight treatment solutions: i) no treatment, ii) pH 7.2 water, iii) 0.1% each alcalase and amylase in water, iv) pH 13 sodium hydroxide, v) commercial solution 1, vi) commercial solution 2, vii) 2000 ppm peroxyacetic acid, and viii) five percent hydrogen peroxide. Treated coupons were rinsed, dried and stained using crystal violet or erythrosine B solutions. Stained coupons were photographed and color differences between background and dyed area evaluated using L*a*b* color space. The experiments were repeated three times on three separate days (n=3).

Results: Results indicate the enzyme mixture removed significantly more of the biomass than the remaining treatments (P<0.05). Neither the pH 13 treatment nor commercial solution 2 were significantly more effective in their ability to remove biomass than DI water alone (P<0.05). Treatments with PAA and hydrogen peroxide left biomass on the coupons comparable to the untreated coupon, and commercial solution 1 was minimally, though significantly, more effective at biomass removal (P<0.05) compared to untreated.

Significance: The present work demonstrates that standard treatments to remove biofilms in a processing environment may be ineffective. Results suggest that a dual-stage process of enzyme treatment followed by sanitation will greatly improve the ability to both remove and disinfect biofilms from stainless steel surfaces.

P2-105 Effect of Dry Sanitization on Biofilm of *Salmonella* Strains Isolated from the Peanut Supply Chain

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Introduction: *Salmonella* is an important foodborne pathogen linked to different foods. In the manufacturing environment of low moisture products it is recommended to employ dry sanitization to avoid *Salmonella* growth, since the increase in humidity may result in cell multiplication and biofilm formation.

Purpose: Evaluate the biofilm formation and the effectiveness of dry sanitization methods on *Salmonella* strains isolated from the peanut supply chain.

Methods: In experiment 1, biofilm formation by six *Salmonella* strains (Muenster, Miami, Glostrup, Javiana, Oranienburg, Yoruba) was assessed in coupons of two different materials: AISI 304 stainless steel (SS) and polypropylene (PP). Coupons were immersed in brain heart infusion broth (BHI), with *ca.* 10^3 CFU/ml of each strain. The bacteria count was determined after zero, eight, 24, 48 and 96 h at 25 and 37°C. In experiment 2, the coupons were inoculated with a *Salmonella* pool containing all the strains. After 24 h at 37°C the coupons were exposed to UV-C radiation, dry air (90°C), ethyl alcohol (70%), and a commercial product based on isopropyl alcohol for up to 30 min.The experiments were repeated three times.

Results: No significant difference (P>0.05) in the biofilm formation was verified between SS and PP, and among the strains. In both materials sessile cell counts of three to four log CFU/cm^2 and five-log CFU//cm^2 were observed after eight h at 25 and 37°C, respectively. A five-log reduction was obtained only with UV-C for 30 min in PP coupons. At the same period of time for the other dry sanitization methods the reductions achieved were around two log and there was no significant difference between the materials tested (P>0.05).

Significance: *Salmonella* strains isolated from the peanut supply chain were able to form biofilm in different materials and showed resistance to some dry sanitization protocols.

P2-106 Synergistic Effect of Sodium Hypochlorite and UV Light on the Survival of *Listeria monocytogenes* Biofilms

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❖ ***Developing Scientist Entrant***

Introduction: In the food industry, inadequately cleaned equipment represents a potential source for *Listeria monocytogenes* contamination. This pathogen has shown niche adaptation to different food processing facilities and its ability to form biofilm is a hurdle for food safety. Even if sanitation practices can minimize the risk of pathogen survival, existing evidence suggests that difficult-to-clean sites in plants are still high risk areas. The combination of sanitizers with UV light might represent an effective way to control pathogen growth.

Purpose: The objective of this research was to study the effect of sodium hypochlorite solution (SHS) and UV light alone or in combination on *L. monocytogenes* biofilms survival on stainless steel (SS) surfaces.

Methods: A Centers for Disease Control and Prevention (CDC) biofilm reactor was used to grow four-day old multi-strain *L. monocytogenes* biofilms on SS coupons. Different conditions were evaluated for optimal growth: temperature (30 and 37°C) and nutrients (tryptic soy broth + 0.6% yeast extract and brain

Poster

heart infusion). SHS (100 ppm for five and 10 min) and UV light (254 nm for 15 and 30 min) were the treatment conditions applied. A control treatment (no sanitizer and/or UV light) was also evaluated. Experiments were run in triplicates.

Results: *L. monocytogenes* biofilm cells on SS coupons ranged from 10^6 to 10^7 CFU/cm^2 for control samples. A significant log reduction of three log CFU/cm^2 was observed when surfaces were treated with SHS for 10 min as compared to controls (P<0.05). After UV exposure for 15 and 30 min, a 1.5 and 1.8-log CFU/cm^2 reduction was reported. A greater log reduction was observed when the treatment of 10 min SHS and 15 min UV light were combined: four log CFU/cm^2 (P<0.05).

Significance: This study demonstrates the enhanced effects of SHS and UV light in combination on the survival of *L. monocytogenes* biofilms on SS surfaces.

P2-107 Bacteriophages as Biosanitizers: Using Lytic Phage to Control and Eradicate *Listeria monocytogenes* Biofilm

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Introduction: *Listeria monocytogenes* is a foodborne pathogenic bacterium that causes invasive listeriosis in people with compromised immunity. It can persist at refrigeration temperature, over a wide range of pH, and at high osmolarity. The propensity of *L. monocytogenes* to produce biofilms and survive in mixed-species biofilms contributes to its persistence in food processing environments. Lytic *Listeria* phages represent a strategy to control sessile *L. monocytogenes*.

Purpose: The purpose of this study is to investigate the biosantiation potential of lytic phages against sessile *L. monocytogenes*

Methods: Strains of different *L. monocytogenes* serotypes (1/2a, 1/2b and 4b) were tested for their ability to form biofilms on polystyrene using a 96-well plate static culture crystal violet biofilm assay. The strains were inoculated at similar levels in TSB supplemented with one percent glucose and two percent NaCl and incubated for 48 hours at 25°C. For isolation of biofilm-degrading *Listeria* phages, 38 and 23 samples were collected from dairy and poultry processing facilities, respectively, and *L. monocytogenes* ATCC 19111 (serotype 1/2a) was used as the enrichment strain. Clear plaques with halos were selected for phage purification and characterization. Moreover, the ability of the commercially available Listex P100 phage to degrade a 24-h *L. monocytogenes* biofilm was investigated at 25°C in static culture.

Results: *L. monocytogenes* ATCC 19111 (serotype 1/2a) produced ten times more adherent biomass than the other strains tested. Our results showed that concentrations of P100 of 10^7 and 10^9 PFU/ml eradicated biomass produced by adherent *L. monoycogenes* to levels comparable to negative controls. Fourteen phage isolates producing plaques with haloes were obtained from effluent samples collected from dairy and poultry processing facilities.

Significance: These results suggest that phages may be effective to control mono-species biofilms of *L. monocytogenes* and provide a baseline for comparison with other *Listeria* phages isolated in this study.

P2-108 Effect of Wash Water Matrix on the Correlation between Free Chlorine and Oxidation-reduction Potential during Fresh Produce Washing Operations

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Introduction: During washing of fresh produce, free chlorine (FC) is added to wash water to avoid pathogen cross-contamination. FC is consumed by organics released into the water from the produce. So, FC dosing during processing is used to maintain sufficient antimicrobial capacity in the wash water. Oxidation-reduction potential (ORP) measurement is used to indirectly assess the FC residual status during wash processes. ORP is appealing to the industry because it is an easy-to-use technology. Despite the industry's interest, the effect of the produce wash operation and organic matter on the ORP is virtually unknown.

Purpose: Assess how water matrix composition (pH, temperature, acidulant, produce) influences the relationship between FC and ORP during fresh produce washing.

Methods: Fresh-cut wash water(500ml per trial) was generated by washing romaine (n=46) or iceberg lettuce (n=67), carrot (n=41), or onion(n=38), and whole produce wash water from tomato (n=101). FC was progressively dosed while ORP was recorded. Influence of pH, temperature and acidulant on ORP were tested in deionized water (n=371). The influence of the water matrix on ORP was tested for all produce wash waters (n=293) and tap water (n=219).

Results: The ORP was correlated with the logarithm of FC under all conditions. A decrease in pH or temperature led to a large and small increase in ORP, respectively. Using tap water instead of distilled water to wash the produce significantly changed the ORP. For all types of tested produce, increasing the product-to-water ratio (e.g., increasing the organics transferred into the water) while maintaining the FC residual led to a decrease in ORP. The choice of acidulant during washing also influenced ORP.

Significance: The ORP can be used for predicting FC when determined for a specific fresh produce operational system and for a specific produce type, but a general guideline/correlation for all wash waters is unrealistic.

P2-109 Multi-Lab Validation for FDA Identification of *Salmonella*, *E. coli* and *Listeria monocytogenes* Using the Vitek-MS System

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Introduction: This study is a continuation of a single laboratory study that was conducted in 2017 for the bacterial identification of *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* using the VITEK-MS instrument.

Purpose: The FDA ORA ORS Pacific Northwest Laboratory (PNL) in coordination with the Center for Food Safety and Applied Nutrition (CFSAN) organized a multi-laboratory study to further validate the technology for these common foodborne pathogens beyond a single laboratory. If successful, this study would support the use of this instrument for bacterial identification of the target organisms.

Methods: A randomized, blinded bacterial panel consisting of 64 traceable, previously characterized environmental and reference isolates composed of *Salmonella* spp. (n=16), *Escherichia coli* (n=16), *Listeria monocytogenes* (n=16) and various exclusionary organisms (n=16) were analyzed in by 18 different collaborators. The standard Biomerieux methodology was utilized.

Results: The results show that this instrument identifies *Salmonella* and *E. coli* species at near 100% accuracy with no exclusionary organisms identifying as these targets. While *Listeria monocytogenes* was identified 96.8% of the time, other *Listeria* species were incorrectly identified as *Listeria monocytogenes* in 13.9% of the tests.

Significance: These multi-laboratory study results strongly support the potential use of the VITEK-MS for the cultural identification of *Salmonella* species and *Escherichia coli* strains. However, for the identification of *Listeria monocytogenes* and other *Listeria* spp., additional refinements to the *Listeria* database may be warranted prior to full application for *L. monocytogenes* identification.

P2-110 Influence of Suspended Particulates from Harvest Debris on *Salmonella* Survival in Chlorinated Whole Tomato Wash Water

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Introduction: *Salmonella enterica* is the main foodborne illness pathogen associated with fresh tomato consumption. During industrial washing, free chlorine (FC) is added to the water to inactivate pathogens and prevent cross-contamination to tomato fruits. However, even maintaining FC residual >100 mg/l (e.g., instantaneously lethal to *Salmonella*), does not completely prevent pathogen cross-contamination. Considerable harvest debris (leaves, stems, soil) enters the wash water along with tomatoes, resulting in suspended particulate matter in the water. Association of *Salmonella* with particulates may contribute to rare occurrences of cross-contamination in high FC residual wash water.

Purpose: Evaluate the survival of inoculated *Salmonella* in FC≥100mg/l tomato wash water containing a range of suspended particulates of different sizes.

Methods: Grape tomatoes, of which 10% were inoculated with *Salmonella*$^{kan\text{-}R}$, were washed (one min) with harvest debris inoculated with *Salmonella*$^{rif\text{-}R}$, in tomato wash water (made by washing one kg/l tomatoes in tap water) (n=6). This experiment was repeated (n=6) with reversed inocula, (tomato, *Salmonella*$^{rif\text{-}R}$; debris, *Salmonella*$^{kan\text{-}R}$).

Results: Inoculated tomatoes (six log CFU/g) and debris (8 log CFU/g) each independently released six log CFU/100 ml *Salmonella* into wash waters. With FC >100 mg/l, three log CFU/100 ml *Salmonella* survived in the wash water. Of these survivors, 50% remained suspended after filtration through 2000 µm, 15% after 300 µm filtration, and seven percent after 60 µm filtration. Only *Salmonella* originating from debris survived, no surviving (one log CFU/100ml detection limit) cells originating from tomatoes were found in water (10 ml sample size).

Significance: *Salmonella's* survival in the chlorinated tomato wash water is associated with particulates, where survivability increases with particulate size. Surviving *Salmonella* cells in tomato wash water are harbored in particulates fragmented from harvest debris during dumping and tank wash processes. No transfer occurs from tomatoes to particulates. The risk of *Salmonella* cross-contamination during fruit washing could be reduced by avoiding the comingling of debris with harvested tomatoes and/or eliminating debris from wash tanks.

P2-111 Efficacy of Ferrous and Alkaline-activated Persulfate to Remove Foodborne Pathogens from Romaine Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: Activated persulfate has shown high efficacy in foodborne pathogen inactivation and has been considered as an alternative sanitizer for fresh produce sanitation.

Purpose: This study aimed to investigate the efficacy of activated persulfate to remove *Escherichia coli* O157:H7 and *Listeria monocytogenes* from romaine lettuce.

Methods: A five-strain cocktail of *E. coli* O157:H7 and *L. monocytogenes* were inoculated on fresh romaine lettuce surfaces. The inoculated leaves were treated with sodium persulfate activated by ferrous sulfate or sodium hydroxide for one, three, and five min. The initial persulfate level was set at 10 to 70 mmol/l or 100 to 700 mmol/l depending on the treatment conditions. The treated leaves were plated on both selective and nonselective agar plates. The wash water was also collected and analyzed. Sodium hypochlorite treatment was applied as a comparison. The effect of activated persulfate treatment on lettuce color quality was also evaluated. The entire experiment was repeated three times.

Results: The results showed both ferrous and alkaline activated persulfate achieved up to 3.5-log CFU/g reductions of both pathogens on romaine lettuce. Higher persulfate level and longer treatment lead to significantly (P<0.05) higher reductions. When persulfate concentration reached 70 mmol/l, no pathogens were recovered from wash water even after enrichment. However, the wash water from ferrous activated persulfate for treating *L. monocytogenes* showed recoveries even the persulfate concentration was increased to 70 mmol/l, which might be due to the higher resistance against activated persulfate treatment. Furthermore, both activation methods showed equal or higher reduction efficacy than sodium hypochlorite at 100 mg/l and pH 6.5. Under the current test range, no significant (P≥0.05) color deterioration was found on lettuce after the treatment.

Significance: This study demonstrated the high efficacy of activated persulfate to remove foodborne pathogens from produce surface and revealed the potential of activated persulfate for food safety applications.

P2-112 Comparison of Sanitation Methods Commonly Used by the United States Fresh Produce Industry or Ghanaian Households for Inactivating

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❖ ***Developing Scientist Entrant***

Introduction: A previous survey conducted by our laboratory revealed poor microbial quality and the presence of *Salmonella* for leafy green vegetables grown or sold in Accra, Ghana.

Purpose: The aim of this study was to compare the effectiveness of sanitation methods commonly used by Ghanaian households (water, citric acid, vinegar and salt water) to several sanitation approaches used by the fresh produce industry in the United States (chlorine, peracetic acid and ozonated water) in reducing the population of *Salmonella* on vegetables.

Poster

Poster

Methods: Cabbage and lettuce artificially inoculated with three *Salmonella* cocktails were treated with sterile water or each of the six sanitizers. The efficacy of the sanitizers in inactivating *Salmonella* was evaluated using standard plate count assay. The effect of the treatments on the overall acceptance of vegetables was evaluated by a consumer panel.

Results: Treatments with chlorine, citric acid, peracetic acid and vinegar resulted in a 2.44- to 2.67-log CFU/g reduction in *Salmonella* counts on cabbage and were significantly ($P \leq 0.05$) more effective compared to the other treatments used in the study. Treatment with citric acid was most ($P \leq 0.05$) effective in reducing the level of *Salmonella* on lettuce (three log CFU/g). Treatments with ozonated water, salt and water were significantly ($P \leq 0.05$) less effective, compared to other treatments used in the study, in reducing *Salmonella* counts on both types of vegetables (0.02 to 1.32 log CFU/g). The consumer panel concluded that citric acid, vinegar and water treated cabbages were the more preferred ($P \leq 0.05$) than chlorine treated ones. Lettuce treated with citric acid and water were more preferred ($P \leq 0.05$) whilst salt-treated lettuce was the least preferred.

Significance: Among sanitation methods commonly used in Ghanaian households, citric acid and vinegar were more effective in reducing microbial counts on vegetables with a lesser detrimental effect on their sensory quality.

P2-113 Influence of Bacterial Competitors on S*almonella enterica* Growth in Microbiological Media and Attachment to Vegetable Seeds

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Introduction: Interest in using biological agents for control of human bacterial pathogens on vegetable seeds is on the rise.

Purpose: The objective of the present study was to evaluate the influence of bacterial competitors on *Salmonella enterica* growth in microbiological media and attachment to vegetable seeds.

Methods: A selected plant pathogen (*Pseudomonas syringae* pv. *Tomato* DC3000), a probiotic bacterium (*Lactobacillus rhamnosus* GG) or bacterial strains previously used as biocontrol agents in plant science (*P. fluorescens* A506, *Bacillus mojavensis* RRC 101 and *Bacillus subtilis* ATCC 6051) were mixed individually with *Salmonella* for competitive growth in microbiological media or attachment to the seeds of alfalfa, fenugreek, lettuce and tomato. The inhibitory effect of metabolites in cell-free supernatants of competitive bacterial spent cultures on the growth of *Salmonella* was also evaluated.

Results: Results showed that the mean population of *Salmonella* in co-culture with *L. rhamnousus* GG at 37°C was 5.36 log lower than the population in the control and 3.53 log lower than the *Salmonella* population in the control at 25°C. The addition of *L. rhamnousus* GG cell-free supernatants to broth resulted in significant ($P<0.05$) reductions in *Salmonella* populations. *Salmonella* cells became undetectable (<10 CFU/ml) at the 12 h sampling point and forward, and a total of five-log reduction was achieved after the 24 h incubation period. Although not as effective as *L. rhamnosus* GG in inhibiting the growth of *Salmonell*a, the biocontrol agents were more effective in competing with the pathogens for attachment to vegetable seeds. When no competitive bacterial strains were present, the mean attachment ratios of *Salmonella* cells to the four types of vegetable seeds were 10.5%. In the co-cultures with *P. fluorescens* A506, *Salmonella* had a lower attachment ratio of 7.0%.

Significance: Results of the study will help to strategize interventions for the production of vegetable seeds with useful microbial qualities.

P2-114 Analyzing Aggregate Environmental Monitoring Data for *Listeria* spp. in Frozen Food Manufacturing Environments

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❖ ***Developing Scientist Entrant***

Introduction: Food processors face serious challenges due to the ubiquity and prevalence of *Listeria monocytogenes* in production facilities. Environmental monitoring for *Listeria* within the industry is important, and detection of *Listeria* spp. is often used as an indicator for the potential presence of *L. monocytogenes* in the food processing environment.

Purpose: Historical environmental monitoring data from the frozen food manufacturing industry was compiled and analyzed to evaluate adequacy of current practices in mitigating the risk of *L. monocytogenes* in the processing environment and to determine if there are trends that could be used to further refine industry practices.

Methods: A method was developed to collect anonymous data for analysis to build a strong aggregate data set from multiple facilities. Information included general descriptions of each facility and specific information about individual environmental monitoring test results. Descriptors were provided to allow for grouping of similar results. The historical data collected spanned six months to a year of environmental monitoring samples.

Results: Twenty-eight facilities provided 47,628 environmental monitoring observations. Removing non-identified data, 38,671 observations were analyzed. More *Listeria* positive samples were detected in zones three and four compared to zone two ($P<0.01$). Pre-lethality production areas had a higher percentage of *Listeria* positive results than other areas ($P<0.01$). There were no significant differences in the frequency of *Listeria* samples during different production shifts and production times ($P=0.5$ and $P=0.3$). Sponges are more likely to be positive than swabs ($P<0.05$). Corrective action samples have higher probability of *Listeria* than routine environmental monitoring samples ($P<0.01$).

Significance: There is variability in sampling plans performed within the industry, indicating an opportunity to assist facilities with incorporating best practices in environmental monitoring for *Listeria*. Prevalence data from processing operations can provide the industry guidance on focused sanitation locations within processing operation to reduce the risk of *L. monocytogenes.*

P2-115 Evaluation of Enzymatic Cleaning on the Microbial Flora of Installations and the Food Products in a Processed Food Industry

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Introduction*:* Biofilms are a permanent source of contamination in food industries and can harbor various types of microorganisms, such as spoilage bacteria. Biofilms are very resistant to conventional cleaning and disinfection methods. New strategies are proposed to eradicate them such, as enzymatic cleaning.

Purpose: The purpose of this study was to evaluate the impact of enzymatic cleaning on the microbial flora on installations in the processed food industry and in the final food products.

Methods: A food company producing Italian dishes was monitored for three months with different cleaning treatments: conventional cleaning, reinforced enzymatic cleaning, and routine enzymatic cleaning. During the period of trials, all factors remained constant and only the cleaning solutions were replaced

in the sanitation treatments. A total of 244 samples were analyzed, including surface samples pipes and finished products (lasagna at day zero and at the end of shelf life) by classical microbiology and 16S rDNA metagenetic. Statistical analysis (non-parametric tests and principal component analysis) was carried out on with R with different packages.

Results: A significant difference in the total flora concentration was observed in the food samples at the beginning of their shelf life between conventional and enzymatic cleaning ($P<0.05$) with a reduction of two log CFU/g. Metagenetic analysis of the food samples at the end of the shelf life showed statistically significant differences of the bacterial flora between conventional and enzymatic cleaning ($P<0.05$) with a decrease of 41% in spoilage bacteria *(Leuconostoc* spp., *Pseudomonas* spp., and *Enterobacteriaceae).* The metagenetic analysis of the surfaces indicates a significant change of the bacterial flora ($P<0.05$) after enzymatic treatment with the removal of the most predominant spoiling bacteria.

Significance: The results indicate a significant decrease in spoilage bacteria in food installations and food products after enzymatic cleaning.

P2-116 Inactivation of *Escherichia coli* O157:H7 in Radish Seeds by Combined Treatments of Gaseous Chlorine Dioxide and Mild Wet Het

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❖ ***Developing Scientist Entrant***

Introduction: A seed decontamination method is important because foodborne pathogens such as *Escherichia coli* O157:H7 exist in seeds of sprout vegetables.

Purpose: This study was carried out to develop a seed decontamination method to inactivate *E. coli* O157:H7 present in radish seeds using a combined treatment of gaseous chlorine dioxide (ClO_2) and mild wet-heat.

Methods: Radish seeds were treated with gaseous ClO_2 (1,000, 2,000, or 3,000 ppm) and mild wet-heat (85% relative humidity (RH) and 60°C) for up to 120 min to determine the conditions which did not decrease the germination rates of radish seeds. Radish seeds were inoculated with *E. coli* O157:H7 (ca. seven log CFU/g), and then the seeds were treated with ClO_2 (1,000, 2,000, or 3,000 ppm) and mild wet-heat for up to 120 min.

Results: The germination rate (97.3 to 99.0%) of radish seeds after combined treatments of ClO_2 and mild wet-heat were not significantly different ($P>0.05$) from those (97.3%) of the control seeds. When the seeds were treated with mild wet-heat for 90 min without gaseous ClO_2, the population of the pathogen was decreased from 6.8 to 4.0 log CFU/g. However, when treated with gaseous ClO_2 (2,000 or 3,000 ppm) and mild wet-heat, *E. coli* O157:H7 was completely inactivated within 90 min.

Significance: It was confirmed that combination of gaseous ClO_2 (2,000 ppm) and mild wet-heat (85% RH and 60°C) can completely inactivate *E. coli* O157:H7 present on radish seeds within for 90 min without decreasing the seed viability.

P2-117 Influence of Types of Abiotic Surfaces on Antimicrobial Activities of Gaseous Chlorine Dioxide Against *Bacillus cereus* Spores

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❖ ***Developing Scientist Entrant***

Introduction: Gaseous chlorine dioxide (ClO_2) has been known to effectively sanitize various abiotic surfaces, but there have been few reports on whether lethality of gaseous ClO_2 against *Bacillus cereus* spores changes with types of abiotic surfaces.

Purpose: This study was performed to investigate the influences of types of abiotic surfaces on the lethality of gaseous ClO_2 against *Bacillus cereus* spores.

Methods: *B. cereus* spores (six log CFU/coupon) were spot-inoculated on the surface of stainless steel, wood, glass, plastic, and carpet coupons (five cm by two cm by one to three mm thick). After inoculation, the coupons were exposed to gaseous ClO_2 (peak concentration: ca. 1,000 ppm) at 85% relative humidity (RH) and 25°C for 30 min and the numbers of viable spores remaining on the coupons were determined.

Results: The initial numbers of *B. cereus* spores attached to various abiotic coupons were 5.7 to 6.3 log CFU/coupon. The numbers of *B. cereus* spores in coupons incubated at 85% RH and 25°C for 30 min without gaseous ClO_2 were not significantly reduced ($P>0.05$). However, when treated with gaseous ClO_2 at 85% RH and 25°C for 30 min, the numbers of *B. cereus* spores on stainless steel, wood, glass, plastic, or carpet coupons were decreased to <1.5, 3.0, <1.5, <1.5, or <1.5 log CFU/coupon, respectively (detection limit: 1.5 log CFU/coupon).

Significance: This is the first study to show that the bactericidal effects of gaseous ClO_2 against *B. cereus* spores are significantly influenced by the types of attachment surfaces. The results of this study may provide useful information in developing a decontamination method using gaseous ClO_2.

P2-118 Inactivation of *Escherichia coli* O157:H7 on Various Abiotic Surfaces Using Gaseous Chlorine Dioxide

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7 on abiotic surfaces, even in low numbers, can act as a persistent source of cross-contamination resulting in causing foodborne diseases.

Purpose: This study was conducted to determine the efficacy of gaseous chlorine dioxide (ClO_2) for inactivating *E. coli* O157:H7 attached to various abiotic surfaces.

Methods: *E. coli* O157:H7 was spot-inoculated with six log CFU/coupon onto stainless steel, wood, glass, plastic, and carpet coupons (five cm width by two cm length by one to three mm height) followed by drying for one h. After drying, the coupons were incubated at 85% or 100% relative humidity (RH) and 25°C for 30 min with or without gaseous ClO_2 (peak concentration: 1,000 ppm). The remaining populations of *E. coli* O157:H7 were determined by spread plating and enrichment methods (detection limit: 1.5 and zero log CFU/coupon, respectively).

Results: The numbers of attached *E. coli* O157:H7 on stainless steel, wood, glass, plastic and carpet coupons were 4.6 to 5.9 log CFU/coupon. In the absence of gaseous ClO_2, the populations of *E. coli* O157:H7 in those coupons were not decreased significantly ($P > 0.05$) during incubation at 85% or 100% RH, 25°C for 30 min. When treated with gaseous ClO_2 (peak concentration: ca. 1,000 ppm) at 25°C for 30 min, regardless of RH, *E. coli* O157:H7 on stainless steel, glass, plastic, and carpet coupons were completely inactivated. However, the populations of *E. coli* O157:H7 attached on wooden surfaces decreased only by 1.3 to 1.6 log CFU/coupon.

Poster

Poster

Significance: The results of this study may provide basic information for developing a disinfection method for abiotic surfaces using gaseous ClO_2 in a short time.

P2-119 *Escherichia coli* O157:H7 Inactivation in Phosphate Buffer by X-Ray with Various Levels of Accelerating Voltage

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Introduction: Outbreaks of illnesses caused by *Escherichia coli* O157:H7 can seriously damage the fresh produce and seafood industries. X-ray irradiation can effectively eliminate pathogens. The energy level of X-ray depends on the accelerating voltage. However, how varying the energy levels affects the efficacy of bacterial inactivation at the same doses has not been reported.

Purpose: In this investigation, we determined the efficacy of inactivation of *E. coli* O157:H7 in phosphate buffer by X-ray with various levels of accelerating voltage.

Methods: The pure culture (phosphate-buffer saline, pH 7.4) was prepared with a three-strain mixture of *E. coli* O157:H7 (F4546, EDL933 and ATCC 43895). Bacterial strains were grown in tryptic soy broth with 0.6% yeast extract and inoculated at 37°C for 24 h prior to use and mixed with an equal volume to give approximately 10^9 CFU ml^{-1}. Pure culture was treated with zero, 250, 500, 750, 1000 or 1250 Gy X-ray at 50, 200 or 350 kV of accelerating voltages with or without one mm aluminum filter.

Results: The result of plate count demonstrated a lower susceptibility to X-ray when no filter was applied on the machine (P<0.05). Treatments with 1250 Gy X-ray achieved the reductions of 5.5 log CFU ml^{-1} at 350 kV or 2.5 and 2.2 log CFU g^{-1} at 200 and 50 kV, respectively. With the use of the aluminum filter, *E. coli* O157:H7 was inactivated to below the detection limit (less than two log CFU ml^{-1}) when X-ray dose was equal or higher than 750 Gy. Treatments with 500 Gy X-ray achieved reductions of 6.6 log CFU ml^{-1} at 50 kV or 5.9, and 4.8 log CFU ml^{-1} at 200 and 350 kV, respectively.

Significance: The degrees of *E. coli* O157:H7 inactivation by X-ray differed significantly by varying accelerating voltages and the inclusion of a filter.

P2-120 Consecutive Treatments with Sterilex Eliminate Biofilms by

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Introduction: Biofilms by *E. coli* O157:H7 and *Salmonella enterica* pose a serious risk to meat safety. The meat industry needs effective sanitizers to eliminate biofilms on contact surface. Sterilex is a sanitizer product consisting of a proprietary hybrid biocide formulation that can be applied as foam or liquid solution. Three consecutive treatments using 10% diluted sanitizer are recommended by the manufacture when active biofilms are present.

Purpose: To evaluate the effectiveness of consecutive treatments by this sanitizer against *E. coli* O157:H7 and *Salmonella* biofilms.

Methods: Biofilms on stainless steel coupons (n=4) were treated with 2.5, 5, or 10% sanitizer applied as foam or liquid solution. Three consecutive treatments at each concentration with various exposure time periods were performed. Bacterial log reductions and post-enrichment prevalence were measured. Biofilm removal and bacterial morphology alternation after treatment were evaluated with scanning electron microscope (SEM).

Results: Compared to positive controls (n=4), viable bacteria at significantly lower levels (P<0.05) were only detected after one or two treatments using 2.5% or 5% sanitizer. Three consecutive treatments at all concentrations reduced both types of bacteria to a non-enumerable level (<2.2 log CFU).

After enrichment, no O157 prevalence was observed after three consecutive treatments at all concentrations. Positive *Salmonella* prevalence was observed after strong biofilm forming strains (greater than seven log CFU) were treated, indicating higher tolerance of the strong *Salmonella* biofilms. SEM revealed significant reduction of the biofilm matrix and a lack of bacterial connecting extracellular polysubstance after the treatments. Instead, disaggregated bacteria with altered morphology and decreased cell length was observed, suggesting membrane damage and loss of cell viability.

Significance: Consecutive treatments with Sterliex is effective against *E. coli* O157:H7 and *Salmonella* biofilms.

P2-121 Comparison of Antimicrobial Activities of Organic Acid Vapors Against *Escherichia coli* O157:H7 and *Listeria monocytogenes* Attached on Stainless Steel

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❖ ***Developing Scientist Entrant***

Introduction: Liquid organic acids have been used as natural sanitizers on food and food-contact surfaces, but the antimicrobial activities of organic acid vapors have not been intensively studied.

Purpose: This study was done to compare the antimicrobial activities of various organic acid vapors against *Escherichia coli* O157:H7 and *Listeria monocytogenes* attached to stainless steel surfaces.

Methods: *E. coli* O157:H7 or *L. monocytogenes* cells were spot-inoculated on the stainless steel coupons (SSC; five by two cm). SSC containing pathogens were incubated at 43% relative humidity (RH) and 25°C with or without acetic, propionic, or formic acid vapor (ca. 100 ppm) for up to six hours. After incubation, the remaining numbers of pathogens on SSC were determined.

Results: The populations of *E. coli* O157:H7 and *L. monocytogenes* inoculated on SSC were 5.3 and 4.6 log CFU/coupon, respectively. Without organic acid vapors, the numbers of pathogens on SSC were not significantly changed during incubation (P>0.05) at 43% RH and 25°C for one hour. However, when treated with propionic acid vapor (100 ppm), the populations of *E. coli* O157:H7 and *L. monocytogenes* were decreased by 3.2 and 0.8 log CFU/coupon, respectively, within one1 hour. After treatment with acetic acid or formic acid vapor at 43% RH and 25°C for one hour, both pathogens were reduced below the detection limit (detection limit: 1.5 log CFU/coupon).

Significance: This study is the first to compare the antimicrobial activities of three types of organic acid vapors against *E. coli* O157:H7 and *L. monocytogenes* on SSC.

Poster

P2-122 Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Formica Coupons by Switchgrass Extractives, a Value-Added Product

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❖ ***Developing Scientist Entrant***

Introduction: Switchgrass, a biofuel feedstock, contains extractives rich in antimicrobial polyphenols that inhibit the enzymatic conversion of carbohydrates. These polyphenols have potential to reduce microbial populations on food contact surfaces. Recent high-profile foodborne outbreaks associated with *Escherichia coli* O157:H7 contaminated lettuce and *Salmonella* contaminated sprouts emphasize the need for improved food safety measures.

Purpose: The objective of this study was to determine the efficacy of switchgrass extractives to decrease *E. coli* O157:H7 and *Salmonella* Typhimurium populations on formica coupons, a model food-contact surface.

Methods: Overnight cultures of *E. coli* O157:H7 and *Salmonella* Typhimurium grown in tryptic soy broth (TSB) at ~seven log CFU/ml were air-dried for 10 min on sterile formica coupons (~five by five cm) and treated with sterile water, 10% household bleach (0.6% sodium hypochlorite), 70% ethanol, or switchgrass extractives for up to 30 min. Bacteria were eluted in TSB-beef extract, serially diluted ten-fold in phosphate buffered saline, surface-spread plated on TSA and incubated at 37°C for 48 h. Data from three replicates of bacterial counts assayed in duplicate were analyzed for comparison of treatments to water controls.

Results: Switchgrass extractives reduced *E. coli* O157:H7 by 1.44±0.54 log CFU/ml on formica after 10 min, by 1.7±0.69 log CFU/ml after 15 min, and to non-detectable levels (≥5-log reduction; detection limit: two log CFU/ml) after 30 min. Bleach (10%) and ethanol (70%) reduced *E. coli* O157:H7 populations to non-detectable levels (≥5 log reduction) after 10 min. Switchgrass extractives reduced *Salmonella* Typhimurium by 1.48±0.48 log CFU/ml, while 10% bleach and 70% ethanol caused reduction to non-detectable levels (≥5-log reduction) after 15 min.

Significance: Switchgrass extractives require longer contact-times to decrease *E. coli* O157:H7 and *Salmonella* Typhimurium populations than 10% bleach or 70% ethanol. Combinations of concentrated switchgrass extractives with other natural disinfectants and/or in hurdle approaches warrant further investigation.

P2-123 Disinfection Efficiency of Slightly Acidic Electrolyzed Water Combined with Chemical Treatments on Fresh Fruits

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Introduction: Potent sanitizers are required for washing farm produce to minimize pathogen contamination and protect human health.

Purpose: In this study, the disinfection efficacy of slightly acidic electrolyzed water (SAEW), fumaric acid (FA) and calcium oxide (CaO) on fresh fruits were investigated.

Methods: The effects of combining SAEW with FA, CaO with SAEW and CaO+SAEW+FA on bacterial coliforms, yeast and mold decontamination on fresh apples, mandarin and tomato were determined. The effects of the treatments on the sensory quality of the treated fruits during storage at 4°C and room temperature (~23°C) were also analyzed. The fruits were washed by a self-developed washing line. Foodborne pathogen inactivation study was also carried out after inoculating the fruit surfaces with *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Results: Treating the fruits with SAEW combined with the two chemical sanitizers resulted in significant (P<0.05) bacterial reduction compared to tap water (control). Treatment with CaO+SAEW+FA resulted in the highest reduction of natural microflora and foodborne pathogens. In all the treatments, the appearance, color, odor and overall quality of fruits did not significantly change (P>0.05) until 12 days of refrigeration storage. Also, the microorganism populations were under the shelf life limits during the storage experiments.

Significance: This data suggests that the combined treatment presents a more efficient means of disinfection and maintaining the quality of fresh fruits.

P2-124 Effectiveness of Blue Light-emitting Diode Illumination in the Inactivation of Histamine-producing Bacteria

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Introduction: Scombroid poisoning is a food-borne intoxication caused by the consumption of seafood containing relatively high histamine content. Visible light-emitting diodes (LEDs) that have antibacterial effects afford a novel method for food preservation. Nevertheless, little information is available on the effectiveness of blue LEDs (470 nm) in the inactivation of various histamine-producing bacteria.

Purpose: The objective of this study was to evaluate the antibacterial effects of blue LEDs on *Enterobacter aerogenes*, *Morganella morganii*, *Raoultella ornithinolytica*, and *Staphylococcus capitis* and on histamine formation.

Methods: A blue LED light source providing a photosynthetic photon flux density of 430 μmol/m^2/s was used for illumination. Bacterial cultures suspended in two percent L-histidine tryptic soy broth (TSBH) was illuminated by 0.38 mW/cm^2 blue LEDs at a distance of 4.5 cm at 10, 20, and 30°C for 12 h.

Results: The initial suspension culture of *M. morganii* (five log CFU/mL) was almost completely inactivated to less than one log CFU/ml. However, regarding the photodynamic inactivation of bacteria, blue LEDs exerted only slight effects on *E. aerogenes*, *R. ornithinolytica*, and *S. capitis* in that they resulted in only 0.73 to 1.29 and 0.93- to 2.75-log CFU/ml reductions in the bacterial populations after 12 h at 10 and 20 °C, respectively. Bacterial cultures (three log CFU/ml) suspended in TSBH broth were also illuminated by 0.38 mW/cm^2 blue LEDs at a distance of 4.5 cm at 30°C. The bacterial populations significantly increased to 7.53 to 8.64 log CFU/ml, but illumination with blue LEDs could significantly inhibit histamine formation. These results thus demonstrate that the antibacterial effect of the LEDs was highly dependent on the bacterial species, illumination time, and temperature. In addition, the blue LEDs exhibited enhanced antibacterial effects under acidic and alkaline pH condition.

Significance: The results of this study elucidate the effectiveness of blue LEDs (470 nm) in inhibiting histamine formation and thus demonstrate their suitability as a novel method for reducing the risk of histamine poisoning.

Poster

P2-125 Efficacy of Novel Photo-chlorine Dioxide against *Clostridium difficile* Endospores

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Introduction: *Clostridium difficile* infections are number one cause of healthcare-associated infections in many developed countries. *C. difficile* endospores pose a significant health risk to the public's health based on their environmental resilience.

Purpose: The aim of this study was to evaluate the efficacy of a novel photo-chlorine dioxide disinfectant against *C. difficile* endospores.

Methods: Chlorine dioxide was generated by mixing one percent sodium chlorite with 10 ppm eosin Y. Photo-chlorine dioxide efficacy was assessed against *C. difficile* endospores in suspension, and on stainless steel under laboratory and greenhouse conditions by following EAP SOP No. MB-31-03 protocol. Surviving endospores were enumerated by plate count assay on brain heart infusion agar supplemented with yeast extract, L-cysteine, and sodium taurocholate (BHIA-YE-CYS-T) with and without organic soiling. All experiments were repeated three times.

Results: In the suspension, *C. difficile* endospores were reduced by 2.25 and 3.65 log CFU within two h with and without soil, respectively. The log reductions of the endospores on stainless steel carriers after two h exposure were less than two $\log_{10}$ CFU with and without soil. Under the greenhouse conditions, the reductions of endospores were ca. three log CFU for both with and without soil conditions after 24 h with four doses of ClO_2 treatment.

Significance: This was the first study to evaluate the efficacy of a novel chlorine dioxide generation system against any human pathogen. Our results suggest this new system is capable of reducing *C. difficile* endospores. However, endospores are more resistant to disinfection especially under the soiled conditions.

P2-126 Ultraviolet Light with Grape Seed Extract and Curcumin Inactivates Aichi Virus on Formica Surfaces

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✦ ***Undergraduate Student Award Entrant***

Introduction: Aichi virus (AiV) causes human gastroenteritis globally due to the consumption of contaminated oysters. AiV of the *Picornaviridae* family (same family as hepatitis A virus) is a 30 nm, small, non-enveloped, single-stranded, positive-sense RNA virus. Novel methods to control the spread of AiV are being researched, as commercial vaccines against AiV are currently unavailable. Photodynamic inactivation (PDI) is a control strategy that utilizes light to inactivate a range of microorganisms including bacteria, viruses, fungi, and parasites, in the presence of oxygen and a photosensitizer. Curcumin and grapeseed extract (GSE) are potential natural photosensitizers that can be used in PDI against AiV.

Purpose: The objective of this research was to determine the ability of PDI-mediated by grapeseed extract and curcumin to inactivate AiV on a model food contact surface.

Methods: AiV at 5.7 log PFU/ml was aseptically dried on sterile Formica coupons (a model food contact surface) in a biosafety cabinet and treated with either water (control), 0.05 mg/ml curcumin, 10 mg/ml GSE, ultraviolet light (UV) light at 254 nM alone, UV with 0.05 mg/ml curcumin or UV light with 10 mg/ml GSE, for up to 30 min at room temperature. Viruses from three replicate treatments were recovered and plaque assayed in duplicate using confluent Vero host cells in six-well plates.

Results: AiV showed reduction of 0.66 and 1.26 log PFU/ml with 0.05 mg/ml curcumin and 10 mg/ml GSE, respectively, while UV light alone caused ~2.7-log PFU/ml reduction after 30 min. Both, GSE with UV light and curcumin with UV light after 30 min showed increased reduction of 2.88 log and 3.41 log PFU/ml respectively, compared to either 10 mg/ml GSE or 0.05 mg/ml curcumin alone.

Significance: Thus, UV light together with natural plant extracts shows promise to decrease AiV transmission from food contact surfaces.

P2-127 Effect of High Intensity Light Pulses on the Reduction of Microbial Load in Chia (*Salvia hispanica L.*) Seeds

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Introduction: The harvest of chia seeds (*Salvia hispanica* L) provides an excellent environment for the growth of microorganisms; the moisture resulting from the irrigation process, the aerobic conditions, pH, and temperatures all contribute to the rapid expansion of microbial populations, including pathogens. Populations are as high as 109 CFU/g have been reported in chia seeds obtained from retail stores.

Purpose: The aim of this study was to evaluate High-Intensity Light Pulses (HILP) in microbial load reduction in chia seeds

Methods: Microbiological load of different samples of chia seeds from local stores in Puebla City (Mexico) was evaluated. The effect of HILP on the susceptibility of a native load of chia was evaluated (aerobic mesophilic bacteria and total coliforms). Twenty-four samples of 50 g each had HILP applied at different time and distance conditions. Response surface methodology (RSM) was used to evaluate the effects of variables, namely the time, distance and their interactions. A central composite experimental design was adopted to derive a statistical model for optimizing the microbiological load reduction in the chia seeds. The energies evaluated in this experimental design ranged from 818 to 4651 J/cm^2.

Results: Reductions of two to four log in aerobic mesophilic bacteria and total coliforms were observed when using HILP. The experimental results showed that the time and the interaction time and distance are significant (P<0.05) in model prediction.

Significance: These findings support HILP as a suitable method for reduction of microbial load in chia seeds

P2-128 Evaluation of Environmental Monitoring Tools for the Release of Microorganisms

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Introduction: Environmental monitoring (EM) is used to determine harborage sites of microorganisms, assess the effectiveness of surface sanitation programs, and prevent transmission of microorganisms. Surprisingly, most EM tools are not well characterized in their ability to recover and release microorganisms for downstream detection.

Purpose: To characterize EM tools for the release of microorganisms.

Methods: Five-hundred microliters of a bacterial cocktail (*Listeria monocytogenes*, *Salmonella* Typhimurium) was directly inoculated onto polyurethane foam and cellulose swabs hydrated with PBS. Inoculum levels ranged from 10^6 to 10^2 CFU per swab. Bacteria were recovered by machine stomaching or manual elution processes without enrichment and plated onto selective agar. Microbial release experiments were replicated using Tulane virus, a human norovirus surrogate, and results were obtained via plaque assay. To study operator variability, 10^4 CFU or PFU of microorganisms were inoculated on both swab types and manually eluted. For each treatment, samples were analyzed in duplicate with one experimental replicate.

Results: Microbial release was more consistent using manual elution methods when compared to machine processing. The manual and machine elution methods resulted in mean log losses of 0.55 and 0.71, respectively. Data indicated significantly (P<0.05) greater release of bacteria compared to Tulane virus with a nearly 20% greater mean release. The mean release of microorganisms from swabs decreased as the inoculum level decreased. For instance, 91.2% of all microorganisms were released when swabs were inoculated with 10^5 CFU or PFU/500 µL compared to 76.3% of microorganisms at 10^2 CFU or PFU/500 µl. Operator variability data indicate that there is no significant (P<0.05) difference amongst operators during manual swab processing.

Significance: Results indicate that the release of microorganisms from EM tools currently used in the food industry varies across microbial type, swab material, and elution process type. This variation emphasizes the need for industry standardization and further EM tool evaluation.

P2-129 Usage Status of ATP Bioluminometers by Dietitians of the Center for Children's Foodservice Management in Korea

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Introduction: Among the 215 Centers for Children's Foodservice Management (CCFSM) in Korea, certain centers have been performing sanitary inspections on children's foodservices with adenosine triphosphate (ATP) bioluminometers to complement the inspection by sanitation checklists.

Purpose: The purpose of this study was to examine usage status of ATP bioluminometers in the inspections for children's foodservices.

Methods: Conducted a survey of 120 dietitians working at 50 CCFSM. Survey questionnaire was composed of items that examined knowledge on principles of ATP bioluminescence (eight items, full points one), types of swabbing surfaces and performance of proper methods (11 items, full points five). The data were analyzed using chi-square test, *t*-test or ANOVA by SPSS 23.0.

Results: ATP usage frequencies identified by 120 respondents were "more than once a month" (48.3%), "more than once semiannually" (32.5%) and "seldom used" (19.2%). Average score for knowledge on principles of ATP bioluminescence was 0.64±0.20 which showed no significant difference. Among the different ATP usage frequency groups: "more than once a month" (0.68±0.19) showed higher knowledge than others (P<0.05). With average performance score on performance of proper methods, "more than once a month" (3.88±0.49) and "more than once semiannually" (3.90±0.53) showed significantly higher scores than "seldom used" (3.54±0.59) (P<0.05). With swabbing surface for ATP, scores were for hand (0.74±0.44), cutting board (0.67± 0.47), knife (0.52±0.50), refrigerator (0.49±0.50) and water purifier (0.31±0.46).

Significance: Scores for knowledge item "ATP count and aerobic plate count are always co-related (0.38±0.48 at full score of one)" and performance item "To swab with a certain area, use a template board. (2.31±0.48 at full score of five)" were too low and there is a need to develop guideline and implement specialized trainings for improvement.

P2-130 Comparison of Sanitary Inspection Results on Knives and Gloves by the Grade of Children's Foodservice

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Introduction: Based on the results of sanitary inspection in the year 2017, Changwon 1st Center for Children's Foodservice Management (CCFSM) in Korea classified 123 children's foodservices which should abide by the Food Sanitation Act (50 to 99 children attending), into two grades: fruit grade (n=22) and bud grade (n=101). During 2018, fruit grades (superb checklist score) had four sanitary inspections while bud grades (average checklist score) had six inspections.

Purpose: This study was performed to examine whether bud grade children's foodservices had improvements in their sanitary status for the use of knives and gloves.

Methods: CCFSM dietitians visited foodservices to monitor the criteria "Use knives separately for each case of handling fish/meat/vegetables" and "Use rubber gloves separately for their different usages (pre-preparation, cooking, cleaning)." Dietitians swabbed 25 cm^2 areas of knives and gloves two times: one for obtaining ATP measurements, and the other for aerobic plate count (APC) using 3M Petrifilm Plates. One-hundred twenty-three samples of knives (for vegetables) and 123 samples of gloves (for cleaning) were taken for APCs. Chi-square test and *t*-test were applied in SPSS 23.0.

Results: The APC test relevance ratio for knives was 90.9% for fruit grade and 92.1% for bud grade, which was not a significant difference. Gloves showed a significantly higher APC test relevance ratio of 90.0% for fruit grade while bud grade was 66.3% (P<0.05). The sanitary status of knives in bud grades achieved improvement up to that of fruit grades; however, for gloves, they need further improvement. For ATP test results, relevance ratios were very low (39% for knives and 12% for gloves); there was no significant difference between the two grades.

Significance: Relevance ratios for both knives and gloves were high for APC while for ATP they were very low. Therefore, more realistic guidelines should be prepared to conduct ATP sanitary monitoring for the food contact surfaces of knives and gloves.

P2-131 The Efficacy of ATP Monitoring Systems for Measuring Organic Load on Postharvest Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: ATP monitoring systems could be a viable method to compliment traditional microbial plate counts to monitor the efficacy of an on-farm sanitation program at removing organic debris and bacteria; That of which would support regulatory expectations mandated by the Produce Safety Rule. However, little work has been done to test the efficacy of these tools under postharvest conditions.

Purpose: Determine if ATP monitoring is applicable for use as a sanitation tool for postharvest farm surfaces.

Methods: Concentrations of produce solids (with and without *L. innocua*) were used as organic load inoculum onto stainless steel coupons and measured using commercially available environmental swab. Stainless steel coupons were inoculated with organic load concentrations (blended produce + DI water) (n=5) then swabbed and analyzed using a commercial monitoring device.

Results: Concentration of produce solids have an effect on ATP response readings. Analysis of variance and Duncan's New Multiple Range Test showed that organic load concentration on a stainless steel coupon has a highly significant effect on the swab reading (P<0.01, n=5) when concentrations exceed six percent. The values over six percent did not respond consistently, suggesting that highly soiled surfaces will interfere with accurate readings of ATP response. Addition of *L. innocua* to produce solids does not cause interference of *L. innocua* detection and there is a highly significant correlation between RLU and CFU response (r=0.70718, P<0.001).

Poster

Poster

Significance: There is a strong need to provide technical support to growers for compliance with the Produce Safety Rule. Identifying food safety tools (such as ATP swabs) specific to the conditions of postharvest farm environments helps to provide resources that can improve food safety practices.

P2-132 Changes in AMP, ADP, and ATP Concentrations over Extended Growth Curves for Bacterial Species Significant to Food Hygiene

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Introduction: Adenosine triphosphate (ATP) is routinely used as a target for rapid assessments of surface hygiene where prokaryotic and eukaryotic cell contaminants are a concern. There is a strong hypothesis and growing evidence that concentrations of ATP will be depleted as cell contaminants metabolize and dephosphorylate ATP into adenosine diphosphate (ADP) and monophosphate (AMP) isomers, thus potentially rendering rapid ATP-based assays less sensitive to contaminants. There are few published reports describing the actual concentrations of adenylate isomers for microbial contaminants.

Purpose: Determine concentrations of ATP, ADP, AMP, and AXP (sum of the concentration of all isomers) over extended growth curves for bacterial contaminants of significance to hygiene control.

Methods: Using a luminometer/luciferin-luciferase based technique, concentrations of adenylate isomers were calculated against curves of authentic adenylate standards. Specific strains of bacteria included *Cronobacter sakazakii*, *Escherichia coli*, *Lactobacillus casei*, and *Bacillus subtilis* grown in recommended media. Replicate (n=3) studies were conducted tracking adenylate concentrations from inoculation to up to several weeks of incubation; in complement, growth was estimated using OD_{600} values. ANOVA was conducted as a means of comparing shifts in adenylate profiles.

Results: In general, initial increases in AXP content followed the growth curve of the organisms. ATP was initially the predominate adenylate following OD adjustments but was soon replaced by AMP as the primary adenylate with intermediate levels of ADP. Each bacterial strain assayed displayed unique profiles of isomer generation and depletion.

Significance: Assays for assessing hygiene solely based on the presence of ATP as a means of assessing prokaryotic contaminants may lose sensitivity as the metabolic state of the bacterium changes. Furthermore, the differences between bacteria used in this study suggest that changes in assay sensitivity would also result from the changes in the genera or species of bacterial contaminant.

P2-133 Sanitation Monitoring of Stainless Steel Surfaces Using the Total Adenylates Hygiene Monitoring Test

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Introduction: ATP rapid hygiene monitoring tests are useful for the implementation of HACCP and HARPC programs. Recently, the ATP+ADP+AMP (A3) test was shown to be a powerful tool to reveal improper cleaning and the presence of contamination that conventional ATP tests missed due to the degradation of ATP to ADP and AMP.

Purpose: The performance of the A3 test kit was verified using simulated sanitation monitoring and comparison with other conventional ATP tests.

Methods: The A3 test (LuciPac A3 Surface/Lumitester PD-30, Kikkoman Biochemifa) and three commercially available ATP tests were evaluated. The dry swabs of the A3 test were moistened using nuclease-free water. Foods (ham, raw chicken, yogurt, beer and orange juice) were homogenized and diluted with nuclease-free water. The aliquots (250 µl) were spread and dried onto stainless steel coupons (10 by 10 cm, n=3) with swabbing and measurements were performed according to the manufacturer's instructions for each test.

Results: The detection sensitivities of the A3 test were superior. The A3 test showed 157,389 relative light units (RLU) for ham (1000-fold dilution). On the other hand, the other commercially-available ATP tests showed between zero and 62 RLU. The results of other foods are as follows: raw chicken (100-fold dilution); 15,872 RLU (A3) and 20 to 173 RLU (ATP), yogurt (100-fold dilution); 18,371 RLU (A3) and 911 to 3,104 RLU (ATP), beer (tenfold dilution); 10,777 RLU (A3) and zero to 200 RLU (ATP), orange juice (1000-fold dilution); 4,568 RLU (A3) and 695 to 1,995 RLU (ATP).

Significance: Food residues on surfaces are the source of nutrients for microorganisms or they can also interfere with the antimicrobial activity of disinfectants. Moreover, they can present a risk of allergen contamination. The results showed that the ATP test may indicate false-negative for food debris and the A3 test is a more accurate tool to verify the levels of hygiene and sanitation.

P2-134 Evaluation of Two Real-time BAX PCR Assays for the Detection of Genus *Listeria* Species and *Listeria monocytogenes*

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Introduction: Detection of *Listeria* species and *L. monocytogenes* in food/environmental samples is of increasing importance highlighted by rising numbers and severity of food safety recalls/outbreaks in recent years. This study aimed to evaluate two real-time PCR methods for their ability to detect both pathogen groups.

Purpose: This evaluation assessed two real-time PCR assays for screening for genus *Listeria* species and *L. monocytogenes*. Assay sensitivity, relative level of detection (RLOD), inclusivity and exclusivity were assessed in pure culture and in food and environmental surface enrichments relative to ISO standard 16140-2:2016

Methods: Fifty target and 30 non-target strains of genus *Listeria* and *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/environmental enrichments from six sample categories (n=442 *Listeria* spp.; n=462 *L. monocytogenes*) in 24 LEB Complete media were prepared. Test kit results were compared to independently spiked or naturally contaminated samples, enriched per ISO.

Results: The assays were 100% inclusive for all species tested; exclusivity was 100% against closely/ distantly related genera. Sensitivity studies showed equal or better performance, compared to ISO method, in all six sample categories. RLOD were all lower than the fixed AL of 2.5 in all categories.

Significance: The real-time PCR assays for genus *Listeria* and *L. monocytogenes* exhibit increased assay sensitivity and faster/easier sample preparation and cycling times, than endpoint PCR detection. These new assays allow for rapid time-to-results for the testing of food/environmental samples, while preserving the ease, accuracy and dependability of the BAX System.

P2-135 The Effect of Food Safety on Customer Satisfaction: Exploring Customer-generated Reviews through Business Intelligence

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✦ ***Undergraduate Student Award Entrant***

Introduction: Due to the low percentage of restaurant customers who report foodborne illness to public health channels, customer-generated content is becoming increasingly important when monitoring the spread of foodborne illness in foodservice establishments. Yelp.com is the predominant online restaurant review forum where customers report their dining experiences, and it has been suggested that information in these reviews could be beneficial to scientists.

Purpose: The objectives were to i) identify customers' voices relating to foodborne illness and restaurant cleanliness issues, ii) quantitatively measure issues related to foodborne illness and restaurant cleanliness, iii) examine the relationship between foodborne illness and restaurant cleanliness issues and restaurant reputation/customer satisfaction.

Methods: Customer-generated reviews from 954 restaurants underwent text mining to record the frequency of terms in dictionaries relating to foodborne illness events and restaurant cleanliness issues. Words for the dictionaries were selected using exploratory text mining and topic modeling from online reviews and through literature-based consultation with a foodborne illness expert. The frequency of key terms was recorded on the individual review level and on the restaurant level. Business intelligence techniques were used to identify trends in the data.

Results: Statistical analysis indicated there were statistically significant inverse correlations between increased frequency of key terms in both categories: average restaurant rating (foodborne illness t=-3.367, cleanliness t= -0.619) and individual review rating (foodborne illness t=-37.924, cleanliness t=-33.863). Of the 231,000 total reviews, 2.53% contained a key term relating to restaurant cleanliness issues and 0.764% contained a key term relating to foodborne illness.

Significance: The correlations between the frequency of key terms and restaurant reputation and customer satisfaction could be used to incentivize restaurateurs to implement safer food practices, as prior literature confirms the link between customer satisfaction and restaurant success.

P2-136 Evaluating FDA Food Recalls with Sanitation as a Root Cause

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Introduction: Cleaning and sanitation programs are a regulatory requirement in the food industry. A quarter of United States food recalls are caused by probable contamination arising from poor plant sanitation and environmental monitoring standards. The study, therefore, proposes that FDA's Food Recall entries should clearly indicate such root cause(s) for each recall, for the benefit of the industry and the public.

Purpose: To study recent pathogen- and allergen-related FDA recalls, and use these as a basis to provide risk-based industry sanitation recommendations.

Methods: Three-hundred eleven relevant data points were evaluated from the FDA's Recall entries from July 2017 to June 2018.

Results: Based on the analysis of evaluated recall entries:

i) Half of the FDA recalls were due to undeclared allergens. Of such recalls, 4.6 to 32.4% may have happened because of allergen cross-contact incidents in plants. Milk, multiple allergen products and tree nuts were the top three allergens implicated in these recalls.

ii) *Listeria* (21.5%) and *Salmonella* (17.7%) were the significant pathogens implicated in recalls. Of *Listeria*-related recalls, 98.5% were due to plant sanitation issues, whereas with *Salmonella*, 63.6 to 96.3% of related recalls may be due to plant issues.

iii) Overall, 37.9 to 59.8% of FDA recalls could be plant sanitation or environmental hygiene-related. This wide percentage range may be attributed to a lack of appreciable clarity on the root cause of contamination of the violative product.

Significance: If such root cause(s) of product contamination were clearly communicated within FDA's food recall entries, then this data could be used by the industry to improve their sanitary design, performance, and maintenance programs. This should eventually help in preventing or significantly minimizing food recalls, inspection violations, and foodborne illnesses.

P2-137 Hand and Glove Surface Cross-Contamination Potential Based on Nitrile and Vinyl Glove Surface Characteristics

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Introduction: Between 2006 and 2015, data assembled indicates that glove-hand cross-contamination (CC) or bare-hand contact was a contributory cause in approximately 16% and 36% of outbreaks, respectively.

Purpose: Recognition of the hazards posed by bare-hand contact and use of disposable gloves as a mitigation strategy is relatively recent, with mechanisms of CC for both needing elucidation.

Methods: In an independent study the surface free energy (SFE) of nitrile (n=8) and vinyl (PVC) (n=7) disposable gloves were determined with Zisman plots of SFE standards. This data was compared to published information on glove CC, transfer coefficients determined for different disposable glove types, the SFE of hands, and surface tensions of foods/human soils to investigate critical correlations. Also investigated were disposable glove puncture characteristics that would affect CC.

Results: While CC potential for disposable gloves resides in glove type and SFE profile, transfer coefficients would be less than dirty bare hands but not necessarily for surfactant cleaned hands, with nitrile advantaged. Nitrile disposable gloves show reduced CC potential as they are more hydrophobic & oleophobic (water & oil repellent) than tested PVC disposable gloves, correlating with lower transfer coefficients (pick-up<deposition). A SFE/surface tension continuum constructed with values for gloves, contact surfaces, hands, food and human soils helps to explain CC as experienced in food environments. Reliability of disposable gloves in reducing CC also requires barrier integrity with puncture directly related to wear duration, process/food rip/tear hazards and durability curve for a disposable glove. Using a Cox survival function plot, nitrile outperforms PVC disposable gloves three to fivefold with respect to cumulative failure. The data provided supports observations related to glove and hand CC.

Significance: The current study identifies some poorly understood factors related to CC involving hands and disposable gloves, that together represent significant contributory causes in foodborne outbreaks linked to faulty food handling behaviors.

P2-138 The Development of a Multiple Hurdle Approach to Improve Microbial Safety of Ground Beef

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❖ ***Developing Scientist Entrant***

Introduction: Cattle are a well-known source of foodborne pathogens. Therefore, beef is the third most common foodborne illness outbreak product.

Purpose: To develop a multiple-hurdle approach to improve microbiological safety of ground beef.

Methods: Beef trim (400 g) were spot inoculated with 200 µl of 7±0.2 log CFU/ml either *E. coli* O157:H7 or *Salmonella* Typhimurium DT 104. Inoculated beef trims were subjected to one of the seven spray treatments; no treatment (NT), deionized water (DI), three percent sodium acid sulfate (SAS), five percent lactic acid (LA), Blitz (PAA), SAS followed by Blitz (15 ml SAS + 15 ml Blitz), and LA followed by Blitz (15 ml LA + 15 ml Blitz). The treated beef was then processed through a meat grinder to inoculate the inner surfaces of the equipment. One thousand g of antimicrobial ice in a combination of 500 ml solution prepared from 325 mg/l PAA solution were processed through an inoculated meat grinder. After the antimicrobial ice treatment, 400 g uninoculated beef trim were processed and analyzed to identify bacterial transfer from the meat grinders.

Results: The SAS treatment was found to be most effective and reduced the pathogen transfer from meat grinder to below the detection limit, while all other spray treatments yielded statistically similar recoveries ranging from 1.95 to 1.44 and 1.99 to 1.64 log for *E. coli* O157:H7 and *Salmonella* Typhimurium DT 104, respectively. The effects of antimicrobial treatments on the color of ground beef were also investigated. Hue, chroma, and a* color analysis was determined over a five-day shelf life study. When looking at a* values, SAS + PAA reported the highest values at 21.75, just behind NT and DI. Similar trend was observed with chroma values with different treatment solutions.

Significance: This hurdle approach, especially with SAS has the potential to reduce cross-contamination and could serve as an easy and rapid antimicrobial intervention for the ground beef industry.

P2-139 Evaluation of a Commercial Enzymatic Drain Cleaner for Food Matrix Digestion

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Introduction: The physical complexity of foods can hinder our ability to detect human pathogens, which can be internalized within food structures. Others have used high-purity enzymes for digestion of foods, however, the cost of this approach may limit its practical use. As an alternative, we investigated the use of an inexpensive commercial enzymatic drain cleaner for pre-analytical digestion of ground turkey.

Purpose: We sought to evaluate the capacity of a commercial powdered enzymatic drain cleaner to physically digest ground turkey, with the ultimate goal of enabling simultaneous bacterial enrichment and reduction in sample complexity prior to downstream analysis.

Methods: A commercial drain cleaner (Zep Drain Defense) containing a mixture of bacterial spores and enzymes (protease, lipase, amylase, cellulase) was used. The product was suspended in either buffered peptone water (BPW) or universal preenrichment broth at up to six times the recommended usage level, then filtered (0.22 mm) to remove bacterial spores. Ground turkey (93% lean) was diluted 1:10 in each medium-enzyme mixture in sterile filter bags (330 micron pores) and incubated at 37°C for up to four h. Visual changes in ground turkey (integrity, color) were observed, compatibility of digested turkey filtrate with centrifugation was examined qualitatively and simultaneous matrix digestion and enrichment of *Salmonella* Typhimurium ATCC 14028 at a high inoculum level (10^7 CFU/ml) was assessed. Multiple experiments were performed during development of this approach. All treatments within each experiment were performed in duplicate.

Results: Increasing concentration of drain product and longer incubation times led to decreased integrity of ground turkey. The BPW-enzyme filtrate was easily centrifuged, unlike the undigested control, which formed compacted particulate plugs. *Salmonella* Typhimurium was enriched by two log in the presence of the enzyme product.

Significance: Our results suggest that this inexpensive household product may be repurposed for preanalytical sample preparation of ground turkey.

P2-140 Microbial Analyses of Dried Crickets Used as a Human Protein Supplement

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Introduction: More than two billion people worldwide supplement their diet or income through entomophagy. This sustainable protein source has recently begun to gain acceptance in the United States. Currently, production and processing practices are unstandardized, and microbial safety and quality of the product has not been well documented. This study assesses the microbial load of cricket flour, a low-water activity, insect-based food for human consumption.

Purpose: The purpose of this study is to observe the continuity of the microbial load of commercial cricket powders across producers and product lots.

Methods: Cricket flour samples (six producers, two lots each, *n*=12) were purchased online. Samples were subjected to cultural analyses for populations including: aerobic mesophiles (AMC), coliforms, *E. coli*, fungi, aerobic and anaerobic spores. Data were analyzed in SPSS v. 25 using Pearson product-moment correlations and MANOVA with Wilk's Lambda and Tukey's HSD post-hoc test.

Results: Both producer and lot exerted significant effects on counts at the model level. AMC values varied from less than one CFU/g to 7.6 log CFU/g and were strongly correlated with high coliform and spore counts. Levels of fungi were largely below detection limit (10 CFU/g) and *E. coli* was not detected in any sample (<10 CFU/g). The highest coliform, aerobic spore and anaerobic spore levels (3.7 log CFU/g, 5.1 log CFU/g and 4.8 log CFU/g, respectively) were obtained from samples originating from a single producer. Variability in microbial load was not related to water activity, which ranged from 0.152 to 0.297 in all samples.

Significance: Incorporation of insect protein into Western diets could increase efficiency of land and water use and reduce greenhouse gas emissions associated with food production. It will be critical, however, to ensure the safety of commercial product as this market expands. At present time, microbial quality appears to be strongly tied to production practices, which may benefit from standardization for best practice.

Poster

P2-141 Development of an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) for the Rapid Detection of Peanut in Processed Foods

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Introduction: Food allergies are problems for many parts of society including sensitive subjects, schools, health authorities, and food industries. The peanut is one of the major food allergens, so a rapid detection method for peanut adulteration in processed foods is necessary to protect consumers who suffer from peanut allergy.

Purpose: The aims of this study were to optimize indirect ELISA based on monoclonal antibodies (MAbs) previously identified as specific to peanuts and to validate the method using processed foods containing peanuts.

Methods: The indirect ELISA-based Mab was optimized by testing key parameters including incubation conditions of coating, blocking, and primary antibody steps, and total 13 processed food samples (10 samples containing peanut and three samples not containing peanut) were used for the validation of the indirect ELISA method.

Results: The optimized indirect ELISA method based on BP 3A1-12 MAb exhibited quite high sensitivity (0.0001%) and showed no cross-reaction to other nuts and food ingredients. The method can successfully detect peanut in processed foods. As results of indirect ELISA, 10 samples containing peanuts were positive and three samples without peanuts were negative

Significance: The optimized indirect ELISA based on BP 3A1-12 MAb can successfully detect peanut in processed food, and this method can be used as an effective tool for monitoring peanut as a food allergen in various processed foods.

P2-142 Food Safety Risk Associated with Dropped Produce on *Listeria monocytogenes*-contaminated Floor Surfaces in Grocery Stores

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❖ ***Developing Scientist Entrant***

Introduction: The handling of fresh produce at grocery stores may pose a significant threat to consumer health. Whether it is a customer or an employee, it is common practice to pick up produce if it falls off a display case or bin in the grocery store setting.

Purpose: To determine the uptake potential of dropped apples, lettuce, and peaches on *Listeria monocytogenes* contaminated commercial floor surfaces.

Methods: Apple, lettuce, and peach were dropped onto two different commonly used grocery floor surfaces (carpet, tile) that were artificially inoculated with *L. monocytogenes* at seven log CFU/ml for five s, one min, 10 min, one h, and four h. Percent transfer was calculated and statistical analysis was performed to test for significance.

Results: A statistically significant difference ($P<0.05$) was observed for percent uptake between the produce types and surfaces. However, percent uptake was not significantly different among the five-time treatments used in this study. The carpet had 3.72% uptake while the tile had 0.65% uptake regardless of the produce type or contact time. Dropped lettuce had the most uptake (5.2%) from both the surfaces combined followed by apple (0.8%) and peach (0.7%) regardless of surface type or contact time.

Significance: Dropping and picking up fresh produce at grocery stores can result in a food safety risk regardless of the contact time. Training is needed for employees and customers concerning the safe handling of fresh produce to enhance food safety and public health.

P2-143 An Approach to Implementing the FDA Recommendation to Verify the Minimization of Contamination and/or Spread of Pathogens in Fresh Cut Processing Food Facilities

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Introduction: Cross-contamination can make a small problem larger and greatly increase the risk of a pathogen outbreak. Verification that a validated process has been executed can minimize or eliminate cross contamination risk to consumers.

Purpose: Demonstrate how a validated calibration system, active process control and efficient data management can ensure a process line is always delivering the desired process.

Methods: Archived calibration and calibration check data assures the quality of process control data. An automated control system with data of known quality provides confidence that the desired process has been achieved. Archived data allows post operation confirmation that processing goals were met at all times.

Results: *Post hoc* analysis of at least weekly calibration and calibration check data for one year for 12 lines (five flume and seven flotation) in a commercial operation indicates that all free effective chlorine measurements have an associated standard error of <±0.4 ppm (Flumes 0.30 and 0.27 for 1° and 2°, respectively. Flotation tanks 0.33 and 0.38 for 1° and 2°, respectively). Continuing this *post hoc* analysis to examine the process data for these same systems indicates > 99.9% delivery of the desired process and a process control of free effective chlorine with a standard error of <±four ppm ((Flumes 3.6 and 3.0 for 1° and 2°, respectively. Flotation tanks 0.33 and 0.38 for 1° and 2°, respectively. Thus, to the extent that the desired process is validated, cross contamination was controlled irrespective of other processing parameters.

Significance: The industry needs performance benchmarks of acceptable practice that all processors should or must meet to minimize outbreak situations such as those experienced with Romaine lettuce. Processors must understand the accuracy and precision of their process control data to ensure that their specified processes are delivered.

P2-144 An Acidic Silver Ion Pretreatment Can Greatly Reduce the Risk of an Illness Outbreak for Fresh Cut Leafy Greens

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Introduction: A pasteurization or kill step for fresh-cut processing would be desirable. Increases in lethality without quality loss move the fresh cut industry in this direction and therefore mitigate outbreak risk.

Purpose: Report the increased lethality of a patent-pending acidic silver ion pretreatment (SmartWash Boost) of cut leafy greens presently being commercialized.

Poster

Methods: Pilot plant studies with and without the pretreatment are used to measure the increased lethality of the proposed pretreatment against inoculation with generic *E. coli*. The magnitude of this increased lethality was optimized by varying the various control parameters including acid content, silver ion concentration and dwell time. Based on models built from case study data and product testing, the impact of increased lethality on outbreak risk can be estimated.

Results: Optimization studies indicate that spraying during cutting with an acidic blend containing about 30 ppm silver ion with a pH of about 2.2 for about 30 s is a preferred pretreatment. Eight independent studies with inoculated product with romaine and iceberg lettuce indicate an overall 0.75±0.30 increase in log lethality over a well-managed chlorine process. In these studies, all inoculations were with a mixed culture of generic *E. coli* and ranged from 10^2 to 10^4 CFU/g. Using all possible case modeling and specific contamination case studies, the potential increase in lethality can provide greater than a 95% reduction in outbreak risk to the extent that *E. coli* models pathogen behavior.

Significance: Processors need to be aware of innovations that are raising the standards for the best practical process. The marketplace has little tolerance for processors who fall behind and therefore put consumers at greater risk. This pretreatment sets a performance benchmark that processors can use as a reference in evaluating their own processes.

P2-145 Growth Kinetics of *Listeria monocytogenes*, Shiga Toxin-producing *Escherichia coli*, and *Salmonella enterica* on Fresh-cut Produce Stored at 5, 10, or 22°C

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Introduction: The risk of pathogen growth on fresh-cut produce at retail is a concern. The FDA Food Code identifies cut tomatoes, melons, and leafy greens as needing time/temperature control (TTC) to ensure safety.

Purpose: We investigated the survival of *Listeria monocytogenes,* Shiga toxin-producing *Escherichia coli* (STEC)*,* and *Salmonella* on cut cucumber, onion, pepper, tomato, and mango stored at five, ten, or 22°C.

Methods: Acid-adapted, single-pathogen cocktails, five to ten strains each, of stationary phase cells served as inocula. Fresh-cut produce was prepared from surface-sterilized whole produce, aseptically peeled and cut into cubes. Cut produce was weighed, 25 g/bag, and 0.25 ml of pathogen cocktail added and distributed by gently massaging. Each bag contained ~four log CFU /g. Inoculated bags were placed at five, ten or 22°C for up to seven days, four days, or 32 hrs, respectively. At regular intervals, a bag was removed, and surviving organisms enumerated. Native microbiota was enumerated from uninoculated samples at each point. Log surviving cells (CFU/g) for each pathogen/produce combination was plotted vs time and curve-fitting applied (DMFit).

Results: Pathogen grew significantly at 22°C, greater than four log CFU/g, with the exception of *L. monocytogenes* on tomato and all three pathogens on mango. Similarly, pathogens grew significantly at 10°C, except that *L. monocytogenes* did not grow on cut tomato, and no pathogen grew on cut mango at either five or 10°C. While decreasing temperature significantly decreased growth rate, *Salmonella* and STEC grew greater than three log CFU/g on cut tomato at 5°C, and *Salmonella* and *L. monocytogenes* grew greater than one log CFU/g on cucumber.

Significance: TTC may reduce the risk of foodborne illness from fresh-cut onion, pepper, and mango, but TTC alone will not prevent the growth of pathogens on other items, e.g., tomato and cucumber. Additional fresh-cut produce items, namely mango, cucumber, onion, and pepper, support pathogen growth when unrefrigerated and should be considered for TTC designation.

P2-146 Comparison of Sodium Nitrite and Natural Celery Nitrite on the Inhibition of Spore Germination of *Clostridium sporogenes* As a Nonpathogenic Surrogate Assay in Meat Products

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❖ ***Developing Scientist Entrant***

Introduction: Nitrite is a regulated ingredient used to prevent the germination of *Clostridium* spores in processed meat products. The use of nitrite as a food preservative got a boost when USDA-FSIS considered vegetable-sourced nitrite (vegetable nitrate fermented to nitrite) as 'natural nitrite' whereby products can be labelled as having 'no added preservatives'. This designation and the labelling allowance provides for a 'clean label' application of nitrite.

Purpose: To identify permissive conditions to facilitate validation of spore inhibition during the comparison of sodium and vegetable (celery) nitrite in cooked meat products.

Methods: A three-strain spore crop from *Clostridium sporogenes* (ATCC 3584, ATCC 19404, and ATCC BAA-2695) was applied during ingredient formulation of low-fat hotdogs and topically applied to chicken breasts in a sous vide process. In both processes, sodium nitrite was compared to comparable levels of celery nitrite. Hotdogs followed a standard preparation and cook process (nitrite was used at 156 ppm). Chicken was vacuum-marinaded and heated by a sous vide process and both nitrites were compared at 100, 150, and 200 ppm. All treatments were performed in triplicate replication and comparisons were analyzed by repeated measures analysis of variance to determine significant differences ($P<0.05$) between treatments.

Results: Heat treatment during cooking initialized spore germination and growth. In our assays, we allowed spore germination to occur at 35°C (without chilling after heating) so that we could best observe inhibition of spore germination by nitrite under the most permissive conditions, compared to controls processed without nitrite. Celery nitrite was as good or better than sodium nitrite in low fat hotdogs, but underperformed comparable levels of sodium nitrite in sous vide chicken.

Significance: The nitrite validation assay described herein allow easy determination if nitrite levels can prevent spore germination under the most permissive conditions to help keep foods safe.

P2-147 Combined Effect of Storage Conditions, Surface Integrity, and Length of Shelf Life on the Growth of *Listeria monocytogenes* and Spoilage Microbiota on Refrigerated Ready-to-Eat Products

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❖ ***Developing Scientist Entrant***

Introduction: Inconsistent date label practices reportedly contribute to food waste in the United States due to consumer misinterpretation. Proposals to unilaterally eliminate date labels may increase the risk from psychrotolerant growth of *Listeria monocytogenes* in RTE foods.

Purpose: The purpose of this study was to evaluate the rate of quality deterioration and food safety risks relative to one another in six product systems (tomatoes, apples, fresh-cut cantaloupe, lettuce leaves, baby spinach, and commercially processed turkey slices) at different refrigeration temperatures, atmospheres, and quality grades.

Methods: RTE food samples inoculated with three-strain cocktails of *L. monocytogenes* were stored in refrigerators at 4±1°C and 9±1°C in triplicate. The length of storage was determined based on the common shelf-life of each product (cantaloupe slices every 24 h for five days; apple slices every 48 h for 12 days; apples, tomatoes, romaine lettuce leaves, and baby spinach leaves twice per week for 27 days; turkey slices once per month for 70 days. Both *L. monocytogenes* growth and spoilage microbiota were tested at each sampling point. A tenfold increase in *L. monocytogenes* levels was used as the cut-off for unacceptable psychrotolerant growth. Products were considered spoiled once visible or aromatic defects in quality were detected.

Results: Generally, conditions that improved microbial quality by extending shelf-life also allowed for *L. monocytogenes* growth of greater than one log CFU/g before deterioration due to microbial spoilage. Modified atmosphere packaging storage enhanced *L. monocytogenes* growth relative to spoilage microbiota in lettuce leaves (one-log growth seven days before spoilage) and turkey slices (greater than one log growth after spoilage). In contrast, the use of secondary quality produce (apples, tomatoes, lettuce) with physical damage reduced shelf life and, consequently, limited the time for *L. monocytogenes* proliferation.

Significance: These data suggest that spoilage cannot be considered a fail-safe indicator or proxy for shelf-life limitation across refrigerated RTE products.

P2-148 Growth and Survival of *Listeria monocytogenes* on Intact Fruit and Vegetable Surfaces: A Systematic Review

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is known to be present in produce associated environments (e.g., fields, packinghouses); thus, it is critical to evaluate *L. monocytogenes* growth and survival data on intact whole produce surfaces.

Purpose: The goal of this study was to perform a systematic literature review to identify and characterize published data on the growth and/or survival of *L. monocytogenes* on fruit and vegetable surfaces.

Methods: Relevant studies were identified by searching seven electronic databases: AGRICOLA, CAB Abstracts, Center for Produce Safety, FSTA, Google Scholar, PubMed and Web of Science. Searches were conducted using the following terms: *Listeria monocytogenes*, produce, growth and survival. Search terms were also modified, exploded, and blasted to find all related subheadings. Included studies had to be prospective, describe methodology (e.g., inoculation method), experimental parameters, and provide quantitative growth and/or survival data. Studies were not included if methods were unclear or inappropriate (e.g. dip inoculation may promote internalization), and if produce was cut, processed, or treated.

Results: Of 3,459 identified citations, 88 were reviewed in full and 29 studies met the inclusion criteria. Studies represented 21 commodities; with the majority of studies focusing on melons, leafy greens, berries, and sprouts. Synthesis of the reviewed studies suggests *L. monocytogenes* growth and survival on intact whole produce surfaces differs substantially by commodity. Parameters, such as temperature, relative humidity and produce surface characteristic had a considerable effect on *L. monocytogenes* growth and survival dynamics. Contaminated produce held at ambient temperatures (≥20°C) had higher growth rates, compared to contaminated produce held at lower temperatures (4±2°C, 10±2°C).

Significance: This review provides an inventory of the current data on *L. monocytogenes* growth and survival on intact whole produce surfaces. Identification of which intact whole produce commodities support *L. monocytogenes* growth and/or survival at various conditions observed along the supply chain will assist the industry in managing *L. monocytogenes* contamination risk.

P2-149 Prevalence of *Salmonella* spp. Isolated from Environmental Food Surfaces from Vegetable Markets in Cambodia

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❖ ***Developing Scientist Entrant***

Introduction: Informal vegetable markets are an important part of the culture and economy in Cambodia. However, their lack of hygiene and sanitation practices, food safety regulations, and infrastructure present a risk of contamination to the vegetables and those who consume them.

Purpose: The aim of this study was to determine the prevalence of *Salmonella* spp. in informal vegetable markets in Cambodia. The effect of location within the market (inside and outside) and surface types (food contact surface, FCS and a non-food contact surface, NFCS) was evaluated.

Methods: Surface types (FCS and NFCS) were collected according to the Food Safety and Inspection Service (FSIS) Directive 10,300.1, Rev. 1, Section VII, A, 12 and analyzed for *Salmonella* spp. by using FSIS Microbiology Laboratory Guidebook 4.09 methods. Data were collected in a nested design and analyzed using a generalized linear mixed-model ANOVA. Significant effects means were compared at α=0.05. Relative risk was calculated.

Results: Preliminary data indicated an overall *Salmonella* prevalence of 50%. There was no significant effect of location ($P=0.90$). A significant effect of surface type was observed ($P=0.01$) with a prevalence of 66% and 36% for FCS and NFCS, respectively. Vegetables exposed to FCS were 27% more likely to be exposed to *Salmonella* when compared to NFCS.

Significance: To the best of our knowledge, this is the first study to investigate the prevalence of *Salmonella* in environmental samples from informal vegetable markets in Cambodia. The availability of accurate data on the prevalence of *Salmonella* in these markets is crucial for effective surveillance, implementation of suitable intervention strategies and prevention of future foodborne illness cases in Cambodia.

P2-150 Prevalence and Quantification of *Salmonella* spp., Generic *Escherichia coli* and Coliforms on Vegetables Sold in Informal Markets in Cambodia

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❖ ***Developing Scientist Entrant***

Introduction: Vegetables in Cambodia are commonly sold in informal markets that escape food safety standards and controls. Consumption of contaminated raw vegetables can potentially cause foodborne illness, however, a full investigation of biological contamination of vegetables in Cambodian informal markets has yet to be conducted.

Purpose: To investigate the prevalence and concentration of *Salmonella* spp., generic *Escherichia coli,* and coliforms on fresh vegetables sold in informal markets in Cambodia.

Methods: A total of $n=52$ lettuce, $n=52$ tomatoes and $n=52$ cucumber samples were collected from informal markets. *Salmonella* spp. qualitative data collection methods included incubating samples at 37°C for 24 h in both a preenrichment of 0.1% peptone water and selective enrichment of tetrathionate

Poster

and Rappaport-Vassiliadis broth. Samples were streaked on xylose lysine tergitol-4 and brilliant green sulfa agar. Quantitative data were collected by plating serial dilutions onto *Enterobacteriaceae* and *E. coli*/coliforms petrifilm. A transfer plating method of petrifilms onto XLT-4 was used to quantify *Salmonella* spp. Latex agglutination was used to identify presumptive positives.

Results: Overall prevalence of *Salmonella* spp. on raw vegetables purchased from informal markets was 18.6% (lettuce=15.4%, tomatoes=19.2%, and cucumber=21.2%). Lettuce had a significantly higher count of *Salmonella* spp., generic *E. coli* and coliforms (6.3, 2.4, and 6.3 log CFU/g, respectively), as compared to tomatoes (5.0, 0.8, and 5.3 log CFU/g) and cucumbers (4.9, 0.9, and 4.3 log CFU/g).

Significance: To our knowledge, this is the first study that explores the biological contamination of fresh vegetables sold in Cambodian informal markets. Preliminary data shows that vegetables sold in informal markets are contaminated with biological hazards and that interventions are necessary to reduce the likelihood of contamination and negative public health outcomes.

P2-151 Validation of the Efficacy of Triple Wash Procedures with Commercial Antimicrobials to Inactivate *Salmonella* and *Listeria monocytogenes* and Improve Microbial Quality of Squashes: Laboratory and Onsite Plant Studies

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❖ ***Developing Scientist Entrant***

Introduction: Although the triple-wash process is recommended for inactivating pathogens on locally grown squashes, it lacks validated data on washed samples.

Purpose: This study aims to evaluate the two triple-wash procedures with antimicrobials to reduce *Salmonella* and *Listeria monocytogenes* on squashes, and evaluate the microbial quality of washed squashes.

Methods: In study I, fresh squashes were dip-inoculated with *Salmonella* Typhimurium and Tennessee, and *L. monocytogenes* (three-strain), followed two triple-wash steps (10 s each) including water dip-antimicrobial dip-water dip (WAW), or water dip-water dip-antimicrobial dip (WWA), then draining (two min) on paper towel. Tested antimicrobials were i) lactic/citric acid blend (LCA; 2.5%); ii) sodium hypochlorite (SH; 100 ppm); and iii) a H_2O_2-peroxyacetic-acid mixer (SaniDate-5.0, 0.0064, 0.25 and 0.50%). Surviving bacteria were recovered on XLT-4 (*Salmonella*) and MOX agar (*L. monocytogenes*). In study II, freshly harvested squash were unwashed or triple-washed (WWA) in water and SaniDate-5.0 (0.0071 and 0.45%) at Preston County Workshop Inc. Reedsville, WV, followed by storage at 5°C for 70 days. Microbial quality of squashes including APCs, coliforms/*Escherichia coli*, lactic-acid-bacteria, yeast/molds, and psychrotrophs were tested every seven days. Data (two replicates with four samples per replicate) were analyzed using the Mixed Model Procedure of SAS (P=0.05).

Results: Counts of *Salmonella* and *L. monocytogenes* on unwashed squash were 2.97 and 3.11 log CFU/g, respectively. Better reductions (P<0.05) of *Salmonella* (2.54 vs 1.84 log CFU/g) and *L. monocytogenes* (2.30 vs 0.72 log CFU/g) were obtained from squashes washed through WWA than the WAW. Antimicrobials reduced (P<0.05) *Salmonella* (1.69 to 2.62 log CFU/g) and *L. monocytogenes* (1.21 to 1.81 log CFU/g) on squashes with the best (P<0.05) reductions showed in SaniDate-5.0 (0.25 or 0.5%). SaniDate-5.0 treated squashes showed lower (P<0.05) counts of APCs, coliform, lactic-acid-bacteria and psychrotrophs than the unwashed and water-treated samples from days 50 to 70.

Significance: Local produce growers should adopt the WWA procedure with commercial antimicrobials (e.g., SaniDate-5.0) to improve microbial safety and quality of squashes during postharvest processing.

P2-152 Cold Plasma Activation (Ionization) Enhances the Efficacy of Aerosolized Hydrogen Peroxide in Reducing Populations of *Salmonella* Typhimurium and *Listeria innocua* on Apples, Tomatoes, Cantaloupe and Romaine Lettuce

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Poster

Introduction: Decontamination technologies are urgently needed to enhance microbial safety of fresh produce.

Purpose: This study investigated whether cold plasma activation affected the efficacy of aerosolized hydrogen peroxide (H_2O_2) against *Salmonella* Typhimurium and *Listeria innocua* on four types of fresh produce items.

Methods: Stem scars and smooth surfaces of grape tomatoes, surfaces of Gala and Granny Smith apples, romaine lettuce and cantaloupe rinds were inoculated with cocktails of two-strain *Salmonella* Typhimurium and three-strain *L. innocua*. The inoculated samples (six pieces each of three replicates) were treated with 7.8% aerosolized H_2O_2 with and without cold plasma activation for various times and cycles.

Results: H_2O_2 activated by cold plasma reduced *Salmonella* and *Listeria* populations by 4.3 log CFU/piece on apple and grape tomato surface after eight s treatment time followed by 30 min dwell time. Without plasma activation, the reductions were less than 2.8 log CFU/piece. On lettuce leaves, *Salmonella* was reduced by 3.01 and 1.70 log CFU/piece, and *Listeria* was reduced by >4.95 and 2.77 log CFU/piece with and without plasma activation, respectively, after 30 s treatment time followed by 30 min dwell time. On cantaloupe rinds, the same treatment reduced *Salmonella* populations by 3.00 and 1.10 log CFU/piece and *Listeria* by >4.91 and 0.97 log CFU/piece with and without plasma activation, respectively. It was more difficult to inactivate bacteria on the stem scar of tomatoes. Even after three cycles of treatment (one cycle was 20 s treatment time followed by 20 min dwell time), *Salmonella* was only reduced by 2.22 and 0.95 log CFU/piece and *Listeria* was reduced by 2.88 and 1.16 log CFU/piece with and without plasma activation, respectively. Statistical analysis indicated that cold plasma significantly (P<0.05) improved the efficacy of aerosolized H_2O_2 against bacteria on all tested fresh produce items.

Significance: Our results demonstrated that cold plasma activation (ionization) of H_2O_2 mist may be used to enhance microbial safety of fresh produce.

P2-153 Surface Survival and Internalization of *Salmonella enterica* Inoculated Onto the Surface of Cucumber Fruit

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Introduction: An increasing number of foodborne illness outbreaks associated with fresh produce have occurred in recent years, with cucumber fruit being one commodity of concern. From 2006 to 2016, 15 cucumber-associated *Salmonella enterica* outbreaks occurred in the United States. A 2014 outbreak of *Salmonella* Newport was traced back to the Maryland Eastern Shore, and a serotype Poona outbreak beginning in late 2015 traced back to a cucumber distributor in Mexico.

Purpose: Determine whether cucumber cultivars display reproducible differences in *Salmonella* surface colonization and the potential for internalization in fruit.

Methods: Cultivar- and serotype-specific associations with the surface of cucumber fruit were assessed. Ten cultivars of field- and greenhouse-grown cucumbers, selected for variability of morphological features, were inoculated with *Salmonella* Newport, Poona, Saintpaul, Typhimurium and Javiana at 10^5 CFU/fruit. *S. enterica* cells were recovered and quantified after 24 hours. To investigate the potential for *S. enterica* to internalize within cucumber fruit, store-bought mini cucumbers were inoculated with 10^8 CFU/fruit of *Salmonella* Newport or Poona. Incubation periods of two, 24, 48, 72 or 96 h were followed by surface sterilization using sodium hypochlorite, crushing of inoculated exocarp, and enumeration of recoverable *Salmonella* cells.

Results: Cultivar-specific differences in *S. enterica* surface survival were detected after 24 hours (P<0.05). Cultivars 'Marketmore 97', 'Diamant' and 'Summer Dance' yielded lower bacterial populations than 'Patio Snacker', 'Corinto', 'Picolino' and 'Excelsior'. The largest populations counted were for serotype *Salmonella* Saintpaul, which was significantly different from *Salmonella* Newport and Poona (P<0.05). *Salmonella* Newport and Poona both penetrated the exocarp. Quantification of internalized bacteria after two h yielded ~two log CFU/fruit, with serotype-specific differences seen only at two h (P<0.05). No significant decrease in internalized cells was seen over 96 h (P<0.05).

Significance: The high degree of differential cucumber cultivar susceptibility and internalization of *Salmonella* reveals a significant food safety risk.

P2-154 Microbial Safety Status of Rape Produced and Sold from Small-scale Farming in South Africa

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❖ ***Developing Scientist Entrant***

Introduction: *Brassica rapa* (rape) is one of the most preferred African leafy green vegetables in traditional dishes. It is locally produced by small-scale farmers and sold at formal and informal markets. Food safety status of this crop has not been investigated in South Africa.

Purpose: Therefore this study assessed the microbial safety of rape in informal supply chains.

Methods: Rape samples (n=40) were collected from six farms during harvest together with retailer samples (n=30). Total coliforms, *Escherichia coli* and *Enterobacteriaceae* were enumerated and the prevalence of *Escherichia coli*, *Salmonella* spp., *Listeria* spp. and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* were assessed.

Results: Coliform counts ranged between 1.40 and 5.35 log CFU/g with most (62 of 70) rape samples exceeding the standard for RTE foods as defined by the South African Department of Health. *Enterobacteriaceae* counts of rape samples ranged from 1.81 to 5.55 log CFU/g. *Listeria monocytogenes* was not detected from rape samples. *Escherichia coli* was detected from rape samples from Farms A, B and F within the field, as well as from retailer samples from Farms A, B, E and F. *Salmonella* spp. were only detected from retailer rape samples originating from Farms C and F. Presumptive ESBL-producing *Rhanella aquatilis, Klebsiella* spp., *Serratia* spp. and *Citrobacter* spp. were detected sporadically from all farms from rape sampled from the field and retailers.

Significance: The occurrence of *E. coli, Salmonella* spp. and presumptive ESBL-producing organisms on rape indicates contamination, requiring the necessity to raise awareness of food safety and educate farmers and retailers of good hygiene practices.

P2-155 Persistence of *Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Salmonella enterica* Typhimurium on Cilantro (*Coriandrum sativum*) and Parsley (*Petroselinum crispum*) When Introduced by Spray Irrigation

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illnesses account for an annual loss of over $15 billion to the United States economy and almost half of these are attributable to fresh produce. In 2017, there were 9,152 cases of foodborne illnesses resulting from either *Cyclospora cayetanensis, Cryptosporidium parvum* or *Salmonella*. Most studies are performed on produce under controlled conditions; however, the survival of foodborne pathogens exposed to natural changing environmental conditions is limited or unavailable.

Purpose: The purpose of this study was to evaluate and compare the survival of *Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Salmonella* Typhimurium on cilantro and parsley under natural and controlled environmental conditions.

Methods: Two separate plots for cilantro and parsley containing ten replicate subplots each measuring two by 0.24 m, were inoculated with 500 µl of *Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Salmonella* Typhimurium by spray irrigation when plant height was about 20 cm. Plant (fresh shoots) samples from both crops were collected for analysis on days ero, one, two, three, seven, 15 and 23; 100 g for *Cyclospora* and *Cryptosporidium*, and 25 g for *Salmonella*. *Cyclospora* and *Cryptosporidium* were analyzed using nested PCR, while *Salmonella* was enumerated on XLT4 agar. A general linear model was used to plot persistence curves and ANOVA for comparison.

Results: *Cyclospora cayetanensis* survived for 23 days, with 10% detection on day 23 for both crops while *Cryptosporidium parvum* could not be detected beyond day three. *Salmonella* Typhimurium persisted for 23 days on both crops grown on the fields and in growth chambers; however, there were statistically significant differences (P<0.05) between persistence pattern observed.

Significance: The extended survival of *Cyclospora* compared to *Cryptosporidium* in the environment suggests that sources (i.e., irrigation water, etc.) other than the field worker may be responsible when *Cyclospora* contamination of produce is suspected.

P2-156 Decontamination of Raw Cucumbers Using Microbubbles

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Poster

Introduction: Raw produce can be contaminated with pathogens such as *Salmonella* during harvesting, packaging or transport. Contaminated produce can pose a greater risk of causing foodborne illness if not properly decontaminated due to these foods being consumed raw.

Purpose: A cavitation process (injection of bubbles into water) was studied to determine the efficacy of microbubbles at reducing the level of *Salmonella* on the surface of raw cucumbers

Methods: Cucumbers were inoculated with *Salmonella* Newport and exposed to microbubbles (~0.5 or ~2 mm dia.) for 30 or 120 seconds, with an air flow of zero, one, seven, or 14 liters per minute (LPM) through an air stone. After treatment, samples of cucumber rinse water and treatment tank water were plated onto XLT4 agar, incubated for 24 h and counted.

Results: Cucumbers treated with larger microbubbles at 14 LPM for 120 sec showed the greatest reduction in *Salmonella* (2.3 log CFU/cucumber greater than no bubble treatment). With smaller microbubbles at one LPM (50 psi compressed air) for 120 sec, mean log reductions were 1.2 log CFU/cucumber greater than when no bubbles were applied. When compared to no bubble application, recovery of *Salmonella* from cucumbers and tank water combined

was 0.5 or 0.2 log lower for small or large bubble treatments, respectively. This suggests that the bubbles not only remove *Salmonella* from cucumbers, but also inactivate the bacteria thus preventing their recovery from the water.

Significance: Microbubbles may be a viable decontamination method for raw produce that requires no chemicals, minimal energy, and no additional water. Since additional pathogen reduction in the tank water was observed, these bubble streams could be used to remove microorganisms from fruit surfaces and deactivate some of these detached organisms.

P2-157 Effect of Storage Temperature on the Survival or Growth of *Listeria monocytogenes* on Whole and Fresh-Cut Produce

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❖ ***Developing Scientist Entrant***

Introduction: Whole and fresh-cut produce are minimally processed and therefore susceptible to microbial contamination.

Purpose: This study examined the survival or growth of *Listeria monocytogenes* on whole and fresh cut produce at different storage temperatures.

Methods: Fresh fruits (cantaloupes, pears, pineapples, papayas, and watermelon) and vegetables (broccoli, cauliflower, lettuce, kale, and green bell peppers) were cut into 25-g pieces and were spot inoculated with 0.5 ml (eight-log CFU/ml) of *Listeria monocytogenes.* Inoculated fresh-cut samples were stored at 4°C or 13°C for six days. To represent whole produce, cantaloupes and green bell pepper disks (20 cm^2) were cut with the rind and spot inoculated on the rind part with 0.5 ml of inoculum and were stored at 24°C for eight d or 4°C for 14 d, respectively.

Results: *Listeria* count on all fresh-cut samples except broccoli and cauliflower remained similar throughout the storage time at 4°C. At 13°C, *Listeria* counts increased significantly ($P<0.05$) within one day on fresh-cut watermelon (1.1 log CFU/g) and cantaloupe (1.52 log CFU/g). Similar results were observed on fresh-cut pear (one log CFU/g), papaya (1.65 log CFU/g), and green bell pepper (1.2 log CFU/g) after two d at 13°C. Pineapple samples did not favor the growth of *Listeria* and a reduction of greater than one log was observed at both storage conditions. *Listeria* levels significantly increased in fresh-cut lettuce 13°C but remained stable on kale, cauliflower and broccoli. *Listeria* growth was not favored on rind samples with levels remained stable on cantaloupe rind (eight d at 24°C) and was below the detectable limit of the test on bell pepper after 14 days of storage at 4°C.

Significance: The results obtained during this experiment serve to establish *Listeria monocytogenes* ability to survive in the fresh-cut produce stored at 4°C, as well as the favorable growth environment created in these surfaces at 13°C.

P2-158 Inactivation of *Salmonella* Typhimurium during Flume Washing of Diced Tomatoes with a Sulfuric Acid/Surfactant-based Sanitizer

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❖ ***Developing Scientist Entrant***

Introduction: As demand for fresh cut produce increases, diced tomatoes have been increasingly linked to salmonellosis outbreaks throughout the United States.

Purpose: This study assessed the efficacy of a new sulfuric acid/surfactant sanitizer against *Salmonella* during simulated commercial washing of diced tomatoes.

Methods: Triplicate batches of Roma tomatoes (9.1 kg) were dip-inoculated in a two-strain avirulent *Salmonella* cocktail (*Salmonella* Typhimurium LT2 and MHM112) to contain five to six log CFU/tomato, air- dried for two h and diced to one-fourth inch (Model H-A dicer, Urschel Laboratories, Inc., Valparaiso, IN). Diced tomato samples were collected during 60 s of washing in a pilot-scale flume system with tap water, 80 ppm peroxyacetic acid (PAA), sulfuric acid/surfactant sanitizer (SS, CMS Technology, Bridgewater, NJ) at pH 1.8 and 2.0, 80 ppm PAA/SS at pH 1.8, or chlorine (five and 10 ppm free chlorine), and again after five, 10, and 14 days of storage at 7°C, and quantitatively examined for *Salmonella* and lactobacilli.

Results: Treating with SS/PAA provided greater reductions in both *Salmonella* and lactobacilli at short treatment times compared to PAA ($P<0.05$) and chlorine. Immediately after dicing, *Salmonella* was present at 3.50±0.40 log CFU/g, with populations decreasing an average of 1.95±0.93, 1.70±1.22, 0.75±0.52, 0.61±0.21, 0.52±0.11, 0.48±0.07, and 0.47±0.07 log after 60 s of washing in 90 ppm PAA, SS/PAA, tap water, 10 ppm chlorine, five ppm chlorine, SS at pH 1.8 and SS at pH 2.0, respectively. After 14 days of storage at 7°C, *Salmonella* decreased 3.62±0.23, 2.57±0.88, 1.53±0.38, 1.28±0.15, 1.24±0.69, 1.22±0.13, and 0.89±0.47 in tomatoes previously processed using SS/PAA, PAA, 10 ppm chlorine, SS at pH 2.0, SS at pH 1.8, five ppm free chlorine, and tap water, respectively.

Significance: Based on these findings, the safety of commercially washed diced tomatoes can be enhanced using SS/PAA.

P2-159 Dieoff of *E. coli* and Attenuated *Salmonella* Typhimurium on Baby Lettuce and Spinach under Field Conditions Following a Standardized Simulated Irrigation Event with Contaminated Water in New York, California, and Spain

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❖ ***Developing Scientist Entrant***

Introduction: Following application of water which exceeds the proposed FSMA standard to preharvest produce, a waiting period is required for pathogens to die off to a level compliant with the standard, assuming a 0.5 log dieoff per day. However, there is limited scientific evidence to support this dieoff rate.

Purpose: This ongoing study evaluates the dieoff rate of *E. coli* and attenuated *Salmonella* on different produce types under field conditions in three geographical locations.

Methods: Standardized replicated field trials were conducted in New York (three trials), California (one trial), and Spain (one trial). For each trial, baby spinach plots ($n=4$) and baby lettuce plots ($n=4$) were grown under field conditions and inoculated with a ~10^5 CFU/ml cocktail of *E. coli* and attenuated *Salmonella* using a backpack sprayer. Five produce samples were collected from each plot at zero, four, eight, 24, 48, 72, and 96 h following inoculation; *E. coli* and *Salmonella* levels were enumerated. Preliminary log-linear and two-part segmented log-linear dieoff models were developed; model fit was evaluated using AIC. Trial, bacteria (*Salmonella* or *E. coli*), and produce type were included as model predictors.

Results: The log-linear model predicted a dieoff rate of 0.45 log/day (95% CI=0.43 to 0.49). The segmented model predicted a dieoff rate of 2.90 log/day (95% CI=2.59 to 3.20) for the first nine h, then 0.26 log/day (95% CI: 0.22 to 0.29). The segmented model (AIC=3309) better fit the observed data than the log-linear model (AIC=3540). Trial ($P<0.001$), bacteria ($P<0.001$), and produce type ($P<0.001$) had a significant effect on dieoff.

Significance: These preliminary results suggest FSMA's log-linear dieoff rate may not effectively describe the true dieoff rate of bacteria on produce under field conditions. Additionally, the significance of trial, bacteria, and produce type indicates the use of a one size-fits-all approach to modelling dieoff may not be appropriate and could result in harvesting contaminated produce.

P2-160 Comparison of Mid-Atlantic Grower Perceptions and Nontraditional Irrigation Water Realities: A Conserve Study

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Introduction: Today, fresh produce growers are concerned with water quality both to protect public health and to comply with buyer requirements and governmental regulations. Concurrently, long-term sustainability of agricultural production may rely on finding alternatives to groundwater, such as surface water and reclaimed water.

Purpose: This study juxtaposes grower perceptions with a two-year profile of generic *E. coli* from nontraditional irrigation water sources in the Mid-Atlantic.

Methods: A 22-question survey was distributed online and in-person over two years (March 2016 to February 2018) to 269 growers in the Mid-Atlantic United States (Delaware, DC, Maryland, Pennsylvania, Virginia, West Virginia). Meanwhile, 12 nontraditional irrigation water sites were quantitatively tested for generic *E. coli* using standard membrane filtration according to EPA method 1604 ($n=227$, collected October 2016 to October 2018).

Results: Most Mid-Atlantic growers surveyed (59%) use groundwater as their primary water source, and 66% of farmers are at least somewhat concerned about water availability. Willingness to use nontraditional water increased significantly if the water quality was proven to be as good or better than farmers' current water sources (75% vs. 52%). Microbial water quality profiles developed over two years during the growing seasons showed that eight of 12 nontraditional irrigation water sources tested would require treatment before unrestricted use under the FDA's proposed Produce Safety Rule (three of four freshwater rivers, two of two brackish rivers, zero of two ponds, two of three reclaimed water, one of one processing water).

Significance: Although use of reclaimed water for irrigation of food crops is uncommon in the Mid-Atlantic, grower interest is high, as long as water quality is acceptable and access is provided. Comprehensive two-year testing of three reclaimed water sites indicate that some sites may require treatment before use as agricultural water. Combined with on-farm treatment solutions or targeted use on crops rarely consumed raw, reclaimed water has promise as a valuable water source for Mid-Atlantic farmers.

P2-161 Enhancing Microbial Safety of Microgreens: Combined Ultrasound and Warm Water Treatment as an Environmentally-friendly Seed Sanitation Method

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❖ ***Developing Scientist Entrant***

Introduction: Microgreens are high-profit-margin produce that is gaining in popularity. However, the microbial food safety aspects of this new product type are not well understood. It is urgent to develop strategies to reduce the risk of microbial contamination in microgreens.

Purpose: This study was performed to develop an ultrasound and warm water combined (US-WW) treatment for seeds to inactivate microorganisms.

Methods: Kale seeds were inoculated with around six log CFU/g of nonpathogenic *E. coli* O157:H7 87–23. The inoculated seeds were treated for 20 min using tap water and 20,000 ppm $Ca(OCl)_2$ solution (at room temperature), or 55°C water with ultrasound at 25% power for five or 10 min, and rinsed with distilled water for another 20 min. The treated seeds were sprouted on filter paper without soil for 24 h in the dark at room temperature, and for another 72 h in light, then stored at room temperature for 96 h. The germination rate and yield were recorded at the end of the sprouting. The *E. coli* survival count was enumerated after prewashing, sprouting, and storage on TSA plates.

Results: The treatments with 20,000 ppm $Ca(OCl)_2$, and the five- and 10-min US-WW significantly reduced the *E. coli* counts in the seeds, by 1.6, 2.1, and 3.1 log CFU/g, respectively. The 10-min US-WW treatment had a significantly higher *E. coli* cell reduction than the five-min US-WW or 20,000 ppm $Ca(OCl)_2$ ($P<0.01$). Only the 10-min US-WW-treated seeds maintained a lowered *E. coli* cell concentration after 96 h sprouting, but the cell amount increased significantly to 3.8 log CFU/g during storage. The germination rates for all samples were above the federal standard.

Significance: The new US-WW treatment may be used as an effective and environment-friendly seed sanitation method for enhancing the microbial food safety of microgreens.

P2-162 Antimicrobial Effects of Spraying Calcium Oxide Solution on Sprouting Seeds

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❖ ***Developing Scientist Entrant***

Introduction: Microgreens are edible seedlings produced by sprouting seeds under conditions favorable for microbial growth. Therefore, special attention must be paid to control microbial growth during microgreen production.

Purpose: This study aimed to develop an antimicrobial spray to inhibit microbial growth during seed sprouting.

Methods: Radish, broccoli, and kale seeds inoculated with *E. coli* O157:H7 87–23 at six to seven log CFU/g were prewashed using tap water or 20,000 ppm NaOCl solution for 20 min, then rinsed for one h with distilled water. The seeds were placed in starting soil at room temperature, kept in the dark for three days, and then given 12 h/day of daylight for four days. The sprouting seeds were sprayed with water and 0.1% or 0.2% CaO solution three times per day for seven days. The microgreens were harvested on day seven by cutting them one cm above the soil, and the germination rates and yields were recorded. The surviving *E. coli* cells were then enumerated on tryptic soy agar plates.

Results: The 20,000 ppm NaOCl prewash significantly reduced the *E. coli* counts in all three kinds of seeds (greater than three log, $P<0.01$). The *E. coli* cell populations on all harvested samples were 1.35-3.93 log CFU/g less than those of the rinsed seeds. In the kale, the 0.1% CaO spray showed a significantly

Poster

higher *E. coli* reduction than the other two sprays ($P<0.05$). In the radish and broccoli seeds, no significant difference in cell reduction was observed between the different sprays. The germination rates in all seeds were above the federal standard. However, the 0.2% CaO spray significantly lowered the yield of broccoli sprouts.

Significance: Antimicrobial spraying is an effective method for reducing the microbial counts on sprouting seeds; however, the effectiveness could be seed-type specific.

P2-163 Washing Techniques to Reduce Microbial Growth Using Different Sanitizers on Fresh Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: Outbreaks of foodborne pathogens from fresh produce is significant concern amongst food industries and markets. Lettuce can be infected easily with foodborne pathogens during growth in field or post-harvest processing, handling, and storage conditions. Washing of produce is most common and convenient method used as a food safety measure to reduce microbial growth.

Purpose: The main objective of this study was to identify the washing treatments and sanitizers that reduce microbial contamination of lettuce.

Methods: Lettuce (Mini Romaine Nevada) was inoculated with *Escherichia coli* (W1485) or *Listeria monocytogenes* and stored at room (22°C) or refrigerated (4°C) temperatures for 24 h. Two different washing techniques (soaking for one, three, or five min or spraying) were performed using water and three different sanitizers at 100 ppm: sodium hypochlorite (SH), acidified sodium chloride (ASC), or peroxyacetic acid (PAA). All the experiments were performed under sterile conditions and repeated three times.

Results: The initial microbial count for *E.coli* (6.29 log CFU/g) and for *L. monocytogenes* (6.35 log CFU/g) was obtained before storage.It was found that *E. coli* growth increased by 2.15 log CFU/g at room temperature only, whereas *Listeria monocytogenes* increased at both room and refrigerated temperatures by 1.69 log CFU/g and 0.81 log CFU/g respectively after 24 hrs of storage. The results show maximum microbial reduction for *E. coli* (3.96 log CFU/g at room temperature, 4.02 log CFU/g at refrigerated temperature) and for *L. monocytogenes* (3.53 log CFU/g at room temperature, 3.46 log CFU/g at refrigerated temperature) by soaking samples in PAA for five min. The maximum reduction with spraying treatments was obtained for *E. coli* (1.24 log CFU/g at room temperature, 1.08 CFU/g at refrigerated temperature) using ASC while the maximum reduction for *L. monocytogenes* (1.67 log CFU/g at room temperature, 2.14 log CFU/g at refrigerated temperature) by using SH.

Significance: Soaking of fresh lettuce with PAA sanitizer could be an alternative and effective method to achieve microbial reduction in lettuce by the fresh produce industry.

P2-164 Survival of *Listeria monocytogenes* in Hydroponic Lettuce Systems

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Introduction: Hydroponic production of leafy greens is on the rise in the US and globally but there is a lack scientific evidence necessary for establishment of water quality and sanitation parameters for hydroponics.

Purpose: We determined the survival of *Listeria monocytogenes* in nutrient flow technology (NFT) system, and the transfer rates of the pathogen to lettuce during the lifecycle of the plant.

Methods: NFT systems (n=4; six lanes), were inoculated with *L. monocytogenes* (~1×10^4 CFU/ml). Irrigation water samples, rockwool medium, roots, and lettuce leaves were collected at seven time points during the plant life cycle for pathogen quantification. Plant health parameters (weigh/size and color) were assessed. Data was analyzed in STATA.

Results: *L. monocytogenes* survived at steady rates in the hydroponic system for the first 24 h post inoculation. The survival rates were similar in the system in the absence of the plants (5.70±0.78 log CFU/ml and 5.32±0.15 log CFU/ml, respectively). While the pathogen was detectible on lettuce roots immediately post inoculation (4.96±0.78 log CFU/ml), and at increasing rates at 12 and 24 h post-inoculation, we were not able to detect it on lettuce leaves until seven d post-inoculation. Color measurements showed no detectible changes on lettuce leaves in the presence of the pathogen.

Significance: The findings will lead to development of management practices to ensure food safety of hydroponic lettuce.

P2-165 Microbial Populations in Recirculating Hydroponic System and Packaged Lettuces

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Introduction: The recent incidence of pathogenic *E. coli* on romaine lettuce has reinforced continuing safety risk associated with fresh produce. Hydroponic produce is thought to pose less risk of hosting, harboring or exposing people to human pathogens due to reduced contact of the edible portion with the substrate/soil. The commonest leafy vegetable produced by the hydroponic system is lettuce, which is consumed mostly in the raw state.

Purpose: The purpose of this study was to assess primary populations and drivers of the microbial population in a commercial, recirculating hydroponic system

Methods: Samples (peat-moss substrate, lettuce leaves, roots) were obtained from an active, commercial hydroponic greenhouse. Water samples were collected from four sites across the recirculation system. Sampling was repeated four times. Samples were culturally enumerated for aerobic mesophiles, coliforms, and fungi. Identification of *Listeria* spp. was conducted by selective enrichment with isolation on MOX and PALCAM media. Data were subject to ANOVA and LSD test for mean separation.

Results: At time of harvest, leaves had significantly lower levels of all bacteria compared to plugs, roots and water. However, counts increased significantly after packaging. This could be attributed to inversion of the product during manual packaging. Fungi counts of up to 6.1 log CFU/g were recorded in the spent substrate, consisting overwhelmingly of *Trichoderma* spp. which is applied as a biocontrol measure. The native peat substrate had the highest count for all enumerated populations, appearing to be the primary inoculant source in the recirculating system.

Significance: Data suggests that there is a high microbial load in the hydroponic system, and that microbes originating in the substrate are transferred to edible plant parts during packaging and distribution. The persistence of coliforms across all samples indicates a potential hazard of hosting of pathogenic *Enterobacteriaceae*.

P2-166 Examination of the Growth and Survival of *Listeria monocytogenes* in Hydroponic Fertilizer Solutions Maintained at Different pH

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Introduction: In a hydroponic system, nutrients are delivered to the roots of the plant by circulating water mixed with water soluble fertilizers. Recent research indicated this as a as a potential source of *Listeria* growth in fresh produce production environments. Water mixed with plant nutrients may develop favorable conditions for the growth of *Listeria*.

Purpose: This study examined the effect of hydroponic fertilizer solutions and pH on the growth/survival of *Listeria monocytogenes*.

Methods: Three varieties of hydroponic fertilizer solutions (strawberry, tomato, and lettuce) were prepared in distilled water and adjusted to an electrical conductivity of 1600 µs/cm. The pH of the solutions were maintained at five, six, and seven using phosphoric acid or potassium hydroxide. Fertilizer solutions were inoculated with a cocktail of *L. monocytogenes* (LCDC (81- 861, 4b), V7 1/2a, 101 M, Scott A) to get an initial level of three log CFU/ml. Inoculated samples were incubated at 21°C and examined for *Listeria* counts at zero, six, 6, 18, 24, 48 and, 72 hours.

Results: ***L. monocytogenes*** levels were significantly increased ($P<0.05$) within 24 hours at all pH levels in lettuce and strawberry fertilizer solutions. After 72 h listeria levels were 4.86 to 5.49 log CFU/ml and 4.67 to 5.31 log CFU/ml in lettuce and strawberry fertilizer solutions respectively. However, in tomato fertilizer solutions *Listeria* levels remained similar at all pH levels during the incubation period. Fertilizer solutions at pH 6 were the most favorable for the growth of *Listeria* with levels increasing to 3.61 (tomato), 5.49 (lettuce) and 5.31 (strawberry) log CFU/ml. For control samples, a dieoff of *Listeria* was observed at all pH levels with levels reduced to 2.20, 2.76 and 2.64 log CFU/ml at pH five, six, and seven, respectively.

Significance: Plant fertilizer solutions are favorable for the growth of *Listeria monocytogenes*. Hydroponic producers must follow appropriate environmental sanitation programs to minimize the risk of *Listeria* contamination.

P2-167 Inactivation of *Escherichia coli* O157:H7 in Spinach Leaves by Nonthermal Pulsed Light and Novel Sanitizer Wash Combination

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Introduction: Spinach provides a good ecological niche for the proliferation of numerous microorganisms, including enteric pathogens. The produce industry uses chlorine-based sanitizer washes, which have only limited efficacy and may cause formation of organochlorine carcinogens. New safe and effective methods are needed. Pulsed light (PL) is an effective non-thermal processing method. However, prolonged exposure to PL to achieve pasteurizing intensity causes quality deterioration. Therefore, a low dose PL treatment in combination with active sanitizer wash may prove appropriate to control microorganisms on produce.

Purpose: The purpose of this study was to investigate the efficacy of an integrated treatment of PL and a new formulation of sanitizer wash (HEN) in inactivating *E. coli* O157:H7 in spinach.

Methods: Spinach leaves were spot inoculated on the surface. A bacterial cocktail containing three outbreak strains of *E. coli* O157:H7 was used as the inoculum. Two different inactivation strategies were explored: PL treatment (one to 63 J/cm^2) followed by HEN wash (PL-HEN) and HEN wash followed by PL treatment (HEN-PL). PL treated or untreated spinach leaves were washed in 250 ml HEN for two min at room temperature (22±2°C). Experiments were conducted independently in triplicate and data analyzed using ANOVA and the Bonferroni LSD method.

Results: Statistically significant ($P<0.05$) increases in inactivation were achieved by a combination treatment of PL and HEN compared to individual treatment. HEN sanitizer (two min) and PL (15 s; fluence, 15.8 J/cm^2) individually inactivated 1.8±0.17 and 2.7±0.25 log CFU/g of *E. coli* O157:H7, respectively, on spinach. However, a treatment of HEN-PL reduced the pathogen population by 4.6±0.42 log while the log reduction achieved by PL-HEN treatment was greater than five log CFU/g, indicating a synergistic activity.

Significance: These data suggest that a nonthermal combination treatment of PL-HEN is an efficacious treatment strategy that may be used to ensure the microbial safety of spinach.

P2-168 Ozonized Water with Plant Antimicrobials: An Effective Method to Inactivate *Salmonella enterica* on Iceberg Lettuce in Produce Wash Water

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Introduction: Postharvest washing of produce is performed to remove physical debris and lower the microbial load.

Purpose: The use of ozone in combination with plant-based antimicrobials was evaluated as an alternative to conventional sanitizers such as chlorine. Plant based antimicrobials that were evaluated in combination with ozone included oregano oil, carvacrol, quillaja saponin and olive extract.

Methods: Ozone was dispersed in phosphate buffered saline, following which individual antimicrobials or their combinations were added. Iceberg lettuce leaves (10-g portions) inoculated with *Salmonella* Newport (6.5±1 log CFU/g) were added to the wash suspension. The leaves were tested for reduction in the *Salmonella* Newport population after 60, 90 and 120 min of treatment.

Results: Exposure to ozonized water for 120 min resulted in a 2.1-log CFU/g ($P<0.05$) reduction in *Salmonella* Newport population. The addition of 0.1% oregano oil to ozonized water resulted in three-log CFU/g reduction after 120 min but a 4.1-log CFU/g reduction after 60 min, indicating that the antioxidant property of oregano oil might have diminished ozone activity and resuscitated injured *Salmonella* Newport cells. The addition of five percent olive extract to ozonized water resulted in 4.2-log CFU/g reduction of *Salmonella* Newport after 120 min ($P<0.05$) of treatment. While five percent olive extract did not confer protection to *Salmonella* Newport cells from ozone, one percent olive extract resulted in higher *Salmonella* Newport survival after 120 min treatment than the 60 min treatment. The use of carvacrol (0.1, 0.3 and 0.5%) in ozonized water reduced the pathogen population to below the limit of detection (10 CFU/g) ($P<0.05$) which was in excess of six log CFU/g.

Significance: These results indicate that the efficacy of ozone is compounded by the addition of certain plant-based antimicrobials. Ozone combined with plant antimicrobials could serve as an effective alternative to sanitizers currently used for washing and processing of produce.

P2-169 Survey of Potential Sources of *E. coli* on Lands Adjacent to Leafy Green Fields

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Introduction: Land use practices, including hobby farms, composting operations, feedlots, dairies, and residences with domestic pets and livestock that are adjacent to produce fields, may play a role in the potential *E. coli* contamination of crops.

Purpose: The purpose of this study was to identify the potential foodborne pathogen risks associated with animal- and manure-related land use adjacent to fresh produce fields, as well as risks related to the presence of flies that could potentially transport pathogens from distant sources.

Methods: From November 2015 to November 2017, we collected feces within five miles of leafy green fields from hobby farms, residences, wild habitat, a feedlot, and a compost facility, and tested them for *E. coli* O157 and non-O157 STEC. We passively collected air and fly samples inside the feedlot, at the perimeter, and at 100 ft, 400 ft, and 1000 ft away from the feedlot using water catchment basins, sentinel spinach plants, and fly traps.

Results: E. *coli* 0157 was detected in 3.77% of fecal samples collected from domestic animals, hobby farm livestock, and wildlife; non-0157 STEC was detected in 5.66% of samples. Five cattle manure samples from the feedlot tested positive for *E. coli* O157 (8.33%), and 41 samples (68.33%) tested positive for non-O157 STEC. Two compost samples (2.78%) tested positive for *E. coli* O157, and seven samples (9.72%) tested positive for non-O157 STEC. Air samples tested positive for *E. coli* O157 up to 400 ft from the feedlot and for non-O157 STEC up to 1000 ft from the feedlot with a decrease in positive samples over distance.

Significance: Animal operations are just one of many land use practices in and around produce production areas that may harbor foodborne pathogens. Location specific assessment may be the key to determine their genuine risk.

P2-170 Isolation and Characterization of *Escherichia coli* from Leafy Green Vegetables Using Molecular and Antimicrobial Disc Diffusion Method

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Introduction:: Protecting our food supply in the face of a burgeoning human population has become a major challenge for many states. One of the many concerns regarding food safety is to minimize the risk associated with the consumption of green leafy vegetables to humans

Purpose: The purpose of this study was to identify *Escherichia coli* from leafy green vegetables to show that it can be isolated from nearby markets

Methods: One-hundreds and thirty-five samples collected from local retail produce distributors in North Carolina were analyzed for verotoxin-producing *Escherichia coli* strains using modified tryptic soy broth with E C Broth as a preenrichment and Rapid *E. coli* agar as a selective media. The isolates were characterized biochemically for the production of beta-D-glucuronidase (MUG), sorbitol, 23S rRNA gene for *Escherichia* genera, and verotoxin (vtx-1 and vtx-2) genes with PCR assay.

Results: A total of 139 isolates were obtained from the 135 samples, of which 138 (99%) were presumptive positive for production of MUG, sorbitol and 23S rRNA gene by PCR. One isolate (0.7%) was positive for verotoxin 1 gene (vt-1) when analyzed by polymerase chain reaction. These isolates were tested for their antimicrobial resistance profile against seven antimicrobial agents using a disk diffusion method. Results indicated that 68% of the isolates were resistant to ciprofloxacin and 61% to meropenem compared to 22% of the isolates which were resistant to streptomycin.

Significance: This study indicated that multi-antimicrobial resistant *E. coli* can be isolated from select green leafy vegetable purchased from local retail distributors.

P2-171 Pilot Plant Simulation of an In-Process Aggregating Continuous Sampling Technique on Spinach

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Introduction: The utility of raw material testing for reducing pathogen risk on produce is limited by sampling. Grab samples even with as many as 60 specimens cannot adequately represent the product that is being evaluated. If a cluster of contamination is not sampled, it cannot be detected.

Purpose: Demonstrate the potential sensitivity and effectiveness of an aggregating continuous sampling technique for spinach, and by extension other produce

Methods: In pilot plant studies using a MicroTally Continuous Sampling Device with swabs, inoculated and uninoculated baby leaf spinach is run over a conveyor for aggregated sampling and testing. Appropriate grab samples of the same materials are collected and tested for comparison. Microbial population compositions were analyzed using a metagenomic sequencing method for representative uninoculated grab samples and surface swab sample.

Results: At low inoculation levels (10 to 50 CFU/g) with generic *E. coli*, most probable number estimates of organism recovery from 120 pounds by surface sampling are comparable to estimates from 100-g grab samples. While APC recovery from grab samples compared well with surface sampling, surface sampling recovery was consistent from 25 to 300 pounds of product (P>0.6). Microbiome analysis of five grabs and five swabs from this study of APC recovery showed great similarity with the swabs reporting 21 of the 23 (91.3%) genera detected. PCA of these samples showed no systematic differences between swabs and grabs. Using both 30 pounds spinach inoculated with 10^4 CFU/g and various amounts of uninoculated spinach to examine the potential for cluster direct, all recoveries were comparable except for when the two products were mixed, which yielded a significantly lower recovery (P<0.05).

Significance: Aggregated sampling collected similar microbial populations to grab samples. By sampling a larger portion of the lot, there is potential to improve detection of significant clusters of contamination relative to traditional multi-specimen grab samples.

P2-172 Performance Evaluation of a GENE-UP Real-Time PCR in a Unit Dose Format (EH1-2) for the Detection of Shiga Toxin-producing *E. coli* in 200 g Spinach

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Introduction: The CDC estimates leafy produce to be responsible for the highest amount of foodborne illnesses, and among the top five causes of foodborne hospitalizations and deaths. Given the perishable nature of leafy produce and the recent testing trends moving towards larger sample sizes, it is critical for a pathogen test to be rapid and maintain a high specificity and sensitivity with a simple workflow.

Purpose: This study evaluated the performance of the GENE-UP Shiga toxin-producing *Escherichia coli* (STEC) stx/eae assay (EH1), and unit dose assay format (EH1-2), a pelleted lyophilized format master mix, for the detection of STECs from spinach in a 200-g sample.

Methods: Unpaired (n=30) replicates were analyzed by EH1/ EH1-2, and the reference method: FDA/BAM Chapter 4A: Diarrheagenic *E. coli*. Samples were tested at low (n=20) and high (n=5) inoculation levels, while n=5 uninoculated samples were also tested. The inoculated organism was *E. coli* serotype O111.

Samples for EH1/ EH1-2 were enriched in buffered peptone water at 42°C for 10 h. All presumptive EH1/ EH1-2 results were confirmed by reference and alternative (biomerieux) culture methods.

Results: The candidate method obtained the following results: zero of five for the uninoculated, 11 fo 20 for the low and five of five for the high. This is compared to zero of five, 15 of 20 and five of five, respectively, for the reference method. The candidate method demonstrated no statistically significant differences between presumptive and confirmed results ($dPOD_{cp}$) , between candidate and reference method results ($dPOD_c$) or between unit dose and multi dose method results ($dPOD_c$). The dPODc and the corresponding 95% confidence intervals is -0.2 (95% CI -0.45 to 0.09).

Significance: These data indicate that i) the unit dose format (EH1-2) performed equivalent to the EH1; and ii) both EH1/EH1-2 performed equivalent to the reference method when testing spinach at 200 g sample size. Both EH1 and EH1-2 provide a viable alternative for STEC detection in spinach.

P2-173 Evaluation of Viability of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Sanitizer-treated Spinach Leaves Using Combined Propidium Monoazide Staining and Quantitative PCR

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial pathogens can enter into a viable but non-culturable (VBNC) state due to sanitizer residues on produce surfaces. Conventional methods have limitations in detecting VBNC cells. Viability PCR could help discriminate live pathogens on produce surfaces providing important information from a public health risk perspective.

Purpose: This study investigated the viability of *L. monocytogenes* and *E. coli* O157:H7 on chlorine and lactic acid-treated spinach surfaces during refrigerated storage.

Methods: Baby spinach leaves were washed with chlorine solution (100 ppm) or lactic acid (0.5%) for three min, and spot inoculated with a cocktail (three strains) of *E. coli* O157:H7 and *L. monocytogenes*. The viability of the pathogens during refrigerated storage (4°C) was examined for 48 h by staining the cells with propidium monoazide followed by a real time PCR analysis. The difference between the amount of total and live-cell-derived DNA was assessed by calculating dCt values (dead cells), and the values were converted to corresponding bacterial counts.

Results: The number of live *L. monocytogenes* and *E. coli* O157:H7 cells decreased with time during refrigerated storage on the spinach leaves (treated and control). After 24 h, the live cell populations of both pathogens on the chlorine and lactic acid treated leaves was significantly lower (P<0.05) compared to the controls. The proportions of dead *E.coli* O157:H7 cells after 48 h of storage were 1.24, 2.80 and 3.54 log CFU/g on distilled water, chlorine and lactic acid treated samples, respectively. Similarly, the proportions of dead *Listeria monocytogenes* cells were 1.21, 2.61 and 3.44 log CFU/g on distilled water, chlorine and lactic acid treated samples, respectively.

Significance: The results indicated that the residual sanitizers could have a role in reducing the number of viable human pathogens on produce surfaces during refrigerated storage.

P2-174 Sodium Bisulfate and Peroxyacetic Acid Reduce *Escherichia coli* O157:H7 Populations on Fresh Romaine When Applied Alone or in Combination as a Postharvest Wash

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Introduction: Romaine lettuce has recently been implicated in multiple outbreaks of *Escherichia coli* O157:H7. Chlorine, commonly used to control foodborne pathogens in fresh-cut romaine wash water, is only moderately effective at decontaminating the product surface. Additional research exploring novel interventions is necessary to improve fresh-cut romaine safety.

Purpose: The purpose of this study was to quantify *E. coli* O157:H7 reductions on fresh-cut romaine using a blend of sodium bisulfate (SBS) and peroxyacetic acid (PAA) as a postharvest wash.

Methods: Fresh-cut romaine was inoculated with an *E. coli* O157:H7 cocktail (~1×10^6 CFU/g), dried for 60 min, and submerged for 30 s in one of six triple-wash treatments (90 s total exposure): ambient water, 80 ppm PAA, 0.75% SBS, 80 ppm PAA+0.75% SBS, chlorine (10 ppm free chlorine wash one, 20 to 30 ppm wash two and three) and an unwashed control. Washed romaine was centrifuged to less than two percent additional moisture by weight, packaged in retail display packages, and stored at 7°C. *E. coli* O157:H7 populations were enumerated on days zero, one, three, five, seven, 10, 12, and 14 by homogenizing 25 g in 225 ml of Dey-Engley neutralizing broth, diluting in 0.1% peptone water, and plating on Sorbitol MacConkey supplemented with cefiximine and tellurite. Colorless *E. coli* O157:H7 colonies were counted following 37°C incubation for 18 to 24 h.

Results: Treatment was a statistically significant variable (P<0.0001), with SBS, PAA, and SBS-PAA significantly more effective than chlorine at reducing *E. coli* O157:H7 (P<0.0001). All treatments achieved significant reductions compared to the unwashed control (P<0.0001). While SBS yielded the largest reduction in comparison to the unwashed control (2.25 log CFU/g), SBS, PAA, and SBS-PAA were not statistically different (P>0.05). PAA was not more effective than washing with water (P=0.0759).

Significance: SBS and SBS-PAA postharvest washes are effective chlorine alternatives for reducing *E. coli* O157:H7 on fresh-cut romaine.

P2-175 Assessing the Role of Phyllosphere Bacteria on Norovirus Stability and Attachment in Romaine Lettuce

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Introduction: Human norovirus (HuNoV) is a leading cause of outbreaks in produce. HuNoV was shown to bind bacteria expressing histo-blood group antigens (HBGAs), the putative HuNoV receptor. Therefore, phyllosphere bacteria which express HBGA may play a role in HuNoV persistence in leafy greens.

Purpose: Assess the role of phyllosphere bacteria on norovirus stability and attachment in romaine lettuce

Methods: The HuNoV surrogate Tulane virus (TV) was inoculated onto pieces of whole head and fresh-cut romaine lettuce at a titer of 4×10^5 PFU and stored at 26 or 4°C. On post-inoculation d zero, one, two, three, and seven, samples were rinsed with 0.1% peptone water and homogenate was plated on TSA for aerobic plate count enumeration. Subsequently, homogenate was centrifuged at 3000×*g* for 30 min to collect supernatant for viral plaque assays and RT-qPCR. 16S rRNA colony sequencing was used to identify select bacteria isolates.

Results: At seven d, 4.61±0.29 PFU/g TV was detected in whole lettuce stored at 4°C. This titer was significantly higher (P<0.0001) compared to other groups and storage temperatures. Sequencing results showed *Shewanella* spp. was unique to this group at three d and seven d. Overall, the TV titer was

Poster

lower in samples stored at 26°C compared to 4°C (P<0.001) at seven d. However, there was no significant difference in bacterial counts at seven d and the count was approximately 1×10^8 CFU/g.

Significance: This work indicates storage temperature may be a strong factor for TV stability on produce over time while culturable bacterial density may not affect TV stability on romaine lettuce. However, TV stability was enhanced in whole head lettuce and therefore, a specific bacteria or bacterial population may be responsible for this increased stability. Sequencing results showed *Shewanella* spp. was unique to this group at three d and seven d, which suggests *Shewanella* spp. may specifically interact with HuNoV and enhance persistence in romaine lettuce.

P2-176 Effect of a Bacteriophage Cocktail against *Salmonella enterica* on Romaine Lettuce Leaves

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❖ ***Developing Scientist Entrant***

Introduction: Multiple outbreaks caused by *Salmonella enterica* have been linked to fresh produce, notably leafy vegetables such as lettuce. Washing in sanitizing solutions has been shown to reduce microbial populations by <90%. Consequently, bacteriophage has been proposed as an alternative means to control *S. enterica* in fresh produce.

Purpose: The purpose of this study was to determine the effect of a bacteriophage cocktail on the fate of three *S. enterica* strains on romaine lettuce.

Methods: Romaine lettuce leaves were cut into four cm^2 pieces and separately inoculated by spotting with i) one of three *S. enterica* strains (*Salmonella* Saintpaul S204 or S205 or *Salmonella* Typhimurium S441) alone, ii) the *S. enterica* strain plus 0.1 M $CaCl_2$ solution, and iii) the *S. enterica* strain plus a cocktail of five bacteriophages lytic to *Salmonella* in a 0.1 M $CaCl_2$ solution. *Salmonella* populations were measured on XLD agar immediately after inoculation and after one and tow days of incubation at 21°C. Three independent replicates were performed with each strain.

Results: Populations of all three strains increased by greater than or equal to two log CFU/ cm^2 on untreated lettuce after two days of incubation at 21°C. Application of the bacteriophage cocktail reduced *Salmonella* populations but the effect was strain-specific. Populations of strains *Salmonella* Saintpaul S204 and S205 were reduced by four log CFU/cm^2 (P<0.05) immediately after inoculation and the difference with untreated controls was maintained over two days of incubation at 21°C. In contrast, populations of strain *Salmonella* Typhimurium S441 were reduced by two log CFU/cm^2 (P<0.05), however the difference with controls was also maintained over two days of incubation.

Significance: The results of this study highlighted strain-specific differences in the susceptibility of *S. enterica* that should be considered in the development of bacteriophage-based control methods for fresh produce.

P2-177 Methods Evaluation to Differentiate Presumptive *Bacillus cereus* on Butterhead Lettuce

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Introduction: *Bacillus thuringiensis* is a naturally occurring, soil-dwelling bacterium, where some strains are used as a biological insecticide, but which is closely related to the human pathogen *Bacillus cereus*.

Purpose: The purpose of this study was to evaluate different methods for identification and differentiation of presumptive *B. cereus* on butterhead lettuce, focusing on differentiation between *B. cereus* and *B. thuringiensis*.

Methods: Fifteen butterhead lettuce samples were plated on MYP-agar and other chromogenic media. Next, different analytical techniques for identification and differentiation in the *B. cereus* group were tested on 20 strains (isolates from culture collection): 16 *B. cereus* group strains, two *B. subtilis*, one *B. amyloliquefaciens* and one *B. circulans* strain, and on 46 isolates from the sampled butterhead lettuce of which 24 were presumptive *B. cereus* isolates. These techniques include colony morphology on chromogenic media and blood agar, identification with MALDI-TOF, PCR on toxin genes, immunological techniques for toxin production, psychrotrophic character and phase-contrast light microscopy for the observation of parasporal crystals.

Results: Presumptive *B. cereus* counts of less than two log CFU/g to a maximum of 4.04 log CFU/g were found. *B. thuringiensis* could not be differentiated from *B. cereus* using colony morphology, presence or expression of toxin genes or psychrotrophic character. MALDI-TOF provides an identification at the *B. cereus* group level, but does not enable differentiation at the species level. Phase-contrast light microscopy is a possible differentiation technique, by observation of the parasporal crystals produced by *B. thuringiensis* during sporulation.

Significance: ***B. thuringiensis*** treated foodstuffs are often rejected because of high *B. cereus* counts, leading to food waste, which is debatable. Differentiation of *B. thuringiensis* can overcome this problem.

P2-178 *Salmonella enterica* Colonization of Kale Leaves is Age- and Drought Stress-dependent

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Introduction: Juvenile kale leaves have become a popular fresh salad ingredient, but kale susceptibility to *Salmonella* is understudied. Moreover, extreme weather events frequently subject crops to periods of drought, which constitutes plant stress that may also affect *Salmonella* colonization.

Purpose: Evaluate the effect of plant developmental stage and drought on *Salmonella* colonization of kale surfaces.

Methods: Kale cultivar 'Improved dwarf' plants were grown for two, three, four, five, six, seven, or eight weeks post-germination in a greenhouse (23°C, 16 h L:8 h D) before being subjected to drought for two to six days depending on age, or regular watering (control). *Salmonella* Newport adapted for rifampicin was grown overnight on tryptic soy agar (TSA) at 35°C, and ~10^6 CFU/ml *Salmonella* were inoculated onto the adaxial side of the third true leaf of plants. Inoculated leaves were clipped 24 h post-inoculation, washed in 30 ml 0.1% peptone water, and serially plated onto TSA with rifampicin. Leaf surface washes from 26-, 33- or 39-day-old control and drought-treated plants were collected by placing plants in 5% methanol and shaken at 150 rpm for 24 h; 200-ml aliquots were filter-sterilized and inoculated with ~10^3 CFU/ml *Salmonella* for growth curve analysis.

Results: In the control group, 20-day-old plants supported significantly higher *Salmonella* growth than 59-day-old plants (P<0.05). Drought significantly affected *Salmonella* colonization in 20- 26- and 33-day-old plants, with higher counts recovered from control (P<0.05). As plants aged, the drought did not cause further restriction of *Salmonella*. Counts on 59-day-old control plants were not significantly different from drought-treated 20- or 59-day-old kale. Growth was significantly greater in washes collected from 26- and 33-day-old control than drought-treated plants, but the reverse was observed in 39-day-old drought-treated plants (P<0.05).

Significance: Baby kale leaves are susceptible to *Salmonella* colonization. *Salmonella* restriction on drought-treated juvenile plants is possibly explained by enhanced exudation of inhibitory phyto-compounds.

P2-179 Isolation and Identification of Molecular of *Pseudomonas* spp. Isolated on Green Leafy Vegetables Purchased from Retail Sources

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Introduction: Green leafy vegetables are commonly found to be a source of foodborne illness caused by *E. coli* O157:H7, *Salmonella,* and *Listeria*. However, underreporting or testing of *Pseudomonas,* an opportunistic bacterium typically associated with prolonged infections, biofilm-forming, and multi-drug resistance can potential pose a threat to our food system.

Purpose: The objectives of this study were to isolate *Pseudomonas* from green leafy vegetables, detect the presence of virulence genes and antimicrobial resistance profile of isolates recovered from selected green leafy vegetables.

Methods: Leafy greens samples (n=135) consisting of spinach, romaine lettuce, collard greens, and kale a long with cilantro, green onions, and alfalfa sprouts were analyzed using a modified BAM isolation method and *Pseudomonas* isolation agar as a selective media. Multiplex polymerase chain reaction was used for species-level identification (PA-GS) and detection of six virulence genes (*exoS,plcN, plcH, lasB, tox A* and *algD)* associated with *Pseudomonas* spp. Antimicrobial testing was performed against seven antimicrobial agents using a disc diffusion method

Results: Of the 135 isolates analyzed, 70 (51.8%) produce colonies on the *Pseudomonas* isolation agar. Using multiplex PCR, 30 (42.9%) produce 618 bp PA-GS, 10 (14.3%) 481 bp plcN and 608 bp plcH, 12 (17.1%) 444 bp exoS, nine (12.9%) 313 bp algB and eight (11.4%) 284 bp lasB. All 30 samples were analysed for their antimicrobial resistance. Of the seven tested antibiotics levofloxacin and amikacin was the most effective having 100% susceptibility for all tested samples. Meropenem was the least effective with a 53% resistance rate among the tested samples.

Significance: ***E. coli*** 0157:H7, *Salmonella,* and *Listeria* are commonly found in fresh produce, but it is also good to test for other potential bacteria as being the source of contamination.

P2-180 Transfer of Indicator *Escherichia coli* to Spinach and Carrots Grown in Organic Soil Amended with Raw Animal Manure in California, 2017 to 2018

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Introduction: Biological soil amendments of animal origin (BSAAO) when inadequately treated are potential sources of zoonotic enteric pathogen. Root crops and vegetables grown close to the ground and fertilized with improperly-treated BSAAOs are at increased risk for direct soil contact and microbial contamination.

Purpose: To investigate the persistence and transfer of indicator *Escherichia coli* from soil amended with untreated animal manures to organic spinach and carrots.

Methods: Chicken litter (CL), dairy manure solids (DMS), and horse manure (HM) were tilled separately into four replicate soil plots (four by one m) at a UC Davis organic certified research farm in fall 2017. Plots were inoculated with a three-strain cocktail of rifampicin-resistant *E. coli* (10^8 MPN/g). Controls included unamended (UA) and uninoculated (UI) plots. Organic spinach and carrot seeds were planted 12 d post-application (dpa). Soil samples were collected from zero to 180 dpa. Spinach leaves were harvested at 98 and 127 dpa and carrots were harvested at 131 and 168 dpa, respectively. *E. coli* populations were quantified using direct plating and Most Probable Number (MPN) methods.

Results: *E. coli* persisted in soil from all manure-amended plots through 180 dpa. Manure and crop type influenced *E. coli* survival. In the spinach field, DMS-amended soil had significantly higher *E. coli* counts compared with UA- and HM-amended plots (P<0.05). In contrast, counts were significantly higher in soil from HM- compared with CL- and DMS-amended plots in the carrot field (P<0.05). Transfer of inoculum to spinach leaves was observed 98 dpa (average, 0.9×10 MPN/g) and 131 dpa (average, 0.01×10 MPN/g). Carrot samples were positive at 127 dpa (average, 1.5×10^2 MPN/g) and 168 dpa (average, 1.6×10^2 MPN/g).

Significance: *E. coli* in manure-amended soil may persist and be transferred to leafy green and root crops in organic production. Findings suggest that current wait-times from application to harvest followed by organic farms may not be sufficient to prevent microbial contamination.

P2-181 *Salmonella* Prevalence, Concentration, and Diversity in Poultry Litter in the Southern United States

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Introduction: Poultry litter is a known source of *Salmonella* and poses a contamination risk to fresh produce when applied as an untreated biological soil amendment of animal origin.

Purpose: The purpose of this research was to quantify *Salmonella* prevalence, concentration and diversity in poultry litter throughout the southern United States.

Methods: Poultry litter was collected from 13 broiler or breeder farms in Alabama (seven), Florida (two), Georgia (two), and Texas (two); 10 farms were visited twice. Seven samples (30 g each) from three to four litter piles at every farm were collected. A total of 490 samples were collected from 37 piles. Samples were selectively enriched for *Salmonella* by cultural methods; presumptive positives were PCR confirmed. The concentration of *Salmonella* was determined through concurrent enrichment and serial dilution in MPN reservoirs, paused using refrigeration until S*almonella* screening was complete. When a sample was confirmed positive, processing of the MPN reservoirs resumed, and presumptive *Salmonella* PCR confirmed prior to MPN calculations. *Salmonella* isolates were serotyped and evaluated by PFGE.

Results: *Salmonella* was detected from six (46.2%) farms, in 13 (35%) piles, and 33 (6.7%) samples. The number of positive samples collected from positive piles ranged from one to seven out of seven collected samples. *Salmonella* was more prevalent in samples collected in the fall/winter months than the spring/summer. Of farms visited twice, one was positive on both samplings. Concentrations of *Salmonella* in positive samples ranged from 1.6 to >280,000 MPN/g with a geometric mean and median of 194 and 120 MPN/g, respectively. Serotypes identified included *Salmonella* Anatum, Braenderup, Kentucky, Kiambu, Mbandaka, Michigan, Newport, Saintpaul, and Seftenberg.

Significance: *Salmonella* was present on half the farms sampled, in less than half of poultry litter samples collected; concentrations were highly variable. Contaminated, untreated, poultry litter may pose a health risk if untreated poultry manure contacts produce and the application-to-harvest interval does not allow for adequate dieoff.

P2-182 Different Soil Contamination Levels of *Salmonella* Newport Influence Internalization during Pepper Transplanting

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❖ ***Developing Scientist Entrant***

Introduction: Internalization of pathogens in produce is a food safety concern due to difficulties eliminating pathogens during post-harvest handling. Prior literature has indicated *Salmonella* may internalize in produce during certain production practices, like transplanting.

Purpose: The objective of this study was to investigate *Salmonella* internalization in pepper plants during transplanting exposed to different levels of soil contamination.

Methods: Pepper plants grown in plugs were transplanted in soil inoculated with *Salmonella* Newport at one of three different contamination levels: high, medium, and low (6.6±0.8, 4.0±0.4, and 2.8±0.2 log CFU/g, respectively) (n=9). Plant sections and soil were sampled one, two, three, and seven d post-transplant. Plants were surface sanitized, aseptically sectioned into root, stem, and leaf, and enumerated for *Salmonella* to determine internalized populations using standard methods. Plant section, population level, and time-point were analyzed for significance using Tukey's HSD test (P≤0.05).

Results: No *Salmonella* was recovered from any leaf sample (zero of 108), regardless of *Salmonella* contamination level (high, medium and low) in the soil. In high *Salmonella* contaminated soils, *Salmonella* internalized in root (35 of 36) and stem (six of 36) samples. Approximately 2.2 and 0.8 log CFU/root and stem, respectively, of *Salmonella* was internalized within 24 h of transplanting in soils with high *Salmonella* contamination. In medium *Salmonella* contaminated soils, *Salmonella* internalized in root (18 of 36) samples only. An average 0.8 log CFU/root of *Salmonella* was internalized in soils with medium *Salmonella* contamination. In low *Salmonella* contaminated soils, no *Salmonella* internalized in root and stem samples (zero of 72).

Significance: Internalization was highly dependent on level of soil contamination, as soils containing higher *Salmonella* populations observed significantly more *Salmonella* internalized in root and stem plant sections. Additionally, *Salmonella* internalization was observed within 24 h post-transplanting. Therefore, it is recommended preharvest practices should be (i) aimed at limiting *Salmonella* populations in soil, or (ii) implemented immediately following transplant to reduce internalization risk.

P2-183 Improving the Microbial Safety of Sprouts Using Lactic Acid Bacteria Cultures

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❖ ***Developing Scientist Entrant***

Introduction: Over the last decade, sprout-related foodborne outbreaks attributed to *Salmonella* and Shiga toxin-producing *E. coli* (STEC) have become a persistent public health concern. Based on epidemiological evidence, seeds used for sprouting were identified as the primary source of pathogens. Furthermore, once harvested, seeds are viable for years providing the opportunity for pathogens to internalize and survive on the seed until germination. Besides contaminated seeds, sprouting occurs in a warm humid environment that favors bacterial growth. Therefore, controlling pathogen contamination on seeds and their eventual outgrowth during sprouting is critical to ensure the microbial safety of sprouts.

Purpose: This study investigated the application of lactic acid bacteria (LAB) as potential biocontrol agents to control pathogens on seeds and sprouts.

Methods: Alfalfa seeds were inoculated with a cocktail of five *Salmonella enterica* serovars or four strains of *E. coli* O157:H7 (six log CFU/g) followed by spray application of LAB cultures (seven log CFU/g of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus reuteri* or *Streptococcus lactis* var Diacetylactis). The seeds were then either stored or set up for germination at 25°C. Stored seeds and germinating sprouts were sampled at regular intervals to enumerate surviving pathogen and LAB populations. Data were analyzed using the PROC MIXED sub-routine of SAS v.9.3.

Results: LAB application significantly reduced pathogen populations in seeds and germinating sprouts (P≤0.05). *Lactobacillus acidophilus* and *Streptococcus lactis* reduced pathogen populations to below detection limits by d nine in germinating sprouts and d 30 in stored seeds. However, approximately five log and eight log CFU of the pathogen was recovered from seed and sprouts, respectively. Additionally, LAB application did not impede seed germination and sprout outgrowth.

Significance: Application of LAB on seeds prior to storage or as a pre-germination spray could serve as a practical and feasible antimicrobial intervention strategy to improve the microbial safety of sprouts.

P2-184 Procedures for Improved Detection and Isolation of *E. coli* O157:H7 from Artificially Contaminated Sprout Irrigation Water

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Introduction: *E. coli* O157:H7 has been implicated in foodborne disease outbreaks with sprouted seeds. Spent irrigation water (SIW) has been tested in compliance programs for detecting *E. coli* O157:H7 in sprouts.

Purpose: This study examined three enrichment procedures and five methods for detection and isolation of *E. coli* O157:H7 from artificially contaminated sprout irrigation water.

Methods: Spent alfalfa sprout irrigation water was inoculated with *E. coli* O157:H7 and stored refrigerated (4°C) for three days. SIW samples (five) were inoculated with eight, 16 and 40 CFU/ml of the pathogen. Uninoculated samples were also examined. Three enrichment procedures were used: i) BAM modified buffered peptone water with pyruvate (mBPWp) for five h at 37°C, followed by addition of acriflavine (A), cefsulodin (C), vancomycin (V) and incubation at 42°C to further enhance selectivity; ii) mBPWp with CV held at 42°C with shaking and iii) mBPWp with CV held at 42°C without shaking.

Enriched samples were examined by i) plating directly onto selective agar plates; ii) acid treatment followed by plating onto selective agars; iii) immunomagnetic separation with Dynabeads MAX *E. coli* O157 followed by streaking onto selective agar plates; iv) immunomagnetic separation followed by acid treatment and streaking onto selective agar plates and (v) real-time PCR. TC-SMAC, R&F *E. coli* O157 chromogenic medium and Chromagar O157 were used for cultural recovery of the pathogen and real-time PCR detection was done on the ABI 7500 Fast platform, screening for *stx*1, *stx*2 and O157*wzy* gene targets.

Results: At the lowest inoculum, test conditions two, three, four, and five were more efficient at recovering the pathogen from spent sprout irrigation water following BAM enrichment than test condition one, which detected the pathogen in only one of five samples. *E. coli* O157:H7 was detected by all methods in the CV at 42°C enrichments. Difficulties in isolating the pathogen from the BAM enrichments were due to excessive growth of competing organisms on the selective plates. Additionally, higher numbers of isolates were seen on the acid-treated samples from the CV at 42°C enrichments (with or without shaking) than from those prepared from BAM enrichments.

Significance: This study demonstrates the importance of optimizing sample processing techniques for detecting pathogenic bacteria from high background food matrices.

P2-185 Determining Water Quality and Bacterial Load on Tomatoes in Flume Tanks from Florida Packinghouses.

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❖ ***Developing Scientist Entrant***

Introduction: Monitoring and maintenance of water quality in flume tanks is crucial to prevent cross-contamination during postharvest washing of tomatoes, but there is limited information of how organic matter influences sanitizer efficacy in the water.

Purpose: The main objective of this study was to monitor water quality in flume tanks and evaluate efficacy of postharvest washing of tomatoes in commercial packinghouses.

Methods: Fresh tomatoes (n=3), both before and after washing, were collected on an hourly basis from four packinghouses in Florida and analyzed for total aerobic plate count (APC), total coliforms (TC) and generic *E. coli* (EC). APC was determined using plate count agar, TC and EC were determined using ECC CHROMagar. Additionally, three flume water samples were collected and tested for APC, TC and EC. Simultaneously, flume tank water samples (n=3) were collected and analyzed for temperature, pH, total dissolved solids (TDS), free chlorine, chemical oxygen demand (COD), oxidation-reduction potential (ORP), and turbidity.

Results: The average APC from four packinghouses were 6.2 log CFU/tomato and six log CFU/tomato for pre- and post-wash tomatoes, respectively. The average TC were 4.37 log CFU/tomato and 3.91 log for pre- and post-wash tomatoes, respectively. No *E. coli* was detected from any tomato samples. APC for water samples had an average of 3.48 log CFU/100 ml and TC average of 1.16 log CFU/100 ml. All water samples were also negative for *E. coli*. Turbidity, COD, and TDS levels in flume water increased over time in all packinghouses. There was a correlation between COD and turbidity (r=0.96), and COD and TDS (r=0.95). No strong relationship was seen between ORP and chlorine (r=0.74).

Significance: There was no significant effect (P>0.05) of postharvest washing on microbiological qualities of tomatoes. Water quality in flume tanks deteriorated over time in all packinghouses during a typical operational day of four to eight h.

P2-186 Investigating the Prevalence, Persistence, and Diversity of *Listeria monocytogenes* and *Listeria* Species in Produce Packinghouses

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* has emerged as a food safety concern for a number of produce commodities. While *L. monocytogenes* contamination can occur throughout the supply chain, contamination from the packinghouse environment represents a particular challenge and has been linked to recalls

Purpose: This project aimed to study the prevalence, persistence, and diversity of *L. monocytogenes* and *Listeria* spp. in produce packinghouses.

Methods: A longitudinal study was performed in 11 produce packinghouses (commodities included micro-green, peach, apple, tomato, broccoli, and cucumber) in three states. In each packinghouse, 34 to 44 sites representing zones two to four were selected and swabbed. Packinghouses were visited four times. Samples were processed for *Listeria* by Food and Drug Administration's Bacteriological Analytical Manual methods. Presumptive *Listeria*-positive isolates were confirmed by PCR. Species and allelic type (AT) were identified by *sig*B sequencing for up to four isolates per sample.

Results: Among 1,584 samples tested, 50 (3.2%), 42 (2.7%), and 10 (0.6%) samples were positive for *L. monocytogenes*, *Listeria* spp. (excluding *L. monocytogenes*), and both *L. monocytogenes* and *Listeria* spp., respectively. Five different species of *Listeria* (*monocytogenes*, *innocua*, *seeligeri*, *welshimeri*, and *marthii*) were identified with *L. monocytogenes* the most prevalent species. The 102 *Listeria*-positive samples yielded 138 representative isolates. Representative isolates were defined as having a unique subtype (AT). Approximately 21 (21%) of 102 of those *Listeria*-positive samples contained two or more different subtypes. A high AT diversity (0.93 Simpson's Diversity Index) was observed amongst *Listeria* isolates. There were 18 instances of *L. monocytogenes* or *Listeria* spp. repeated isolation (site testing positive more than two times). Upon analysis of subtype data, only three sites tested positive for the same *Listeria* AT more than two times.

Significance: Data showed in this longitudinal study that *Listeria* prevalence and persistence in packinghouses was low (less than four percent prevalence). Therefore, sanitation program development and implementation in packinghouses are critical to limit *Listeria* harborage and residence.

P2-187 Detecting *Listeria monocytogenes* in a Variety of Individually Quick-Frozen Vegetables Using the BAX System Real-time PCR Assay

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Introduction: *Listeria monocytogenes* is typically associated with processed RTE foods, meats and dairy products but in recent years, numerous sporadic cases and outbreaks have involved moderate and low-risk foods including intact fruit and vegetables. In 2018, one of the latest outbreaks involved packages of individually quick frozen (IQF) vegetables.

Purpose: Since *L. monocytogenes* can survive freezing temperatures, there are increased food safety concerns in frozen products. For this reason, a real-time PCR assay for *L. monocytogenes* was evaluated in a variety of frozen vegetables to minimize the risk that contaminated product will reach consumers.

Methods: Four frozen vegetables; broccoli, carrots, corn and peas, were thawed and divided into 125-g samples for the test method and 25-g samples for the reference method. For each vegetable and method, samples were inoculated with a low and high level of *L. monocytogenes*. Additional samples were left uninoculated for negative controls. All samples were held at -20°C for two weeks and thawed before enrichment. Test method samples were homogenized with 1125 ml of 24 LEB Complete media and incubated at 35°C for 24 to 48 hours. Sample aliquots were then analyzed using real-time PCR. The reference method samples were enriched and plated according to the procedures in the FDA BAM Chapter 10.

Results: For the low inoculation level, the real-time *L. monocytogenes* PCR assay returned positive results for eight samples of broccoli and corn, 10 samples of carrots, and 11 samples of peas. All presumptive results were identical to culture. When compared to the reference method, POD analysis indicated no significant statistical difference.

Significance: The results of this study demonstrates the ability of the BAX System to accurately detect *L. monocytogenes* in a variety of 125-g samples of frozen vegetables equivalent to the reference method.

P2-188 A Multi-regional Study of Generic *Escherichia coli* Persistence in Soils Amended with Raw Manure and Produce in Organic Farming Systems

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Introduction: Animal manure is commonly used to improve soil fertility and is particularly important in organic agriculture. Crops grown in soils amended with raw manure may become contaminated by microbial pathogens and fecal indicators (e.g., generic *E. coli*) present in manure. Reducing the risk of microbial contamination of crops is based on wait-times between manuring and crop harvesting.

Purpose: To determine the dieoff of generic *E. coli* in manured soil and potential transfer to fresh produce considering different conditions (management practices, farm, regions, sample types) in soils amended with raw manure in certified organic farms.

Methods: Seventeen USDA certified organic farms in different United States regions (California, Maine, Maryland, Minnesota) were enrolled in a two-year longitudinal study. Samples (soil, fresh produce and water) were collected during the 2017 to 2018 growing seasons and were quantified for generic *E. coli* populations by Most Probable Number (MPN) and confirmed via PCR.

Results: A total of 53.7% of samples were positive for generic *E. coli* (1560 of 2905): 60.3% soil, 22.5% produce, and 23.7% water. Average log MPN values by region were: California, 0.60 log MPN/gdw soil (range zero to 6.50), 0.11 log MPN/100 g produce (range zero to 4.50); Maryland, 1.06 log MPN/gdw soil (range zero to 5.46), 0.14 log MPN/100 g produce (range zero to 1.37); Maine, 0.55 log MPN/gdw soil (range zero to 4.94), 0.40 MPN/100 g produce (range zero to 4.85); Minnesota, 1.19 log MPN/gdw soil (range zero to 6.44), 0.23 log MPN/100 g produce (range zero to 4.20). Populations of generic *E. coli* decreased substantially by 120 days post manuring on 10 of 17 farms. Quantitative differences in mean generic *E. coli* populations occurred between years, regions, manure and produce type.

Significance: Results from this study provide science-based information to identify potential factors influencing pathogen survival in preharvest produce production environments for soils amended with raw manure on organic production systems.

P2-189 Multi-regional Prevalence and Persistence of Four Foodborne Pathogens in Manured Soils in Certified Organic Farms

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Introduction: Soil amendment with untreated manure may contribute to contamination by foodborne pathogens. Certified organic farms follow the USDA-National Organic Program (NOP) standards for wait-times (90 to 120 days) between the application of raw manure and crop harvest.

Purpose: To assess the prevalence and persistence of foodborne pathogens in untreated manure and amended soils from NOP farms in four United States regions.

Methods: A longitudinal, multi-regional study was conducted on seventeen certified organic farms in four United States regions (nine in California, three in Maine, four in Minnesota and one in Maryland). Untreated manure, soil, and agricultural water collected during the 2017 to 2018 growing seasons were cultured for Shiga toxin-producing *Escherichia coli* (non-O157 STEC), *E. coli* O157: H7, *Listeria monocytogenes,* and *Salmonella*.

Results: In manure samples, 18 (7.9%) of 228 were positive for non-O157 STEC, three (1.32%) of 228 for *E. coli* O157:H7, 15 (6.6%) of 228 for *Salmonella*, and nine (3.9%) of 228 for *L. monocytogenes*. Prevalence of non-O157 STEC in soil samples was 73 (10.4%) of 700, 80 (7.7%) of 1043, ten (5.2%) of 196, and 16 (4.1%) of 386 in Minnesota, Maryland, California, and Maine, respectively. *L. monocytogenes* was detected in 31 (7.3%) of 700, 47 (5.5%) of 1043, 25 (4.4%) of 196, and one (4.2%) of 196 soil samples from Minnesota, California, Maine and Maryland, respectively. *Salmonella* was detected in 26 (1.1%) of 2295 soil samples (Maine, Minnesota, and California). The overall prevalence of *E. coli* O157:H7 in soil was one (0.04%) of 2325 at 60 days post-manuring. Non-O157 STEC and *L. monocytogenes* were detected in soil up to 30 and 90 days post-manuring, respectively. Foodborne pathogen prevalence in soil and manure depended on the pathogen, year, manure type, and region. All water samples were pathogen-free (zero of 38).

Significance: Results from this study provide multi-regional baseline data relating to current NOP wait-time rules and thus a framework for risk mitigation strategies to reduce pathogen persistence that may contribute to contamination of fresh produce typically eaten raw from NOP-certified farming systems using untreated manure.

P2-190 Decontamination of Pathogens on Produce Using Chlorine Dioxide Gas Generated by Sodium Chlorite Acid Reaction

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Introduction: Produce surfaces are prone to harbor foodborne pathogens. Chlorine dioxide (ClO_2) gas is a potential surface decontaminant for produce. Among various methods of generating ClO_2, the reaction of sodium chlorite ($NaClO_2$) with acids is of low cost and suitable for small-scale decontamination operation.

Purpose: The objective of this study was to determine the treatment parameters for using ClO_2 gas generated from $NaClO_2$ and acids for decontamination of tomatoes, blueberries, and baby-cut carrots.

Methods: The stem scars of beefsteak tomatoes, calyces of blueberries, and surfaces of baby-cut carrots were inoculated with *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* to inoculation levels of five to 7.5 log CFU/g. The inoculated samples were placed among 45 kg tomatoes in perforated boxes in a 1246-l chamber and exposed to various concentrations of ClO_2 gas generated by mixing two ClO_2-generating dry media for five h or intermittently dosing 3.6 or 7.2 N HCl to $NaClO_2$ for 15 h at 15°C and 60 to 85% RH. The ClO_2 concentrations (ppm) during treatment were recorded to calculate the cumulative ClO_2 exposure (ppm-h), and the populations of pathogens (log CFU/g) before and after treatments were enumerated to determine the log reductions.

Results: Generally, the increase in cumulative ClO_2 exposure increased the log reductions of pathogens. The cumulative exposures of 1000, 800, and 1200 ppm-h caused four-log reductions of *Salmonella*, STEC, and *L. monocytogenes*, respectively, on tomatoes, whereas 2000, 3000, and 2000 ppm-h were needed for achieving similar log reductions on blueberries. The decontamination was most effective against the pathogens on baby-cut carrots, to which 600 and 400 ppm-h caused six-log reductions of *Salmonella* and STEC, respectively.

Significance: This study identified the treatment parameters for using $NaClO_2$-acid-generated ClO_2 gas for surface decontamination of tomatoes, blueberries, and baby-cut carrots to improve the microbial food safety of these products.

P2-191 Role of the Dormant State in the Persistence and Resistance of Shiga Toxin-producing *Escherichia coli* in the Fresh Produce Chain

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❖ ***Developing Scientist Entrant***

Introduction: STEC, especially *E. coli* O157:H7, have been implicated in over 40 foodborne illness outbreaks linked to contaminated leafy greens such as romaine lettuce. The underlying reason for the pairing of STEC-leafy greens remains unclear. In the following, aspects of the role of the dormant (persister) state in the survival and resistance of STEC within the fresh produce chain was studied.

Purpose: To assess the factors that modulate the persister (dormant) state within members of the top seven STEC and influence on the survival in the soil environment along with tolerance to hypochlorite.

Methods: The STEC were representative strains of O157, O121, O111, O103, O45, and O26. The levels of persisters were determined by cultivating the bacterium in M9 media with the test modulator (indole, bile derivatives, EDTA, soil extracts, seed exudates) up to a mid-exponential phase. Ampicillin was added to the culture and incubated for eight h. The residual persister population was then enumerated by plating and via viability counts. The role of lettuce exudates in resuscitating persister cells was assessed by using an MPN technique coupled with extended incubation at 25°C. The relative hypochlorite resistance of persisters was determined using the dilution test.

Results: *E. coli* O103 formed a significantly ($P<0.05$) higher proportion of persisters compared to the other serotypes tested. From the inducing agents tested, only inclusion of indole (universal signal molecule) promoted an increase proportion of persisters. Persister cells exhibited extended survival in soil, in addition to higher tolerance to hypochlorite compared to normal STEC populations. Extracts of romaine lettuce increase the rate and extent of persister revival when plated onto modified M9 media.

Significance: The persister state in STEC needs to be considered when assessing the survival and resistance of the pathogenic group within the fresh produce chain.

P2-192 Detection of Low Levels of *Salmonella* spp. in Sprout Rinse Water Using the RapidChek SELECT *Salmonella* Test Method

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Introduction: Contaminated seeds have been identified as a major source of sprout-associated pathogen outbreaks. While seeds are decontaminated prior to sprouting, there is no inherent step in the growth process to reduce or eliminate pathogens in raw sprouts. Testing for the presence of *Salmonella* spp. in sprout rinse water will ensure that the released product is safe for human consumption.

Purpose: To validate RapidChek SELECT *Salmonella* for the detection of *Salmonella* spp. in sprout rinse water samples.

Methods: RapidChek SELECT Salmonella vs. FDA BAM

Ssamples of mung bean sprout rinse water (375 g) were inoculated with a low level of *Salmonella*. Samples for the test method were enriched in one liter of pre-warmed primary media, transferred to secondary media, evaluated with test strips and plated on selective agar. Samples for the FDA BAM reference method were enriched in 3.375 L lactose broth, transferred to TT and RV media, and plated on selective agar. Non-inoculated samples were run with both methods as negative controls.

Results: There was no statistical difference between the test method and the FDA BAM reference method ($P=0.52$). The test method demonstrated 100% specificity and 100% sensitivity when inoculated at one cell per sample. There were no false negative results observed with the inoculated samples and no false positive results observed in non-inoculated negative control samples.

Significance: RapidChek SELECT *Salmonella* detected *Salmonella* spp. in sprout rinse water with 100% sensitivity and specificity. This test method allows for reduced enrichment volume which saves time, money and incubator space. As such, RapidChek provides users with a rapid, reliable, cost-effective tool for monitoring *Salmonella* spp. in the sprout rinse water.

P2-193 *Salmonella* Detection Via Immunomagnetic Separation and Liquid Crystal Technology

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Introduction: Liquid crystal technology is designed to improve critical run times and sensitivity while increasing capability for detection of *Salmonella* in specialty crops. The use of immunomagnetic separation (IMS) in complex food samples can be a useful tool to concentrate low levels of target bacteria for further analysis and detection.

Purpose: This study's objective was to provide a detection platform and assay to screen for *Salmonella* in apples, increasing available technologies while improving food safety.

Methods: Matrix limit of detection and validation studies were conducted in apples by using immunomagnetic separation and liquid crystal technology. Samples were inoculated at levels of zero, one, 10, 100 and 1000 CFU/g with *Salmonella* spp. strains and non-target strains which were cold stressed overnight. Seven varieties of apples were analyzed at each inoculum level. Buffed peptone water was added at 400 ml per apple, followed by a 15-h incubation at 42°C and centrifugation. A 1.3-ml aliquot of the liquid eluate was removed and analyzed using liquid crystal technology.

Results: The limit of detection exhibited some degree of variance between each fortification evaluated in both *Salmonella* spp. inoculated samples and non-target samples. Each apple variety analyzed during the validation demonstrated 75% sensitivity (nine of 12 apples) at both low and high inocula of *Salmoella* Enteritidis and 100% specificity (six of six apples).

Significance: The data from this study suggests that liquid crystal technology may offer a suitable screening method for the detection of *Salmonella* in specialty crops.

Poster

Poster

P2-194 Antimicrobial Effect of Natural Fruit Extracts against *Salmonella* on Cucumbers

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✦ ***Undergraduate Student Award Entrant***

Introduction: Salmonellosis outbreaks linked to the consumption of contaminated cucumbers have highlighted the need to develop effective interventions for improving the microbial safety of cucumbers.

Purpose: The efficacy of fruit extracts, namely lemon, yuzu, grapefruit (naringin), and grape (resveratrol) for reducing *Salmonella* on cucumbers was investigated.

Methods: Fresh Persian cucumbers (*n*=128) purchased from the local retailer were submerged in 70% ethanol for one minute to remove surface wax. Each cucumber was marked with two one by five 5 cm areas and spot-inoculated with 20 µl of nine log CFU/ml of *Salmonella* cocktail (*Salmonella* Newport, Poona, and Typhimurium) to obtain five log CFU/cm^2 on cucumbers. After air-drying for two hours, each inoculated area was sprayed with one 1 ml treatment solution, including controls (water, 15% ethanol, and water adjusted to pH 3.0), 10% lemon, 10% yuzu, one percent naringin, or one percent resveratrol. After treatment, cucumbers were stored at 10°C or 22°C for seven days. On days zero, two, five, and seven, each marked area was swabbed with cotton applicator; swab was pummeled for two min in 10 ml of buffered peptone water, and then plated on XLT4 agars.

Results: There was no significant difference in *Salmonella* populations among the controls (P>0.05). At 22°C, *Salmonella* populations recovered from naringin and resveratrol-treated cucumbers were 3.5 and 3.0 log CFU/cm^2, respectively, compared to five log CFU/cm^2 *Salmonella* from controls on day two (P<0.05). Fruit extracts exerted higher antimicrobial activity against *Salmonella* on cucumbers stored at 10°C; lemon and yuzu extracts reduced *Salmonella* populations by ~2.5 log CFU/cm^2 on day seven as compared to control (P<0.05). The antibacterial effect of these extracts was marginal at 22°C.

Significance: The results of this study reveal that fruit extracts could potentially be used to decrease *Salmonella* levels on cucumbers. The storage temperature may influence the effectiveness of these extracts on cucumbers.

P2-195 Carvacrol Nanoemulsion Controls *Escherichia coli* O157:H7 on Fresh Produce

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Introduction: Fresh produce has been implicated in recent foodborne illness outbreaks associated with the consumption of *Escherichia coli* O157:H7 (EHEC).

Purpose: We investigated the efficacy of nano-emulsified carvacrol, a natural phytochemical, as a wash treatment for reducing EHEC on fresh produce.

Methods: Fresh spinach, romaine lettuce, and iceberg lettuce leaf cores (three cm diameter, 216 samples) were spot-inoculated with 25 µl of a five-strain cocktail of nalidixic acid (NA) resistant EHEC at eight log CFU/ml. After air-drying for one h, 20 pieces of each inoculated produce leaves were immersed in water-based treatment solutions (200 ml/group), including water, 25 or 50 ppm free chlorine, and 0.25 or 0.75% (w/w) carvacrol nanoemulsion (CRN) for two minutes, followed by rinsing with 100 ml neutralizing broth for one min and drying in spinning salad bowl for one min. Inoculated produce leaves without any treatment were served as baseline. Produce leaves were stored at 10°C and EHEC populations were enumerated on days zero, two, seven, and 14 by plating on CT-sorbitol MacConkey agar with NA.

Results: A 0.75% CRN wash treatment significantly reduced EHEC populations on spinach, Iceberg lettuce, and romaine lettuce to 3.6 log, 4.0 log, and 4.5 log CFU/cm^2, respectively, compared to ~six log CFU/cm^2 of EHEC recovered from the baseline on day zero. During storage, the antimicrobial activity of CRN against EHEC was more pronounced on romaine lettuce, followed by iceberg lettuce and spinach; populations of EHEC on romaine lettuce were reduced by four log CFU/cm^2 as compared to the baseline after 14 days. Antimicrobial properties of CRN at 0.75% against EHEC were superior to 25 and 50 ppm chlorine on both lettuces on day 14; recovery of EHEC was ~one log and ~two log CFU/cm^2 lower in 0.75% CRN treated romaine and iceberg lettuce samples, respectively, as compared to chlorine-treated samples (P<0.05).

Significance: Results of this study support the potential use of CRN as water-soluble antimicrobial washing treatment for controlling EHEC on fresh produce.

P2-196 on Fresh Strawberries by Lactic Acid Bacteria

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Introduction: Foodborne illness outbreaks associated with the consumption of raw fruits have gained public attention in recent years. *Listeria monocytogenes* as an important foodborne bacterial pathogen may contaminate strawberries and threaten public health.

Purpose: The purpose of this study was to investigate the effect of two canine-feces origin lactic acid bacteria (LAB), namely *Lactobacillus plantarum* and *Pediococcus pentosaceus,* as post-harvest biocontrol agents against *L. monocytogenes* on fresh strawberries.

Methods: Strawberries (*n*=144) purchased from the local grocery store were sorted for identical sizes and then dip-inoculated with a three-strain cocktail of *L. monocytogenes* in phosphate buffered saline (PBS) to obtain 6.0 log CFU/g of *Listeria* populations on strawberries. After *Listeria* inoculation and air-drying for one hour, strawberries were dipped in MRS broth containing nine log CFU/ml cocktail of *L. plantarum* and *P. pentosaceus*. *Listeria*-inoculated strawberries with no further treatment or dipped with fresh MRS broth alone were included as controls. After treatment, strawberries were stored at 4°C or 10°C for seven days. Surviving *Listeria,* LAB, and total yeast and mold populations on strawberries were determined on zero, one, three, and seven days-post-treatment by serial dilution and spiral plating on Rapid'*Lmono,* MRS, and potato dextrose agars, respectively.

Results: There was no significant difference in *Listeria* populations between the control and MRS control, in which ~five log CFU/g of *Listeria* were recovered from the controls on day seven. At both 4°C and 10°C storage, LAB treatment significantly decreased *Listeria* populations by 1.5 log, 2.0 log, and 2.5 log CFU/g as compared to the controls on days one, three, and seven, respectively (P<0.05). LAB populations remained at ~ 7.5 log CFU/g levels on strawberries at both storage temperatures throughout the entire study. Total yeast and mold populations and visual appearances of strawberries with MRS or LAB treatment at both temperatures were comparable to the control during storage.

Significance: Results of this study indicate that LAB can potentially be used as post-harvest biocontrol agents against *L. monocytogenes* on fresh strawberries.

Poster

P2-197 Efficacy of Benzyl Isothiocyanate for Controlling *Salmonella* on Alfalfa Seeds

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Introduction: Salmonellosis outbreaks associated with the consumption of alfalfa sprouts have occurred with increased frequency worldwide. It is critical to identify effective treatments for controlling *Salmonella* contamination on alfalfa seeds.

Purpose: The purpose of this study was to investigate the efficacy of benzyl isothiocyanate (BIT), a cruciferous vegetable-derived compound, for controlling *Salmonella* on alfalfa seeds.

Methods: Alfalfa seeds (150 g) were immersed in 300 ml of sterile deionized water containing individual *Salmonella* serotypes Newport, Typhimurium, and Tennessee at two inoculation levels of four or six log CFU/ml for two min. After overnight air-drying, 15 g of inoculated seeds were treated with 30 ml of zero, 0.5, 1, 1.5, or 2% BIT solutions for 30 min. Inoculated seeds with no treatment (baseline) or 20,000 ppm of total chlorine were included as controls. After treatment, seeds were stored at 25°C for seven days, and surviving *Salmonella* populations were determined on days zero, one, five, and seven by plating on XLT4 agars. The experiment at each inoculation level was repeated four times in this study (*n*=672). The effect of BIT on germination of alfalfa seeds was studied using uninoculated seeds with automated sprouting equipment.

Results: The antimicrobial effects of BIT against three serotypes of *Salmonella* on alfalfa seeds was concentration-dependent. The anti-*Salmonella* activity of BIT at 1.5 and 2% was comparable to 20,000 ppm chlorine on days five and seven. At the high inoculation level, ~4.5 log CFU/g *Salmonella* were recovered from the baseline on day seven; however, BIT at 2% significantly decreased pathogen populations to <1.7 log CFU/g. *Salmonella* Tennessee was the most sensitive among serotypes evaluated in this study. The germination rate of 0.5 to 2% BIT treated alfalfa seeds (85 to 87%) was not significantly different from control or chlorine-treated seeds (~90%).

Significance: Results of the study indicate that BIT can potentially be used to control *Salmonella* contamination on alfalfa seeds.

P2-198 Growth Potential of *Listeria monocytogenes* in Apple Flesh and Juice

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Introduction: Recent outbreaks of listeriosis and associated recalls raised questions regarding survival and growth potential of *Listeria monocytogenes* in acidic fruits like apples.

Purpose: Understanding *L. monocytogenes* survival/growth in apple varieties and possible virulence mechanism implications.

Methods: The growth potential of *L. monocytogenes* (a rifampicin resistant serotype 1/2b strain) on artificially inoculated cut chunks and in juices from seven apple varieties [Braeburn, Fuji, Gala, Golden Delicious(GD), Granny Smith(GS), McIntosh and Red Delicious(RD)] was tested following incubation at 10°C. The bacterial populations were measured using plate counts. The transcriptional profiles of inoculated *L. monocytogenes* in different apple juices were compared by a *Listeria*-specific DNA microarray. Biochemical parameters (pH, acidity, sugar, polyphenol, and antioxidant) of apple varieties were also measured by standard methods.

Results: *L. monocytogenes* grew ~one to two log in the chunks of Breaburn, Fuji, Gala, and GD but not in GS, McIntosh and RD. Inoculated *L. monocytogenes* did not grow in any apple juices; rather, the *L. monocytogenes* population decreased ~4-5 log in 48 hours in GS, McIntosh and RD. The pH of different apples varied between 3.2 to 4.5. *L. monocytogenes*, inoculated to apple juices adjusted to pH 6.8, decreased ~one to two log in 48 hours in GS and McIntosh but maintained their inoculated numbers in other apple juices. The polyphenol, antioxidant, and acidity of GS were significantly higher than those of other apple varieties (P<0.05). Microarray analyses revealed 163 *L. monocytogenes* genes (cut-off value 2) were either up- or down-regulated in various apple juices. Of interest, the *gadB* and *inlA* genes were over-expressed in *L. monocytogenes* when exposed to GS and McIntosh.

Significance: *L. monocytogenes* exposed to GS or McIntosh may acquire advantages over other apple varieties through increased expression of *gad*B (acid tolerance-related) and *inl*A (invasion-related) genes. Understanding the growth/survival of *L. monocytogenes* in apple and its role in virulence gene expression may have important implications in risk assessment.

P2-199 Impact of Field Debris on Tomato Wash Water Quality Deterioration

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Introduction: Tomatoes have been linked to multiple salmonellosis outbreaks in the United States during the past decades. Science-based information is needed to evaluate current packinghouse wash operation practices, and to develop an effective, practical food safety standard for tomatoes.

Purpose: To assess factors impacting wash water deterioration during a simulated dump tank washing process, and to determine the effect of decreased chlorine concentration on the survival of indigenous microorganisms in water and on tomatoes or leaf-stem debris.

Methods: Freshly harvested grape tomatoes (2000 g) intermixed with leaf-stem harvest debris (6.4 g) were sequentially washed in water (20 liters) in multiple (*n*=12) batches. Water samples, collected after washing each batch, were assessed for common water quality parameters including pH, total dissolved solids (TDS), turbidity, chemical oxygen demand (COD), and chlorine demand. Microbial populations (aerobic bacterial and yeast-mold plate counts) in water and on tomatoes as impacted by chlorine concentration and water filtration (300 µm) were also determined.

Results: Water quality deterioration was proportional to the cumulative quantity of tomatoes (g/liter) and debris (g/liter) washed per minute. Chlorine demand was ≤75 mg/l when the cumulative ratio of tomato to water reached 1.2:1. Field debris, which accounted for less than one percent of the total weight of tomato harvest, contributed 37.8% of TDS, 46.15% of turbidity, 48.8% of COD and 50.6% of chlorine demand in the wash water. Field debris and defective tomatoes were the major contributors of microbial counts in the wash water. Removal of the debris and its fine fragments by filtration through a 300 µm nylon mesh significantly reduced microbial populations.

Significance: Results show that removing field debris from harvested grape tomatoes prior to washing reduces the deterioration of wash water quality and microbial load in post-harvest tomato washing. This information is fundamental to the development of science-based packinghouse practices to ensure food safety.

P2-200 Impact of Irrigation with Wastewater and Roof-harvested Rainwater on the Persistence of *Escherichia coli S*urrogates on Lettuce Cultivars in the Field

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Introduction: Secondary-treated wastewater (STWW) and roof-harvested rainwater (RHW) may serve as alternative irrigation sources for small growers to decrease the pressure on freshwater. Further investigation is needed to evaluate the microbiological safety of fresh produce irrigated with these waters.

Poster

Purpose: The effect of STWW and RHW on the persistence of *Escherichia coli* surrogates on different cultivars of lettuce in the field was investigated.

Methods: Four-week old, field grown lettuce plants (Annapolis, Celinet, and Coastline cultivars) were spray-irrigated with well water (control), STWW, or RHW containing five log CFU/ml of a mixture of non-pathogenic surrogates: nalidixic acid-resistant *E. coli* O157:H12 and chloramphenicol-resistant *E. coli* K12. At zero, one, three, seven and 10 days post-irrigation, four replicate lettuce samples (20 g/sample) from each group were collected and pummeled in 80 ml of buffered peptone water for two minutes, followed by plating on sorbitol-MacConkey agar with antibiotics.

Results: *E. coli* H12 persisted at a significantly higher level than *E. coli* K12 on lettuce regardless of the water sources and lettuce cultivars. Recovery of H12 and K12 strains on STWW- and RHW-irrigated lettuce was not different from control-irrigated lettuce on day 10; however, H12 populations increased by 1.6 log CFU/g in the RHW- and STWW-irrigated Annapolis lettuce on day seven compared to the control. The difference in leaf characteristics of lettuce cultivars influenced the persistence of these surrogates on leaves. On day three, the recovery of *E. coli* H12 on RHW-irrigated Celinet lettuce was significantly higher (three log CFU/g) than *E. coli* H12 recovered from other two cultivars (one log CFU/g). *Listeria monocytogenes* and *E. coli* O157:H7 were not detected in lettuce irrigated with STWW or RHW.

Significance: Bacterial pathogens may persist at higher levels on lettuce irrigated with STWW and RHW. The bacterial strains and lettuce cultivars could influence the bacterial persistence on the irrigated lettuce.

P2-201 Potential for *Salmonella* Cross-Contamination during Tomato Washing and Pre- and Post-Wash Commingling

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Introduction: *Salmonella* is a major foodborne pathogen associated with consumption of fresh tomatoes. Cross-contamination of *Salmonella* during postharvest harvest handling have been suspected for multiple tomato-related outbreaks. Chlorine-based dump-tank flume washing systems are commonly used for tomato processing.

Purpose: To examining *Salmonella* cross-contamination potentials at different stages of tomato processing, including pre-washing commingle, washing in chlorinated water, and post-washing commingle.

Methods: Commercially harvested fresh grape tomatoes and debris (non-consumable pant materials harvested with the tomatoes) were differentially inoculated with *Salmonella* cocktails (8 or 6 log CFU/ml) carrying distinguishable antibiotic resistance markers. Freshly harvested grape tomatoes were washed along with inoculated tomatoes and debris in simulated tomato dump-tank wash water at designated free chlorine levels. Non-inoculated tomatoes (350 g) were allowed of instantaneous contact with the inoculated tomatoes (50 g) and debris (0.5 g) during washing, as well as during pre-washing and post-washing commingle. The levels and sources of *Salmonella* contamination on the non-inoculated tomatoes were determined by plate counting and most probable number methods. Three replicates were tested for each treatment. The experiment was repeated twice.

Results: *Salmonella* cross-contamination during washing (the stage of submersion in wash water) in chlorinated wash water was extremely rare even at high inoculation levels. Pre-wash comingle of non-inoculated tomatoes, inoculated tomatoes, and debris resulted high level of contamination which was not effectively remedied by the subsequent washing (1.67-48.33% cross contamination). Debris was the main source of contamination in these cases. Post-washing contacts between non-inoculated and inoculated tomatoes did not significantly increase *Salmonella* cross contamination probability comparing to washing treatments without commingling.

Significance: This study demonstrated the critical role of direct contact with debris as the major source in cross-contamination during tomato processing. The information is useful for developing improved strategies of foodborne pathogen mitigation during tomato processing.

P2-202 WITHDRAWN

P2-203 *Salmonella* Inactivation and Cross-Contamination on Cherry and Grape Tomatoes during Washing in Simulated Commercial Wash Water

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Introduction: In recent years, *Salmonella* has been implicated in numerous outbreaks associated with tomatoes, including outbreaks linked to packinghouse contamination. Recently, there has been a concerted effort by the fresh produce industry to establish science-based national standards for fresh produce processing that comply with FDA rules pertaining to FSMA regulations. Few studies have examined the inactivation and cross-contamination of *Salmonella* on fresh tomatoes in flume wash systems that can be used for supporting the establishment of new standards.

Purpose: To examine the inactivation and potential for cross-contamination of *Salmonella enterica* from two potential sources: tomato fruit and field debris (mainly dry leaves and stems), in a simulated flume setup with different concentrations of free chlorine.

Methods: Cherry or grape tomatoes and debris were inoculated by submersion with differentiable *Salmonella* cocktails, and incubated overnight at 15°C. Inoculated tomatoes and debris were washed with uninoculated tomatoes in various concentrations of free chlorine for one minute in a simulated wash setup. After wash, inactivation of *Salmonella* on tomatoes and debris was evaluated by plate count enumeration on XLT4, and uninoculated tomatoes were evaluated for detection of *Salmonella* cross-contamination.

Results: Washing inoculated tomatoes in 25 mg/l or higher concentrations of free chlorine reduced population of *Salmonella* on tomato by at least 2.9 log, which was significantly higher than the log reduction (one-log) obtained by washing in unchlorinated water (P<0.02). Washing did not significantly reduce the population of *Salmonella* on debris, regardless of the presence or absence of chlorine in wash water (P=0.36). When wash water was chlorinated, 11 cross-contamination events occurred with 10 positive samples originating from debris and one positive sample originating from tomato out of 720 individual tomatoes tested.

Significance: These observations highlight the role that contaminated field debris may play as a potential source for cross-contamination during the tomato washing process.

P2-204 Evaluation of Viral Infectivity during the Frozen Storage of Berries

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✦ ***Undergraduate Student Award Entrant***

Introduction: Raspberries and strawberries are vulnerable to contamination by human norovirus (HuNov) and hepatitis A virus (HAV). Viruses are generally unaffected by frozen storage conditions; however, data gaps remain regarding long-term storage of frozen berries.

Purpose: This study assesses the effects of frozen storage on the infectivity of HAV and norovirus-surrogate Tulane virus (TV) on strawberries and raspberries along with acute physical changes to frozen berries.

Methods: Composite samples were prepared in duplicate (average weight 15 g, one strawberry or three raspberries) and inoculated with six log $TCID_{50}$/ml of TV or HAV using a 0.1 ml volume. Samples were stored in a frost-free freezer (-12±3°C) and processed every 30 d. Berry pH and color were measured in triplicate. Viruses were recovered by rinsing two combined samples with 10 ml PBS 30X and infectivity determined using $TCID_{50}$ with LLC-MK2 cells for TV and FRhK-4 cells for HAV. Data were analyzed using a one-way ANOVA in JMP statistical software and the significance threshold was set to 0.05.

Results: After 150 days, no significant changes in pH were detected for raspberries (P=0.46) or strawberries (P=0.74); however, raspberries maintained a significantly lower average pH of 2.78±0.07, compared to strawberries (P<0.0001) which averaged a pH of 3.21±0.08. No significant differences in color of either berry type were detected (P=0.78). After 240 days, HAV infectivity from frozen raspberries (P=0.87) or strawberries (P=0.88), was unchanged with virus titer at an average of 6.1±0.4 log $TCID_{50}$/ml. A significant decrease was observed in TV infectivity on strawberries (P=0.05) after 240 days with a remaining titer of 3.6 log $TCID_{50}$/ml; however, no significant changes were detected in infectivity from frozen raspberries (P=0.40) with virus titer at an average of 4.5±0.9 log $TCID_{50}$/ml.

Significance: These data revealed the persistence of viruses in frozen berries after a six-month storage period. These findings reinforce the importance of good hygiene and preharvest sanitation.

P2-205 Steady State Gaseous Chlorine Dioxide Treatment for the Inactivation of Tulane Virus on Berry Fruits

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Introduction: Pathogenic human viruses are a substantial problem for the fresh and frozen berry industry, principally because they are environmentally prevalent and very difficult to inactivate. Gaseous chlorine dioxide (ClO_2) may be a potential intervention but much research has focused on sachet or bolus type applications in which a high concentration of gaseous chlorine dioxide charge is administered and permitted to dissipate with time. The use of direct constant level of ClO_2 as opposed to bolus type sachet treatments, may circumvent issues regarding oxidative burden of different produce products permitting equal treatments regardless of the lot size being treated.

Purpose: To determine the effectiveness of constant levels of ClO_2 against a human norovirus surrogate (Tulane virus; TV) on berries

Methods: ClO_2 was generated within a 270-l glove box to treat 50-g batches of blueberries, raspberries, and blackberries as well as 100-g batches of strawberries that were immersion coated with TV. Concentrations of ClO_2 ranging from 0.63 to 4.40 ppm-h/g berries were evaluated.

Results: As compared to untreated TV-contaminated berries, log reductions of TV were >2.9 for all berry types and conditions tested indicating the gaseous ClO_2 was highly effective. Average log reductions with strawberries, raspberries, blueberries and blackberries, treated with 0.63 ppm-h/g, the lowest ClO_2 concentration tested, were 2.98, 3.40, 3.82, and 4.17 respectively.

Significance: Overall results suggest that constant levels of ClO_2 are quite effective against a foodborne virus surrogate on the surface of strawberries, blueberries, blackberries and raspberries. This work should serve as a guideline for the industry regarding treatment of berries with chlorine dioxide gas.

P2-206 The Effect of Edible Nano Coating for Improving Shelf Life and Food Safety of Raspberries

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❖ ***Developing Scientist Entrant***

Introduction: Raspberries have gained recent attention because of their richness in beneficial phytonutrients for human health. However, high perishability, a short growing season and high vulnerability to fungal spoilage limit their availability. Moreover, microbial contamination in handling may lead to food safety issues.

Purpose: The goal of this study was to prolong the shelf life of raspberries and ensure food safety by application of an edible nano-coating (ENC) containing the antimicrobial agent limonene without compromising beneficial attributes of raspberries.

Methods: ENC was prepared with soy lecithin nano-liposomes impregnated with limonene using a thin layer hydration technique followed by sonication. For comparison, two percent chitosan coating and uncoated berries were used as a control. One of three coating treatments – ENC, chitosan, or no coating – were randomly applied to freshly harvested raspberries where each treatment had three replicates with 50 berries, and subjected to shelf-life study. At each sampling time of zero, seven, 14, 21, and 28 d, berries were analyzed for decay incidence, weight loss (WL), yeast and mold count, total bacterial count, pH, titratable acidity, total phenolic content (TPC), total anthocyanin (TA), firmness and surface color. General linear model procedures were performed (P<0.05) for all the treatments at different sampling times.

Results: Limonene ENC reduced physical fruit loss as well as showing significantly lower mold deterioration, lower bacterial count, and less color change ($F_{3,8}$; P<0.05) throughout storage. Also, less WL and higher TPC and TA were significantly different characteristics compared to uncoated. The coating did not significantly affect firmness, titratable acidity, pH, and soluble solid of berries. Less than 50% fruit loss was observed in ENC treatment after 28 d compared to uncoated.

Significance: As an edible coating, liposome encapsulated limonene could be an excellent alternative postharvest treatment for extending the shelf life of short-season berries.

P2-207 Effective Pack Practices: Use of Antifungal Packaging Films with Cinnamaldehyde Nanoemulsions to Control Postharvest Diseases in Strawberries

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❖ ***Developing Scientist Entrant***

Introduction: Every year 40% of the food items intended for human consumption never reach the table. Produce can be lost due to inadequate storage conditions, package failure, spoilage, or degradation. Fruits account for 12.3% of food loss in the United States and berries tend to be the most susceptible to waste. Produce growers need cost-effective technologies to help decrease loss and preserve quality and safety until reaching consumers' tables.

Purpose: The objective of this study was to evaluate the effectiveness of previously formulated active packaging systems to extend shelf-life and enhance strawberries' quality.

Methods: Pullulan films incorporated with cinnamaldehyde nanoemulsions were selected for this field study. Fresh strawberries were harvested from a local farm in Kansas and separated into two groups (control and treatment). For each group, 10 strawberries were placed into a molded fiber berry basket and a pullulan film (seven by eight cm) was added at the bottom of each container for the treated group. Samples were stored for up to 10 days at 3°C and 12°C. Every two days microbial, visual, and physiological quality parameters were evaluated. The experiment was run in triplicate.

Results: For treated strawberries stored at 3°C, a reduction of two log CFU/g in yeast and mold population was observed over the 10-day period ($P<0.05$), as compared to the control. No significant difference ($P>0.05$) in yeast and molds was seen between controls and treated berries stored at 12°C. No significant differences ($P>0.05$) were reported for either group or storage temperature in aerobic plate counts. A significant improvement in visual quality was noted for treated berries at both storage temperatures as compared to the control group ($P<0.05$).

Significance: This study demonstrates the effectiveness of pullulan films incorporated with cinnamaldehyde nanoemulsions to reduce fungal decay in strawberries and promote desired quality during storage.

P2-208 The Influence of Water Antimicrobials and Low Temperature Storage on Inhibiting *E. coli* O157:H7 and O26:H11 on Strawberries

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Introduction: Foodborne illness caused by O157 and non-O157 Shiga toxin-producing *E. coli* (STEC) on fresh produce remains a concern worldwide. Fruits contaminated at the point of production, transportation and retail serve as a vehicle for STEC transmission. In this study, commercial and home washing and low-temperature storage practices of strawberries were evaluated for survival of O157:H7 and O26:H11 STEC.

Purpose: Evaluate practices that facilitate STEC inhibition to improve microbial safety of strawberries.

Methods: Strawberries were spot inoculated to achieve six log CFU/g of O157:H7 and 5.3 log CFU/g of O26:H11. The inoculated strawberries were washed for 90 s using tap water, electrolyzed water (50 ppm free chlorine) or 50 ppm free chlorine at 20°C. After washing, the strawberries were separately stored at 4°C for two d, or -20°C/-80°C for 30 d. Samples were processed and plate counting was conducted to determine the population of O157:H7/O26:H11 remaining on strawberries at predetermined days of storage.

Results: Washing strawberries in water containing chemical antimicrobials significantly decreased the level of STEC by two log. Using water alone had limited effect. Frozen storage (-20°C and -80°C) had a significant effect on inhibiting both strains on strawberries. As time went by, the number of STEC showed a declining trend. After 15 d frozen storage, the STEC level was below the detection limit (1.7 log CFU/g) for the strawberries washed by water antimicrobials. However, 2.5 log CFU/g of STEC still remained on the strawberries washed by water alone. The results showed a hurdle effect could lead to a greater log reduction of STEC populations.

Significance: Under proper frozen storage for a long time, the risk of O157:H7/O26:H11 remaining on frozen strawberries is low. It is recommended to wash strawberries using water antimicrobials prior to consumption/further processing.

P2-209 Effects of X-Ray Irradiation on Pathogen Contamination and Quality Aspects of Fresh Blueberries

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❖ ***Developing Scientist Entrant***

Introduction: Ionizing irradiation has been utilized for phytosanitary treatment of fresh fruits, including blueberries. However, fresh blueberries contaminated with *Salmonella* and *E. coli* has recently led to multiple foodborne outbreaks. Therefore, microbial and quality assessment for irradiated fresh blueberries will benefit processors.

Purpose: This study aimed to quantify the effects of X-ray irradiation for inactivation of *Salmonella* and *E. coli* on fresh blueberries and their physical quality during storage.

Methods: For the inactivation study, fresh blueberries were spot-inoculated with a 0.1 ml inoculum of *Salmonella* Enteritidis PT 30 or a cocktail of *E. coli* O157:H7, and dried for three hours. Samples were irradiated in an X-ray food irradiator at 70 kV with doses from zero to 0.5 kGy, plated, incubated, and enumerated for survivors in triplicate. For the quality study, samples of 15 berries were irradiated with center doses from zero to 0.4 kGy, and assessed in terms of firmness (n=15 berries), bioyield point (n=3 berries) and Kramer shear force (n=1) at zero, one, three, and seven d of storage in duplicate.

Results: Radiation D-values (dose required for 90% reduction in population) with 95% confidence intervals were 0.333±0.076 and 0.362±0.193 kGy for *Salmonella* and *E. coli*, respectively. Irradiation and storage had no significant ($P<0.05$) impact on firmness (2.03±0.21 N/mm, n=32), but bioyield point (2.93±0.53 N at zero kGy) showed significant ($P<0.05$) negative correlation with X-ray dosage (r=-0.757, up to 59.6% decrease). Although Kramer shear test results indicated significant changes ($P<0.05$), no conclusive trend was observed.

Significance: Low-energy X-ray can be utilized to deliver a typical phytosanitary dose of 0.4 kGy for fresh blueberries while reducing risk of foodborne illness and maintaining quality.

Poster

P2-210 Efficacy of Gaseous Chlorine Dioxide in Reducing *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* on Strawberries and Blueberries

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Introduction: Use of aqueous sanitizers affect the quality and shelf life of berries. Gaseous chlorine dioxide, a strong oxidizing agent at low concentrations, may be utilized as an economical and effective sanitizing agent when delivered as dry material sachets.

Purpose: This study evaluated the efficacy of low concentration (less than five ppm) gaseous chlorine dioxide (ClO_2) treatment with gas permeable sachets in reducing the levels of *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* on strawberries and blueberries.

Methods: Strawberries and blueberries were spot inoculated (10^7 CFU/g) with multi-strain cocktails of *Salmonella enterica*, *E. coli* O157:H7, and *Listeria monocytogenes* and dried for 120 minutes inside a biosafety cabinet. The inoculated samples were placed in sealed five-liter chambers containing gaseous ClO_2 sachets (63 mg/l for strawberry chambers and 30 mg/l for blueberry chambers) for one, two, and three h with control samples treated with no gaseous ClO_2 for three h. Gas overhead concentrations were measured using a C-16 PORTASENS II gas detector at 20 minute intervals.

Results: Gaseous chlorine dioxide (ClO_2) treatments for three h on strawberries reduced *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* levels by 2.0±0.83, 3.5±0.18, and 2.9±0.20 log CFU/g, respectively. *Listeria monocytogenes* levels after three-h treatment were reduced by >1.5 log CFU/g compared to one and two h treatments. Three h of gaseous ClO_2 treatment on blueberries reduced *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. levels by 2.3, 2.1±0.28, and 2.2±0.63 log CFU/g, respectively. Reductions were similar for *E. coli* O157:H7 and *Salmonella* spp. in both strawberry and blueberry samples for all treatment times. Control treatments reported insignificant reductions for all pathogens, averaging 0.20 log CFU/g of pathogen reduction.

Significance: The findings of this study indicate that gaseous ClO_2 delivered in gas permeable sachets at low concentrations (less than five ppm) can significantly reduce pathogens on strawberries and blueberries during post-harvest sanitation.

P2-211 Inactivation of Murine Norovirus and Hepatitis A Virus on Strawberry, Blueberry and Raspberry by High Pressure Processing

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Introduction: Multiple outbreaks associated with foodborne viruses have occurred in recent years due to the consumption of contaminated berry products. High-pressure processing (HPP) has been recognized as a reliable nonthermal processing technology for the food industry capable of inactivating a wide variety of foodborne pathogens including viruses while retaining the organoleptic quality.

Purpose: The objective of this study was to evaluate the efficacy of HPP on inactivating murine norovirus (MNV-1) and hepatitis A virus (HAV) artificially inoculated onto fresh strawberries, blueberries and raspberries.

Methods: Fresh berries (25 g) were spot-inoculated with MNV-1 or HAV to 10^3 to10^4 PFU/sample and packed in high barrier bags filled with 30 ml sterile water. Berries were HPP treated at 200-600 MPa for three minutes with an initial process temperature of 4°C. After treatment, viruses were extracted from the samples and quantified by viral plaque assay.

Results: The initial inoculum level was approximately four and three log PFU/sample of MNV-1 and HAV, respectively, on berries. Water in the package removed 0.6 to 2.1 log of viruses. With water immersion, the efficacy of HPP was significantly enhanced in both strawberries and blueberries compared to treatment at the same pressure level without water e.g., vacuum packed ($P<0.05$). Pressure treatment at 350 MPa was able to reduce MNV-1 by >3.9 log, and treatment at 400 MPa archived >2.4 log reduction of HAV in raspberries immersed in water during HPP. At 400 MPa, both MNV-1 and HAV was reduced to below detection level in strawberries, blueberries and raspberries.

Significance: HPP can be a preventive control for berries while retaining sensory characteristics. However, process optimization will be needed for large scale production due to the need for berries to be immersed in water.

P2-212 Behavior of Two Serotypes of *Listeria monocytogenes* from Outbreaks and Recalls on the Surface of Stone Fruits during Refrigerated Storage

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Introduction: In 2014, a multistate outbreak of foodborne illnesses associated with stone fruits contaminated with *Listeria monocytogenes* was reported. Due to this outbreak and research suggesting that the surfaces of stone fruits should not support the growth of *L. monocytogenes*, the behavior of *L. monocytogenes* strains isolated from stone fruits was investigated.

Purpose: To investigate the effects of strain genotype and fruit cultivar on the survival of *L. monocytogenes* in stone fruit.

Methods: Two *L. monocytogenes* strains (4b and 1/2b), isolated from recalled stone fruits from the 2014 investigation were used. Yellow and white peaches, and yellow nectarines were inoculated by submersion into water containing *L. monocytogenes,* at one of two inoculation levels, for five minutes. Fruits were air dried for 30 min at room temperature and stored at 4°C for 26 days. *L. monocytogenes* levels were enumerated by a rinsing and direct plating method developed in our FDA laboratories. Counts were obtained by plating onto ALOA (Biomérieux) and RAPID'*L.mono* (BIO-RAD) agars. Twelve treatments (four replicates per treatment) were conducted.

Results: The *L. monocytogenes* populations sharply declined in the first three d and then declined more slowly until day 21. The maximum decline was 1.77 log CFU/fruit on yellow peaches inoculated with the serotype 4b strain at ~5000 CFU/fruit. On Day 21, the highest level of *L. monocytogenes* (2.20 log CFU/fruit) was observed on yellow peaches that had been inoculated with a high level of the serotype 4b strain. The *L. monocytogenes* remained viable until the end of storage (day 26), but the levels were not significantly different from those on day 21. The highest level of *L. monocytogenes* on day 26 was observed on white peaches inoculated with the serotype 1/2b strain at the high level (2.50 log CFU/fruit).

Significance: Despite the lack of growth, on stone fruits under the refrigerated conditions, *L. monocytogenes* did survive for an extended time, demonstrating that stone fruits can carry a potential risk for causing listeriosis in susceptible populations.

Poster

P2-213 Characterization of Tree Fruit Bacterial Communities during Harvest

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Introduction: Under FSMA the tree fruit industry is required to validate onsite sanitizing wash water systems, but food safety issues limit using actual foodborne pathogens for testing. Therefore, we hypothesize that the native microbiome present on tree fruits can be utilized as surrogates to validate sanitization.

Purpose: Currently, tree fruit microbiome data is limited, particularly throughout a growing season, thus we are establishing the microbiome of three tree fruit types, apples (pome), peaches (stone), and navel oranges (citrus) with the goal of establishing an onsite validation bacterial index.

Methods: Fruits were harvested during the early, middle, and late parts of the growing season and rinsed with a solution (0.15 M NaCl, 0.1% Tween 20) to remove bacteria. Viable and total community composition were differentiated by treating half the rinsate with propidium monoazide, which removed 'relic' DNA from non-viable cells, and the untreated other half represented the total community. DNA was extracted from both rinsate samples, and 16S rRNA gene sequencing was performed using two sets of barcoded primers, 799F – 1115R (chloroplast excluding) and 515F – 926R. Barcoded amplicons were sequenced on an Illumina MiSeq, and the UPARSE pipeline used for operational taxonomic units (OTUs), QIIME calculated alpha and beta diversity metrics, and taxonomy assigned using the RDP classifier against the GreenGenes database.

Results: Total DNA (viable cells and relic DNA) analysis demonstrated that peaches have the highest amount of bacterial diversity followed by oranges, then apples. We observed significant shifts in overall bacterial community composition during the growing season regardless of fruit type. Additionally, the overall bacterial community composition for each tree fruit type was unique.

Significance: Among the viable communities, *Planococcaceae*, *Sphingomonas* spp., *Bacillus flexus,* and several other types of bacteria, were consistently present on all fruits, and these taxa represent potential surrogates for onsite validating tree fruit sanitizing systems.

P2-214 Microbial Quality of Peach Wash Water and Gloves Worn by Packers in Peach Packinghouses

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❖ ***Developing Scientist Entrant***

Introduction: Postharvest water quality and workers' hygiene are important factors to comply with the Produce Safety Rule under the Food Safety Modernization Act.

Purpose: This project evaluated the microbial quality of peach wash water and gloves worn by packers in selected peach packinghouses.

Methods: Incoming and outgoing peach wash water (one liter each), and gloves at manual sorting and packing areas (three pairs each) were collected from four peach packinghouses before packing started in the morning (AM), at lunch break (N) and the end of a packing day (PM). Each glove sample was rinsed with 100 ml phosphate buffered saline, followed by hand massage and stomaching. Incoming wash water (100 ml) was concentrated through membrane filtration while outgoing water and glove rinsate were plated directly to determine the levels of total aerobic bacteria, yeasts and molds and coliforms, as well as the presence of fecal coliforms and enterococci.

Results: The mean microbial loads in the outgoing water were significantly higher (P<0.05) than the incoming water. While the average total yeast and mold and coliform counts in the AM, N and PM water samples were similar (P>0.05), the aerobic counts in the AM samples were significantly higher than the PM samples. Approximately 43% of outgoing water samples tested positive for fecal coliforms and 29% for enterococci. The N and PM glove samples had similar mean total aerobic, yeast and mold, and coliform counts which were significantly higher than the clean AM glove samples. The mean microbial counts on gloves from sorting and packing areas were not significantly different (P>0.05). Approximately 38% of the glove samples tested positive for fecal coliforms and sven percent for enterococci.

Significance: The evaluated incoming wash water met the regulatory standard. The presence of fecal coliforms and enterococci on packers' gloves and in outgoing water indicates a potential risk of contamination on peaches.

P2-215 Assessment of the Efficacy of Rapid Tests on Predicting Bacterial Growth on Apple Packinghouse Equipment Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: The 2015 listeriosis caramel apple outbreak, linked to apples contaminated during packing, has increased the focus on the prevalence of pathogens within tree fruit packinghouses and the need for reliable rapid testing methodologies to quickly respond with recleaning during a sanitation event.

Purpose: To assess the prevalence of common microbial indicator species on zone 1 (food contact) surfaces in four apple packinghouses, compare prevalence between facilities, and assess utility and accuracy of frequently used rapid testing methods on zone 1 surfaces.

Methods: Food contact surfaces were sampled (n=156) over a 100 cm^2 area before processing to quantify *Enterobacteriaceae* spp., coliforms, generic *E. coli,* total aerobic bacteria, ATP and carbohydrate residue. The biological samples were stored in Dey Engley neutralizing buffer at 4°C for 24 h before plating on *Enterobacteriaceae*, coliform/*E. coli*, and APC Petrifilm plates. Rapid test results (carbohydrate residue (SpotCheck Plus) and ATP (UltraSnap)) were read immediately. A larger area (0.92 m^2) was sampled for the presence of *Listeria* spp., enriched in 90 ml Buffered Listeria Enrichment Broth and confirmed with PCR via the *iap* gene.

Results: No significant relationship was found between rapid tests and the number of *Enterobacteriaceae* spp., coliforms, generic *E. coli*, or total aerobic bacteria recovered. No samples were positive for *Listeria* spp. Recovery of total aerobic bacteria was significantly different at sites along the processing line (P=0.018). Recovery (CFU/100 cm^2) was higher at spray bar brushes (mean=3,255) than at dryer (999), dump tank (718), or sorter (484) sites but indistinguishable from packaging (1,735) or wax brush (1,227) sites.

Significance: The rapid tests were not closely linked to the recovery of common indicator species, suggesting that current methodologies are poorly suited for determining microbial load. This emphasizes the need to utilize both rapid tests which can facilitate response to residual soil load, along with frequent indicator organism testing to determine trends.

P2-216 The Use of Advanced Oxidation Process to Degrade Chlorpyrifos and Reduce Colonies of *Escherichia coli* O157:H7 on Apples

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❖ ***Developing Scientist Entrant***

Introduction: Pesticide residues and presence of pathogens such as *E. coli* O157 on apples remains a significant food safety issue. Specifically, there are concerns with chlorpyrifos, one of the most widely used insecticides in North America, which has been linked to detrimental neurological effects in children. There has been several foodborne illness outbreaks linked to *E. coli* O157:H7-contaminated apples. Consequently, risk controls are required to reduce the carriage of pesticides and pathogens on apple fruit.

Purpose: To validate an advanced oxidation process (AOP) on the degradation of chlorpyrifos and inactivation of *E. coli* O157:H7 on apples.

Methods: Response surface methodology utilizing a full factorial central composite design was used to validate AOP treatment with the effect of UV dose, temperature of hydrogen peroxide (H_2O_2) and concentration of H_2O_2 being assessed. Apple skins were spiked with 200 µg of chlorpyrifos then treated using one of 20 treatment combinations and suspended in 20 ml of acetonitrile. The residual chlorpyrifos was then quantified using HPLC. The optimized treatment was then assessed with regards to *E. coli* O157:H7 inactivation on the surface of apples.

Results: Samples (n=176) were analyzed using Design Expert® Software 11.1.0. It was determined that the extent of chlorpyrifos degradation was significantly (P<0.05) correlated to UV dose and hydrogen peroxide temperature. An optimal UV dose of 62.3 kJ/m^2 was shown to support the degradation of 120 µg (six ppm) whereas a UV dose of 11.7 kJ/m^2 reduced 62 µg (3.2 ppm). The optimal treatment of 62.3 kJ/m^2 UV-C and 1.22% hydrogen peroxide at 70°C could ensure reduction of chlorpyrifos and greater then six log CFU reduction of *E. coli* O157:H7.

Significance: The optimized AOP based process can be applied by apple and other fresh produce processors to decrease pesticides and inactivate pathogens as part of a risk control program.

P2-217 Attachment Strength of Foodborne Pathogens on Different Melon Varieties from Various Regions in the United States

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Introduction: Foodborne pathogens may contaminate melons during any production step from farm to fork. It is essential to understand the initial stages of foodborne pathogens attachment to melons so that effective mitigation strategies could be implemented post-harvest.

Purpose: The attachment strength of *Salmonella* Newport and *Listeria monocytogenes* on six different melon varieties grown in seven locations from six states (Arizona, Indiana, North Carolina, Georgia, Texas-Uvalde, Texas-Weslaco, and California) was investigated.

Methods: Melon discs were separately inoculated with overnight cultures of either *Salmonella* Newport or *L. monocytogenes* (approximately seven log CFU/ml) for 30 min. Inoculated discs were placed under a biohood for 30 min to allow the bacteria to attach on the rind surface. The samples were vortexed for 15 s to recover loosely attached bacterial cells and sonicated for two minutes to recover strongly attached bacterial cells. Samples were serially diluted and plated on xylose lysine desoxycholate (XLD) agar to enumerate *Salmonella* and on modified Oxford formulation (MOX) agar to enumerate *L. monocytogenes*. Attachment strength was calculated using the formula: Attachment strength= (strongly attached bacteria) / (loosely attached bacteria + strongly attached bacteria).

Results: Variety #2 (OC164) had the lowest average attachment strength for *Salmonella* (0.23). Variety #5 (HD252) had the highest average attachment strength for *Salmonella* (0.30). Variety #3 (HD150) had the lowest average attachment strength for *L. monocytogenes* (0.16). Variety #4 (Infinite Gold Sakata) had the highest average attachment strength for *L. monocytogenes* (0.26). In general, the attachment strength of *Salmonella* and *L. monocytogenes* on cantaloupes (0.27 to 0.28) was higher than on honeydews (0.23 to 0.30). *Salmonella* showed a stronger attachment to melon surfaces than *Listeria*.

Significance: The results could help determine what melon varieties may pose a food safety risk due to a stronger bacterial attachment in case of a contamination event from *S. enterica* and *L. monocytogenes*.

P2-218 Survival of *Salmonella* spp. on Cantaloupe Field Pack Food Contact Surfaces

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Introduction: Limited information on the survival of *Salmonella* on food contact surfaces during field packing operations exists.

Purpose: The survival of *Salmonella* on cantaloupe food contact surfaces, using two different inoculation methods, was evaluated.

Methods: Five food contact surfaces (cotton, nitrile, rubber gloves, cotton rags, and stainless steel) coupons were autoclaved (clean) or rubbed with a cantaloupe leaf for 20 s (fouled), and inoculated with a five-strain cocktail of *Salmonella* (six log CFU/ml or g). The wet inoculum was spot inoculated (100 µl) onto coupons and dried for one h. The dry inoculum was prepared by mixing aqueous inoculum with sterile sand and drying for 24 h at 40°C, then mixing coupon with 100 g of sand for two min. Coupons were held at 35°C (34% RH) for eight h. Samples were stomached for two min with 0.1% peptone (100 ml), except stainless steel, where a rub-shake-rub was used, and plated onto selective and non-selective media in triplicate experiments with duplicate replicates *(n*=6*).*

Results: Under all conditions, *Salmonella* population reductions following a wet inoculum were significantly higher (P<0.05) than those following a dry inoculation over eight h (iclean cotton gloves, 3.30 and 0.75 log reduction for wet and dry, respectively). The exception was fouled cotton gloves, wet (0.80-log reduction) and dry (1.32-log reduction). *Salmonella* population decline on clean surfaces, regardless of inoculum type or material, were greater than fouled surfaces over eight h. For example, reductions on dry rubber glove (1.25 and 0.33 log), and wet nitrile glove (3.02 and 1.0 log) for clean and fouled, respectively. The exception was cotton gloves, clean (0.75 log reduction) and fouled (1.32 log reduction). Population declines for all materials and cleanliness, ranged from 0.33-log reduction (dry fouled rubber gloves) to 3.3-log reduction (wet clean cotton gloves).

Significance: Inoculum type and surface cleanliness impacts the survival of *Salmonella* spp. on field pack food contact surfaces.

P2-219 Aqueous Chlorine Dioxide Inactivates *Salmonella* on Whole Papaya

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* may attach to the surface of papaya through soil, animal feces, and post-harvest handling. Chlorine dioxide (ClO_2) is a strong oxidizer and produces few carcinogenic byproducts. Washing papaya with ClO_2 may reduce the risk of *Salmonella* contamination.

Purpose: This study aimed to evaluate the efficacy of aqueous ClO_2 in reducing *Salmonella* on whole papaya.

Methods: *Salmonella* was spot inoculated on fresh green papayas. The bacteria were collected by excising the inoculated area and homogenizing in pH 7.0 buffer. Bacterial levels were determined by overlaying selective XLD agar on nonselective plate count agar after the bacteria being recovered for one h. Papayas inoculated with *Salmonella* were stored at 21 and 4 °C, and bacterial populations were enumerated at zero, one, seven, 10, and 14 d. ClO_2 was generated by mixing HCl or citric acid with $NaClO_2$. The release kinetics of ClO_2 were determined for 72 h. *Salmonella*-contaminated papayas were submerged in five and 10 ppm ClO_2 generated by HCl or citric acid with $NaClO_2$ for five min and then tested for bacterial levels.

Results: With 5.05 log CFU initial inoculum on green papayas, *Salmonella* grew by 2.13 log CFU at 21°C within 14 days, while it remained stable at 4°C. HCl and citric acid produced 780 ppm and 270 ppm of ClO_2, respectively, and the reactions were completed within 72 h. Compared to tap water treatment, five ppm of aqueous ClO_2 produced by HCl and citric acid reduced *Salmonella* by 0.49 and 0.02 log CFU, respectively. Ten ppm of ClO_2 produced by both acids achieved at least 4.51 log CFU reduction of *Salmonella* on papaya.

Significance: There is a great growth/survival potential if *Salmonella* contaminates papaya. ClO_2 offers an effective and environmentally friendly alternative to chlorine bleach for controlling *Salmonella* on papaya.

P2-220 Prevalence, Virulence and Antimicrobial Resistance of *Salmonella* Isolated from Mango "Ataulfo"

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Introduction: *Salmonella* is one of the mayor foodborne pathogens in the world, causing a number of outbreaks related to fresh produce. In 2012 a food outbreak linked to mangoes imported from Mexico occurred. In Mexico, information regarding the presence of the pathogen in mangoes at the point of sale and the characteristics of the isolated strains has not been reported.

Purpose: The main goal of this study was to detect, quantify and characterize *Salmonella* strains isolated from Ataulfo mangoes purchased in Queretaro, Mexico.

Methods: During July and August 2018, 60 samples of Ataulfo mangoes were collected from retail shops, supermarkets and markets located in Queretaro City, Mexico. The detection of *Salmonella* was conducted using the 3M Detection Molecular System and positive samples were confirmed by traditional method established in the Bacteriological Analytical Manual, and quantified by the most probable number technique. In the isolated *Salmonella* strains the presence of thirteen virulence genes (*hilA, agfA, orgA, sipA, sifA, sseF, sseL, invA, ssaQ, sspH1, sopE, spvC, pefA)* were conducted by four-multiplex PCR. In addition, their antimicrobial susceptibility to twelve antibiotics (amikacin, ampicilin, carbenzilin, cephalotin, cefoxitin, ciprofloxacin, cloramphenicol, gentamicin, netilmicin, nitrofuratoin, norfloxacine, trimetoprim/sulfamethoxazole) using the disk diffusion method of the Clinical and Laboratory Standards Institute were also evaluated.

Results: The pathogen was present in two (3.33%) of 60 samples and the average concentration was 11 MPN/mango. From positive samples 10 strains were isolated; all of them have the same virulotype *(agfA-orgA-sipA-sifA-sseF-sseL-invA-ssaQ-sspH1)* and only two had antimicrobial resistance to nitrofuratoin.

Significance: The data obtained in this study is important to establish the risk to the Mexican population associated with the consumption of fresh mango. Decontamination methods should be evaluated to control the presence of *Salmonella* in mango.

P2-221 Internalization of *Salmonella* spp. in Mangoes (*Mangifera indica*) Variety Tommy Atkins

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Introduction: In recent years, significant cases of salmonellosis caused by contaminated fruits were reported. Cross-contamination of fruit surfaces and post-harvest internalization of pathogens may result in increased risks to consumers.

Purpose: This study investigated the internalization capacity of *Salmonella* spp. in mangoes variety Tommy Atkins, under different experimental conditions.

Methods: Mature mangoes were pre-evaluated for absence of *Salmonella* spp. on the surface using the swab technique. *Salmonella* negative fruits were challenged with *Salmonella typhymurium* ATCC 14028 *and Salmonella enteritidis* ATCC 13076 and their internalization was evaluated after spot contamination of the peduncle region (six log CFU/ml) and after immersion in contaminated cooling water (six log CFU/ml- 21.1°C for 10 min) of fruits submitted to the classical "hot water treatment" (46.1°C for 90min). Mangoes were kept up to 10 days under refrigeration (8°C) and at controlled room temperature (25°C). Counts of *Salmonella* were performed after 24 h, five and 10 days, in three regions of the challenged fruits: stem scar "SS", middle side "MS" and blossom end "BE").

Results: Internalization of *Salmonella* spp. was detected after 24 h, specially on the stem scar area. On the 10th day at 8°C, the counts (log CFU/g±sd) in surface inoculated fruits were SS=3.9±0.2; MS=2.8±0.1; BE=2.1±0.1. For fruits at 25°C, the counts were SS=6.2±0.2; MS=5.8±0.1; BE=4.2±0.2. Fruits immersed in contaminated water presented the following counts on the 10th day at 8°C: SS=4.3±0.3; MS=3.5±0.3; BE=3.1±0.1 and at 25°C: SS=4.5±0.1; MS=4.0±0.1; BE=3.5±0.2.

Significance: The findings indicate that *Salmonella* spp. is able to internalize and spread through the pulp of mangoes. These data may help producers and health agencies to develop quantitative risk assessments and establish proper measures to prevent outbreaks.

P2-222 *Listeria* Contamination and Identification of Potential Growth Niches in a Ready-Meal Manufacturing Small- and Medium- sized Enterprise: A Case Study

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Introduction: Growth niches are locations which still harbour the microorganism after the completion of routine sanitation in the area. In order to comply with applicable regulations and also adhere to safety standards, the manufacturer is required to document the identification and successful control of *L. monocytogenes* through their environmental monitoring programme.

Purpose: This study aims to evaluate *Listeria* spp. contamination and identify potential growth niches in a ready-meal manufacturing small and medium sized enterprise.

Methods: Microbiological laboratory reports from 2012 to 2018 were reviewed to determine *Listeria* spp. contamination of food products due to potential growth niches in a ready-meal manufacturing business. In total 4401 test results were analysed including finished products (*n*=2548), raw materials (*n*=136), and environmental samples (*n*=1717). In conjunction with laboratory results, cleaning reports were examined in order to isolate final product contamination data attributed to potential growth niches. Results attributed to factors unrelated to environmental contamination were disregarded.

Results: A total of 14 environmental *Listeria* spp. positives, including one *L. monocytogenes*, resulted in the identification of two distinct growth niches during two separate time intervals. Analysis indicated such growth niches were potentially attributed to an uncleanable location and a damaged floor, with

periods of two and six months, respectively, elapsing until an increase in unattributed *Listeria* positive samples led to investigative actions and mitigation. Three *Listeria* spp. finished product positives were reported prior to the mitigation of the first niche compared to none for the second one.

Significance: The case study highlights finished product contamination via potential growth niches attributed to two different root causes, showing the impact over the finished product's microbiological quality and the need for reliable and effective strategies to identify and mitigate growth niches.

P2-223 Stress and Sanitizer Resistance Characterization of Persistent and Transient *Listeria monocytogenes* Isolates from a Cold-Smoked Salmon Processing Facility

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* isolates can persist in food processing facilities over a long period of time (>10 years). Persistent isolates may harbor genetic features that allow for survival and growth in the food processing environments, such as sanitizer resistance genes.

Purpose: Assess the phenotypic differences across persistent and transient *L. monocytogenes* isolates collected from a cold-smoked salmon processing facility (CSSPF) as part of academic research projects.

Methods: WGS was performed on 42 *L. monocytogenes* isolates with the same ribotype collected over a period of 18 years from a CSSPF. Phylogenetic clustering was established using a k-mer based approach. Isolates were screened for the presence of sanitizer and stress resistance genes. Selected isolates were further assessed for growth at low concentration of four quaternary ammonium compound (QAC) sanitizers, under five stress conditions (15°C, 40°C, 0.95 a_w, 6% NaCl, pH 5.5), and for survival under oxidative stress.

Results: Isolates fell into three clusters. The sanitizer resistance gene *bcrABC* was found among all cluster 3 (*n*=33) and cluster 2 (*n*=2) isolates while the sanitizer resistance gene *qacH* was only found in one isolate from cluster 1. Stress survival islets 1 (SSI-1) and 2 (SSI-2) were only found among cluster 3 and 1 isolates (*n*=6), respectively. Isolates carrying *qacH* or *bcrABC* showed reduced sensitivity to QAC sanitizers. SSI-1 and SSI-2 did not confer growth or survival advantage under different stresses when compared with isolates lacking these islets.

Significance: Isolates with the same ribotype and collected from the same CSSPF showed diverse genetic features associated with sanitizer and stress resistance. Sanitizer resistance genes *qacH* and *bcrABC* conferred a growth advantage when exposed to low sanitizer concentrations but the presence of stress resistance genes may not be used as predictors of an isolate's stress survival ability.

P2-224 Blue Light Exposure Kills *Escherichia coli* Cells Treated at Close Range and May Enhance Microgreen Food Safety

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Introduction: Blue light in the range of 400 to 470 nm has been demonstrated to have antimicrobial effects and is also important for vegetative plant growth and enhanced production of various phytonutrients in specific plant species during cultivation.

Purpose: The current study investigated the use of blue light to inactivate three strains of *E. coli* (MW423, MW416, and MW425) known to grow well on plants.

Methods: *E. coli* cultures grown overnight in tryptic soy broth (TSB) with nalidixic acid were resuspended in PBS. The inoculum was placed in four identical glass petri dishes each containing a mini-stir bar. Each petri dish was placed on a stir plate under a lamp with 400 nm, 460 nm, 460 nm + 600 nm, or held in the dark. The inoculum was sampled from each dish and plated on TSA and MacConkey agar at intervals for up to 24 hours.

Results: In this study, over the course of six trials, 400 and 460 nm blue light were shown to injure and kill *E. coli* cells in PBS at close range. Of three strains tested on TSA using 400 nm light, MW423 (three trials) was reduced by four log within seven hours, MW416 (two trials) was undetectable within eight hours, and MW425 (one trial) was undetectable within six hours. The reductions were considerably less for 460 nm light treatment but still significant compared to the dark control. During each trial the inoculum was sampled at six to 10 time points.

Significance: This study suggested that blue light could be an intervention to reduce bacterial populations on microgreens grown from inoculated seed. Optimization of this intervention could provide a means of reducing food safety risks related to microgreens while simultaneously enhancing food quality.

P2-225 Effects of Switchgrass Fast Pyrolysis Biochar Generation Temperature on Survival of *E. coli* O157:H7 in Soil

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Introduction: Previous studies have demonstrated that biochar can inactivate bacterial pathogens in soil. The antimicrobial efficacy of slow- or fast-pyrolysis generated biochar varies based on biofeedstock, processing temperature and residence time.

Purpose: A study was conducted to determine the biocidal activity of fast-pyrolysis biochar processed at varying temperatures in a newly-constructed pyrolyzer.

Methods: Switchgrass biochar was generated in a newly-constructed fast-pyrolysis reactor at three temperatures (450, 500 and 600°C), and added to a simulated crop soil at concentrations of zero, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5%, respectively. Biochar-amended soil was then inoculated with ca. 7.6 log CFU/g of a three-strain composite of non-toxigenic *E. coli* O157:H7 and held for seven weeks at 22°C in polypropylene bottles. Bottles were rotated on bottle rollers prior to each sampling period to evenly distribute inocula. The second set of ten slow pyrolysis biochars were treated as described above except that biochar was added to the soil at a 10% concentration and one-g samples were analyzed weekly over four weeks. Three independent replicate experiments were conducted.

Results: Pathogen populations in fast-pyrolysis biochar-amended soil (450 and 500°C biochars) did not decline when compared with the zero percent biochar control over seven weeks. However, when soil was amended with fast-pyrolysis biochar generated at 600°C, pathogen populations after only one week of storage were (biochar concentrations in parentheses) 6.93 log CFU/g (zero percent), 5.89 log (one percent), 3.80 log (1.5%), 3.78 log (two percent), 3.25 log (2.5%), 1.46 (three percent), and 0.00 (3.5%). Only one of ten slow-pyrolysis generated biochars reduced pathogen populations over four weeks (oilseed rape straw pellets generated at 700°C), with a difference of only 1.37 log CFU/g.

Significance: These results indicate that fast-pyrolysis biochar processed in the newly-built pyrolyzer can generate biochar with biocidal activity against bacterial pathogens in the soil when generated at 600°C.

Poster

P2-226 Survival of Desiccation-resistant *Salmonella* on Apple Slices after Dehydration and Following Antimicrobial Immersion Treatments

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Introduction: *Salmonella* is capable of surviving dehydration processes of various foods including dried fruit. Dried fruit, including apple slices, have been the subject of recalls due to possible contamination with *Salmonella*.

Purpose: A study was conducted to determine the survival of *Salmonella* on apple slices of six varieties after dehydration as well as survival following antimicrobial immersion treatments and dehydration.

Methods: Six varieties of apples (Envy, Gala, Red Delicious, Fuji, Pink Lady, Granny Smith) were cored and sliced into 0.4-cm rings, inoculated with a five-strain composite of desiccation-resistant *Salmonella* and dehydrated at 60°C for five hours. Subsequently, Gala apple slices were treated in 0.5% solutions of one of eight antimicrobial rinses for two minutes and then dehydrated at 60°C for five hours. Antimicrobial solutions were potassium sorbate (PS), sodium benzoate (SB), ascorbic acid (AA), propionic acid (PA), lactic acid (LA), citric acid (CA), fumaric acid (FA), and sodium acid sulfate (SAS).

Results: Reduction of *Salmonella* populations varied according to apple variety. Inactivation rates (log CFU) were Envy (1.69), Gala (2.09), Red Delicious (2.77), Fuji (2.93), Pink Lady (3.15), and Granny Smith (3.77). Greater numbers of *Salmonella* ($P<0.05$) were inactivated on Granny Smith, Pink Lady and Fuji apples than on Gala and Envy apples. Survival of *Salmonella* on Gala apple slices (log CFU) following antimicrobial treatments and dehydration were untreated control (5.58), PS (4.76), SB (3.90), AA (3.29), PA (3.13), LA (2.89), CA (2.83), FA (1.76), and SAS (0.0). Lower survival rates were achieved by pretreating apple slices with either FA or SAS before dehydration than all other antimicrobial treatments.

Significance: These results may provide a means for increasing the inactivation of *Salmonella* during the dehydration of apple slices applicable to the food industry.

P2-227 Prevalence and Distribution of *Listeria monocytogenes* in Public Watersheds of the Central California Coast Near Leafy Green Growing Areas from 2011 to 2016

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Introduction: Several outbreaks and recalls of produce have been attributed to contamination by *Listeria monocytogenes*. Avenues of pre-harvest contamination include water, livestock, and wildlife. Public access waterways may be a central reservoir for *L. monocytogenes* contamination.

Purpose: We report the results of a five-year study (2011 to 2016) on the prevalence and ecology of *L. monocytogens* in public access watersheds along the Central California coast.

Methods: Between December 2011 and September 2016, 2,922 Moore swabs were collected bimonthly from 30 sites in lakes, streams, ponds, and rivers over a 500 square mile region, and enriched for *L. monocytogenes*. After immuno-magnetic separation (IMS) of the non-selective enrichment and both direct plating of IMS beads and secondary enrichment in Fraser broth, final plating was done on Brilliance *Listeria* Agar. PCR targeting the *hlyA* gene was used to confirm presumptive positives, and all strains were serotyped, and whole genome sequencing (WGS) is currently being done.

Results: Of the 2,922 Moore swabs, 42.5% were positive for *L. monocytogenes.* An isolate was collected from each positive sample, and with multiple isolates in some samples, the total number of isolates was 1,376. Prevalence varied by season with higher prevalence in winter/spring than in summer/fall. The most common serotypes recovered were 4b (1143 isolates), 1/2a (95 isolates), and 1/2b (83 isolates). Core genome MLST (cgMLST) analysis is in progress. Sequence types recovered include established epidemic, clonal groups, CC1 and CC2, CC4 (an emerging hypervirulent strain), Sequence Types (e.g. ST382, CC5) reported in previous outbreaks, and novel types not yet described.

Significance: The data indicate the *L. monocytogenes* is widespread and persistent in the watersheds of this region, and that the waters could serve as a source of preharvest contamination.

P2-228 Establishing Baseline Inhibition of *Escherichia coli* in Aqueous Dairy Manure Prior to Treatment by a Fungal Biocontrol Agent

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❖ ***Developing Scientist Entrant***

Introduction: Biocontrol strategies should be explored as preharvest mitigation steps for microbial reduction in soil amendments of animal origin. It is essential to establish survival of *Escherichia coli* in liquid manure before assessment of biocontrol strategies, including white-rot fungi.

Purpose: This study evaluated the persistence of *E. coli* and *E. coli* O157:H7 in liquid dairy manure, before land application, to address produce safety concerns.

Methods: *E. coli* (TVS355) and *E. coli* O157:H7 (4407) were inoculated into liquid dairy manure, obtained locally, at four and eight log CFU/ml, incubated at 37 or 22°C, and sampled at zero, 24, 48 and 72 h. Bacteria were enumerated on MacConkey agar with rifampicin (MACR) or nalidixic Acid (MACN). Bacterial survival was evaluated using a one-way ANOVA across 10 trials, with $n=4$ per treatment. *Phanerochaete chrysosporium* and *Pleurotus ostreatus* were obtained locally and maintained on one percent malt agar at 37 or 25°C.

Results: Liquid manure remained at pH 8.0 to 8.2 across the sampling time, with a consistent water activity of 0.99 and a C:N of 4.6%. In all studies *E. coli* did not grow in manure; however, greater dieoff was observed by *E. coli* O157:H7 compared to *E. coli* ($P>0.05$). Regardless of inoculum level, *E. coli* O157:H7 decreased by 0.71 log CFU/ml from zero to 24 h and by 1.46 to 1.57 log CFU/ml from 24 to 48 h. Comparatively, *E. coli* decreased by 0.21 to 0.19 log CFU/ml from zero to 24 h and by 0.46 to 1.11 log CFU/ml from 24 to 48 h. *E. coli* O157:H7 was not detected at 72 h, but *E. coli* survived in liquid manure at 72 h at 6.6 log CFU/ml for higher inoculum and 2.3 log CFU/ml for the lower inoculum. In the presence of *P. ostreatus*, *E. coli* at the higher inoculum was reduced by greater than four log at 72 h.

Significance: While *E. coli* survives in manure, reduction may be increased by application of *P. ostreatus* and deserves further attention.

Poster

P2-229 Bioremediation Practices to Reduce Human Pathogen Contamination for Agricultural Soils

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Introduction: Currently there are no sustainable and effective bioremediation practices capable of reducing or eliminating the presence of enteric pathogens in agricultural soils.

Purpose: To develop bioremediation practices that can reduce *Salmonella and Listeria* spp. contamination from organic and conventional farm land.

Methods: Organic and conventional soil (series Wickham) were used in all evaluations. At each location a randomized complete block design was established consisting of four melon (cv Athena) blocks and six treatments consisting of a control (soil), muscadine pomace and mushroom compost, bisulfate of soda (SAS), and a cover crop (mustard). Soils were drench inoculated (two liters) with an avirulent rifampicin resistant *Salmonella* Typhimurium at five log CFU/ml. Recovery of *Salmonella* Typhimurium and naturally occurring *Listeria* spp. was performed at zero, seven, 14, 35, 77, 81 (SAS application – cover crop), 84 and 112 d post-inoculation. *Salmonella* and *Listeria* spp. were confirmed by probe-based PCR.

Results: In both organic and conventional systems after 35 d post-inoculation there was a five-log reduction in the population of *Salmonella* Typhimurium after the application of SAS in all treatments. However, in organic systems *Salmonella* Typhimurium after seven d post-inoculation was below the limit of detection (1.08 log CFU/g) in the control and mushroom compost treatments when compared with the same treatments in conventional soils (remaining population 1.85±0.54 log CFU/g) ($P>0.05$). In both systems faster dieoff was observed in the control after four d post-inoculation when compared to other treatments ($P>0.05$). Cover crop used alone reduced the population of *Salmonella* by 1.5±0.02 log CFU/150 g after 35 d post-inoculation. The application of SAS to all treatments reduced the population of *Listeria* spp. by 92 and 69% at four and seven d post-inoculation.

Significance: Bisulfate of soda was able to generate a five-log reduction in the populations of *Salmonella* Typhimurium after 35 d post-inoculation. The use of compost and a mustard cover crop marginally reduced the population of *Salmonella* Typhimurium.

P2-230 Effects of Manuring on Survival of *E. coli* in Certified Organic Field Soils and Transfer to Fresh Produce in the Delmarva Region

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❖ ***Developing Scientist Entrant***

Introduction: Key data are needed to ensure safe production of fresh produce in certified organically-managed crop systems using untreated animal manure. Current National Organic Program (NOP) standards stipulating a 90 to 120 day waiting period between manuring and crop harvest remain a concern.

Purpose: To evaluate *Escherichia coli* survival in soil and transfer to fresh produce harvested 90 to 120 d after application of raw manure to certified NOP field soil.

Methods: From 2017 to 2018, certified NOP silty-loam field plots were amended with dairy manurev(DM), horse manure (HM), poultry litter (PL), or left unamended (UnA), before spray-inoculating with a mixture of rifampicin-resistant *E. coli* ($EC^{rif\text{-}R}$) at six log CFU/ml. Composite core samples were enumerated (MPN) at d zero, 30, 60, 90, and 120 post-inoculation, with harvested produce enrichments on d 90 and 120.

Results: In radish soils, $EC^{rif\text{-}R}$ populations declined from 100% positive ($n=32$) on d zero to zero to 20% positive for all treatments by d 90 to 120 ($n=64$). However, there was 100% persistence on bulbs for all 2017 treatments. In 2018 PL and DM amendments were 50% positive ($n=16$) for transfer of $EC^{rif\text{-}R}$ to 120 d bulbs. For tomato soils, $EC^{rif\text{-}R}$ populations were 100% positive ($n=32$) at d zero (2017, 2018). They declined to 20% for all manured treatments by d 90. However, 30 and 40% were positive by d 120 in DM and PL 2017 plots, respectively. Army worm-frass resulted in 100% contamination of all tomato fruits ($n=32$). In 2018, $EC^{rif\text{-}R}$ declined to 10-20% by d 90 to 120 with no transfer to tomato fruits. Spinach soil $EC^{rif\text{-}R}$ populations varied substantially during 2017 to 2018 with 100% transfer to harvested 30-g samples per plot ($n=32$) in 2017; the 2018 crop failed.

Significance: These first *E. coli* survival data for certified-NOP coastal Delmarva soils and produce will aid risk-based assessments of manure application-to-harvest wait-times for fresh produce safety. This study paves the way for future evaluations of microbiological and physicochemical factors contributing to organic food safety practices.

P2-231 Strain, Soil Type, and Irrigation Regime Influence *Salmonella* Survival in Poultry Litter Amended Sandy and Clay Soils

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Introduction: Data is needed on pathogen dynamics in amended soils to assess risk and inform policy for the application of untreated biological soil amendments of animal origin (BSAAO) for growing produce.

Purpose: This study investigated the variability in growth and survival of 12 *Salmonella* strains in poultry litter (PL) amended sandy-loam (SL) and clay-loam (CL) soils.

Methods: Greenhouse experiments were arranged in a randomized complete block design totaling 90 pots (three replicates×12 strains getting daily H_2O+three strains getting weekly H_2O)×two soil types). Each strain (four to five log CFU/g) was homogenized with each soil type and PL. Soil moisture was maintained for all 12 strains via daily irrigation, while three strains were additionally selected for weekly irrigation. Soil was sampled at zero, 0.167, 1, 2, 4, 7, 14, 21, 28, 56, 84, 112, 168, 210, 252, 336, 392, and 430 d post-inoculation, or until after two consecutive negative samples by 25-g enrichment. Population levels were enumerated on selective agar. MPN was used to detect lower levels (-0.6 log MPN/g). Significance was analyzed using Tukey's HSD ($P<0.05$).

Results: While all 12 *Salmonella* strains had variable growth and survival curves, generally, an initial decrease in *Salmonella* populations was observed within four h, followed by growth of one to three log CFU/g, and then a population decline starting at four to 10 d. Most strains survived 112 and 168 d in SL and CL soils amended with PL, respectively. Additionally, *Salmonella* populations exposed to weekly irrigation exhibited extended survival (average of 252 d), compared to the same strains exposed to daily irrigation (average of 112 d).

Significance: PL supported population growth and increased survival time of *Salmonella* in soil. This study characterized strain variability and the influences of soil-type and irrigation regime on the population dynamics of *Salmonella*. Data can assist in risk assessment and identifying best practices for the application of BSAAO to produce fields.

Poster

P2-232 A Longitudinal Study Using 16s rRNA Gene Sequence Analysis of Soil Amended or Unamended with Heat-treated Poultry Pellets Contaminated with *Salmonella* Newport

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Introduction: *Salmonella* Newport is a foodborne pathogen frequently associated with outbreaks in produce. Several factors, such as the use of manure, temperature, and soil moisture have been shown to impact *Salmonella* survival in soil.

Purpose: The purpose of this study was to investigate soil microbiomes by 16s rRNA amplicon sequencing, over a two-month period, in heat-treated poultry pellets (HTPP)-amended or unamended soils contaminated with and without *Salmonella* Newport.

Methods: *Salmonella* Newport was inoculated at ~7.5 log CFU per gram dry weight into a total of six planters, three containing HTPP-amended soil and three without amendment. Six similar planters were also set up without *Salmonella* Newport inoculation. Soil samples were collected in duplicate from each planter on days zero, 21, 35, and 63, and six replicate samples from bulk and rhizosphere soil were also collected at days 35 and 63. 16s rRNA microbiome sequencing was performed on extracted metagenomic DNA.

Results: Amended and unamended soils harbored very diverse microbiomes, with >1,500 taxa identified over all samples. The addition of HTPP introduced, or added certain taxa; namely, *Pseudomonas* (*P. entomophila* and *P. fuscovaginae*) and *Bacilli* (such as *B. aryabhattai*, *B. asahii*, *B. arbutinivorans*, and *Rummeliibacillus stabekisii*) to the soil. Some taxa remained constant during the 63 days of the study, such as *Granulicella tundricola*, and members of the *Actinomycetales* and the *Rhodoplanes*, while others increased in proportional abundances, such as *Niastella*, *Devosia*, *Brevundimonas* spp., and *Sphingobacterium multivorum. Salmonella* persisted longer in amended soils, ranging from 5% relative abundance on day 0 to 0.1% on day 63. In unamended soils, *Salmonella* was undetected after day 21. *Salmonella* was also detected in rhizosphere soil at day 35 but declined to undetectable levels by day 63.

Significance: The soil amendment resulted in an increase in abundance of some members of the microbiome without an overall impact on the diversity and promoted the survival of *Salmonella*.

P2-233 Factors Affecting *Salmonella* Newport Survival in Soil and Subsequent Transfer to Spinach Plants

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❖ ***Developing Scientist Entrant***

Introduction: Biological soil amendments such as untreated poultry litter can harbor pathogens like *Salmonella* leading to potential transfer from soils to produce. Heat-treated poultry pellets (HTPP) can provide produce growers with a slow release fertilizer with a minimized risk. However, the impact of HTTP-amendment on the survival of *Salmonella* in soil is unknown.

Purpose: The purpose of this experiment was to assess the impact of HTPP as an amendment on the survival of *Salmonella* Newport in the soil, and the contribution of factors, such as irrigation, presence/absence of spinach plants, *rpoS* gene, and potential formation of viable but non-culturable cells on survival. We also determined the populations of *Salmonella* Newport surviving on spinach leaves after simulated transfer.

Methods: *Salmonella* Newport wild-type (WT) and *rpoS*-deficient (*ΔrpoS*) strains were drip-inoculated in HTPP-amended and unamended soil in planters and spinach seeds were sown. Survival of *Salmonella* Newport was quantified by culture method and PMA-qPCR over 91 days in three experimental replicates. A simulated 'splash' transfer of *Salmonella* Newport from contaminated soil to nine spinach plants were evaluated at 35 and 63 days post-inoculation (dpi) and survival on spinach was quantified for 48 h.

Results: *Salmonella* Newport WT and *ΔrpoS* reached the limit of detection (one log CFU/g) in unamended soil at 35 dpi, whereas two to four log CFU/g was observed for both strains at 91 dpi in HTPP-amended soil. No significant differences in survival were observed between the strains. Cell densities determined by PMA-qPCR and culture methods were similar *(P*>0.05*),* as was survival in soils with/without spinach plants. Higher populations of *Salmonella* Newport from HTTP-amended soil transferred to and survived on spinach leaves than that from unamended soils (*P*<0.05).

Significance: HTTP-amended soils provided a favorable environment for *Salmonella* Newport survival in soils and transfer to spinach plants when introduced via irrigation during spinach cultivation.

P2-234 Serotypes and Antimicrobial Resistance of *Salmonella* Recovered from Chicken Litter in Florida Operations

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❖ ***Developing Scientist Entrant***

Introduction: The development of antimicrobial resistance (AMR) in foodborne pathogens is a major public health concern, and identifying the *Salmonella* serovars present in chicken litter and their AMR profiles may aid in assessing the risk of this biological soil amendment of animal origin (BSAAO).

Purpose: The purpose of this study was to serotype and develop AMR profiles for *Salmonella* recovered from chicken litter in Florida.

Methods: *Salmonella* isolates (*n*=47) recovered from raw chicken litter were sent to the USDA National Veterinary Services Laboratories for serotyping. Antimicrobial susceptibility testing (AST) was performed using a microbroth dilution method. Minimum inhibitory concentrations (MICs) were determined for a panel of 14 antimicrobial agents: amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, meropenem, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/ sulfamethoxazole. MICs were determined using the Sensititre National Antimicrobial Resistance Monitoring System (NARMS) Gram-negative panel (product code: YCMV4AGNF; Remel, Lenexa, KS).

Results: Twelve unique *Salmonella* serovars were identified with the most common serovars being *Salmonella* Typhimurium (13), Kentucky (eight), Enteritidis (seven), and Mbandaka (seven). AST demonstrated that 23 (49%) of 47 isolates were sensitive to all antimicrobials tested, while the remaining 24 (51%) of isolates showed intermediate sensitivity or resistance to at least one antimicrobial agent. The only resistances identified were to tetracycline (14 isolates), sulfisoxazole (11 isolates), and streptomycin (seven isolates). Resistances to two antimicrobials were observed in 14 (30%) of 47 isolates and none were resistant to more than two antimicrobials. Of the 14 multidrug-resistant isolates found, 11 of the 13 *Salmonella* Typhimurium were resistant to sulfisoxazole and tetracycline, and three of eight Kentucky were resistant to streptomycin and tetracycline.

Significance: This data will provide information for risk assessments of chicken litter as a BSAAO used in the production of fresh fruits and vegetables.

Poster

P2-235 Survival of *Escherichia coli* O157 Recovered from Bovine Manure in Autoclaved and Unautoclaved Florida Sandy Soil

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❖ ***Developing Scientist Entrant***

Introduction: The influence of soil microbiome on *Escherichia coli* O157 survival is not well understood, which makes setting an application interval for untreated biological soil amendments of animal origin difficult.

Purpose: The objective of this research was to determine the survival of *E. coli* O157 strains with different *stx* profiles in unautoclaved and autoclaved sandy soil from Florida.

Methods: Soil was sieved, and autoclaved twice for one h. Soil moisture was adjusted to a 10% volumetric water content. Triplicate, one-kg mesocosms were individually inoculated with rifampicin-resistant *E. coli* O157 strains (*stx1*+*stx2*+, *stx1*-*stx2*+, and *stx1*-*stx2*-) isolated from bovine manure in Florida. Mesocosms were incubated at 30°C, and subsamples were analyzed on day zero, one, three, seven, 14, 28, and monthly (until extinction) to determine death rates (significance between soils determined with Welch's two sample t-test (*P*= 0.05). Soil (10 g) was added to 90 ml peptone water, stomached, diluted and plated on tryptic soy agar (TSA), and TSA was supplemented with rifampicin (80 ppm) for inoculated mesocosms. Dynamics of microbial populations in soils was determined by 16S rRNA gene-based metagenomic analysis.

Results: Microbial population levels for unautoclaved and autoclaved soils ranged from 5.9 to 7.6 log CFU g^{-1}. The average linear death rate (log CFU g^{-1} day^{-1}) among all *E. coli* O157 strains was significantly higher in unautoclaved (0.18) versus autoclaved (0.10) soil (*P*=0.0084), and similar death rates (log CFU g^{-1} day^{-1}) were observed between *stx1*+*stx2*+, *stx1*-*stx2*+, and *stx1*-*stx2*- strains in unautoclaved (0.20, 0.16, and 0.19) and autoclaved (0.09, 0.11, and 0.09) soils, respectively. Microbial populations in soils shifted over time with a higher abundance of *Actinobacteria* and *Acidobacteria* observed in unautoclaved soils, and a predominance of *Firmicutes* and *Proteobacteria* in autoclaved soils.

Significance: This study provides insights on *E. coli* O157 survival in sandy soil based on natural microbial populations.

P2-236 Bacterial Survival as a Factor of Variation in Extrinsic and Intrinsic Soil Parameters with Biological Soil Amendments of Animal Origin

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Introduction: Complex parameters within soil may affect pathogen survival in conjunction with application of Biological Soil Amendments of Animal Origin (BSAAO), which are used to enhance soil nutrients and moisture, and may contribute to contamination.

Purpose: Extrinsic and intrinsic factors of soils amended with BSAAO were evaluated for their influence on *E. coli* survival.

Methods: Four different amendments were applied to twenty plots ($3m^2$), composite soil samples collected every 10 days (n=234) and evaluated for *E. coli* along with soil extrinsic (temperature, soil moisture) and intrinsic factors (conductivity, soluble carbon). Amendments included poultry litter (PL), heat-treated poultry litter pellets (HTPP), composted PL (CPL), or unamended inorganic fertilizer (UN). Plots were spray-inoculated with *E. coli* TVS 355 (1L of 10^6 CFU/ml applied per plot). Data were analyzed using one-way ANOVA (P<0.05).

Results: Average values for soil moisture (15±4.0%), temperature (27.2±7.0ºC) and solids content (85±4.0%) did not vary significantly by amendment type over the 120-day trial. Significant (P<0.05) decreases in soil conductivity (1.5 to 0.1 mmhos/cm) and soluble carbon (1984.4 to 103.2 mg/kg) levels were observed between days 0 and day 120 across all amendment types. At day 60, PL and HTPP plots had higher soluble carbon levels (175.6 and 171.3 mg/kg) and also supported *E. coli* survival for longer durations at higher populations (2.4-5.3 logs CFU/g dry weight) compared to CPL and UN plots (<0.4 MPN logs) with lower soluble carbon levels (95.3 and 76.8 mg/kg), suggesting this factor could be critical for bacterial survival in soil.

Significance: Identifying and measuring soil parameters may clarify their role as potential indicators of pathogen survival in manure-amended soils. Results suggest soluble carbon could be a critical factor to understanding potential for contamination.

P2-237 Application of Rhizobacteria as a Biocontrol by Tackling Plant-Pathogen Interactions

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Introduction: *Salmonella* contamination of plants can involve leaf colonization and internalization, depending on flagellar and virulence genes. Preharvest control is difficult, making rhizobacteria that disrupt *Salmonella* persistence a potential biocontrol.

Purpose: Evaluate the role of specific *Salmonella* traits in lettuce leaf colonization and internalization and the subsequent effects of rhizobacteria as a biocontrol.

Methods: Roots of lettuce plants **(***Latuca sativa*) were inoculated with live cultures of *Bacillus subtilis* UD1022 (eight log CFU/ml) and maintained in a biogrowth chamber for 48 h. Plant leaves were inoculated (nine log CFU/ml) with *Salmonella* Typhimurium (ATCC 14028s) or its mutants: Δ*invA* (*Salmonella* Pathogenicity Island-1; SPI-1), Δ*hilD* (SPI-1 transcriptional regulator), Δ*sseB* (SPI-2), Δ*fliC* and Δ*fljB* (flagellin subunits), and control *Salmonella* Newport. Leaf samples were collected on days zero, one, three, five, and seven, and processed to enumerate populations, internalization, stomatal closure (width of apertures), and plant immune response (semi-quantitative PCR). Data were analyzed using one-way ANOVA (*P*<0.05).

Results: *Salmonella* populations were significantly (*P*<0.05) reduced in all UD1022 treated groups by d seven except Δ*fliB* and Δ*invA*. The Δ*fliB* and Δ*invA* showed similar survival (4.3 and 2.7 log CFU/g, respectively) to their untreated-control groups. Other mutants Δ*fliC,* Δ*hilD,* and Δ*sseB* were reduced to undetectable levels in UD1022 treated plants compared to controls, by d three. *Salmonella* Typhimurium and Newport populations decreased (2.2 to 2.8 log CFU/g) significantly (*P*<0.05) in UD1022 treated compared to untreated plants. *Salmonella* internalization was not detected in plants after UD1022 treatment and had significantly (*P*<0.05) higher stomatal closure rate (aperture width=2.34µm) by d one, compared to controls (8.5 µm). However, Δ*hilD* and Δ*sseB* mutants were not internalized in both treated and untreated controls (means=1.49 and 1.26µm), suggesting that these genes are vital for *Salmonella* internalization in lettuce.

Significance: *Salmonella* survival on plants is complex, involving flagella. Biocontrol UD1022 reduces *Salmonella* populations and internalization suggesting its utilization as a preharvest biocontrol.

Poster

P2-238 Estimating *Salmonella* and *Campylobacter* Cell Density in Animal Feces and Their Potential to Lead to Significant Contamination Events

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Introduction: Significant foodborne outbreaks have been linked to the presence of wildlife and/or domesticated species in and around agricultural environments. Despite these linkages, there is little information about the likelihood of pathogens to be associated with certain species nor about the load that may be present in feces.

Purpose: To estimate the quantity of *Salmonella* and/or *Campylobacter* in animal feces to identify species and/or individual animals that could contribute to the contamination of agricultural products.

Methods: Dropped feces were identified and collected as part of wildlife research looking at the intersection of free-range cattle, mule deer, and rocky mountain elk in southeastern Oregon (*n*=27 per species). Total DNA was extracted using a modification of the Qiagen DNeasy Blood and Tissue kit. DNA extractions served as the template for two real-time SYBR green PCR assays targeting the *inv*A gene of *Salmonella* (invA_176F 5'-CAACGTTTCCTGCGGTACTGT; invA_291R CCCGAACGTGGCGATAATT)and the 16S rRNA gene of *Campylobacter*(R-camp-F2 CACGTGCTACAATGGCATAT; R-camp-RS GGCTTCATGCTCTGCAGTT). Ct values were compared to a standard curve to estimate cell density in the original fecal sample (detection limit ~3.7 log CFU/g).

Results: Cattle displayed the highest *Campylobacter* prevalence with 10 (37%) of 27 samples, followed by deer and elk, with seven (26%) of 27 for each. *Campylobacter* levels were estimated at between 3.8 and 8.8 log CFU/g of feces. *Salmonella* was far less prevalent in these animal feces, in five (six percent) of 81, and was the most prevalent in rocky mountain elk at three (11%) of 27, with levels ranging from 4.4 to 5.9 log CFU/g.

Significance: These results confirm that cattle, deer, and elk can be significant carriers of *Campylobacter* and *Salmonella;* however, various species and individual animals differ in their potential for contaminating agricultural products. Further work is necessary to understand how these and other species contribute to *Campylobacter* and *Salmonella* contamination in production environments.

P2-239 Isolation and Characterization of Extended Spectrum β-Lactamase (ESBL) Producing Non-Shiga-toxigenic *Escherichia coli* (nSTEC) from Healthy Food Animals and Their Environment

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Introduction: The occurrence and dissemination of extended spectrum ß-lactamase (ESBL) producing *E. coli* among food animals is a global public health concern.

Purpose: The purpose of this study was to determine the prevalence of multidrug resistant ESBL *E. coli* from apparently healthy food animals and their associated environment in west-coastal India.

Methods: A total of 253 faecal swabs were collected from healthy young calves (*n*=23), goats (*n*=155), pigs (*n*=20), buffalo (*n*=7), ducks (*n*=30) and their associated environment (*n*=18) in west coastal region of India. Isolation, characterization and antimicrobial susceptibility testing of ESBL *E. coli* was carried out according to USDA/WHO recommended guidelines using four µg/ml cefotaxime in MacConkey for the screen. Multiplex-PCR assay was optimized to detect ESBL ($^{blaCTX, blaTEM, blaSHV}$) and virulence ($stx_{1, stx2, eaeA}$) genes.

Results: Overall, the prevalence of ESBL *E. coli* was 110 (43.47%) of 253 among fecal swabs of animals. The prevalence of ESBL *E. coli* in young calves was 18 (78.26%) of 23, goats 47 (30.32%) of 155, pigs six (30%) of 20, buffalo three (42.85%) of seven, duck cloacal swab 23 (76.66%) of 30, and the associated environment was 13 (72.22%) of 18. Antimicrobial susceptibility testing of all isolates (*n*=110) showed a higher frequency of resistance to penicillin (110 of 110) followed by ceftazidime in 76 (69.09%) and ceftriaxone in 68 (61.81%). Multidrug resistance was observed in 48 isolates (43.63%) of 110. The most common MDR pattern was Aamoxicilin-cefotaxime-ceftazidime-ceftriaxone. Multiplex-PCR assay of 110 isolates detected ESBL resistance determinants ^{blaCTX} in 67 (60.91%), ^{blaSHV} in eight (7.27%), and ^{blaTEM} in 10 (9.09%). All isolates were negative for presence of virulence gene stx_2. However, *stx1* was found in three (2.72%) and *eae*A in five (4.54%) wfrom goat and cattle fecal swabs.

Significance: The study highlights that apparently healthy food animals are potential reservoirs of non-virulent multi drug-resistant *E. coli* which harbours ESBL genes which may pose a risk to humans via food chain.

P2-240 Incidence of Coagulase-positive Staphylococci and *Staphylococcus aureus* on Flies from Cattle Sources

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❖ ***Developing Scientist Entrant***

Introduction: Human staphylococcal food poisoning is sometimes caused by consuming foods originated from animals that are reservoirs of coagulase-positive staphylococci and *Staphylococcus aureus*, such as cattle. Flies can be transmitters of these pathogens from cattle to human.

Purpose: The purpose of this study was to identify the prevalence of coagulase-positive staphylococci and *S. aureus* in flies captured from cattle farms.

Methods: Flies (50 from each farm) were captured using fly tapes at nine cattle farms interspersed through Georgia (United States). Coagulase-positive staphylococci and *S. aureus* were isolated from the internal surface and external tissue of the flies following a method outlined in the FDA Bacteriological Analytical Manual with slight modification. Presumptive coagulase positive staphylococci and *S. aureus* colonies were subsequently confirmed.

Results: Coagulase-positive staphylococci were isolated from all nine cattle farms (100%) where 92 (20%) of 450 captured flies were found to be positive for coagulase-positive staphylococci. *S. aureus* was isolated from seven (78%) of the farms where 10 (2%) of 450 flies were found to carry the pathogen. The incidence of coagulase-positive staphylococci and *S. aureus*-positive flies varied from farm to farm ranging from two to 44% and zero to six percent, respectively. The top three incidences for coagulase positive staphylococci were 44%, 32%, and 24%, and those for *S. aureus* were six, four, and two percent, respectively.

Significance: These data suggest that flies are active carriers of coagulase-positive staphylococci and *S. aureus* on cattle farms.

Poster

P2-241 Whole Genome Sequence Analysis of Seven Broad Host Range *Salmonella enterica* bacteriophages

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❖ ***Developing Scientist Entrant***

Introduction: ***Salmonella*** spp. cause 1.3 million illnesses and 470 deaths annually in North America. Ongoing salmonellosis outbreaks linked to poultry products have necessitated the need to develop approaches to reduce the presence of *Salmonella* in these foods. Bacteriophages are emerging as a processing aid to reduce *Salmonella* on poultry.

Purpose: Whole genome analysis of seven broad host range phages was conducted to determine their appropriateness for control of *Salmonella* on poultry products.

Methods: Genomic DNA of seven *Salmonella* phages (SB3, SB6, SB9, SB10, SB13, SB18, SB28) isolated from raw sewage was extracted using the Promega Wizard DNA kit and purified by Ethanol precipitation. Whole genome sequencing (WGS) was accomplished on an Illumina MiSeq platform with 300-bp paired-end libraries and 30X coverage, followed by raw read assembly using the A5 pipeline, and gene annotation using PATRIC.

Results: The genome sizes of the phages ranged from 41 (phage SB3) to 114 kb (SB9). Four phages (SB6, SB9, SB10, SB13) had large genomes (111-114 kb) that were similarly sized. Whole genome blast indicated that six phages were *Siphoviridae*, while SB18 was observed to be a Myophage. These observations were in agreement with phenotypic (TEM) analysis of morphology. Additionally, six phages had high homology (95% or greater homology over 79% or more of the genome) with previously identified *Salmonella* phages, except for phage SB18 which had high homology (98% identity, 98% genome coverage) to the *Erwinia* phage phiEa21-4. No virulence or antibiotic resistance genes were observed except for the presence of a dihydrofolate reductase (dfr)-gene, encoding resistance to trimethoprim, in phage SB18.

Significance: The results of this study demonstrate that, with the exception of phage SB18, all of the phages characterized in this study are appropriate for use in a phage cocktail to reduce the presence of *Salmonella* in poultry and other foods.

P2-242 Addition of Probiotics Affects the Physicochemical and Microbiological Properties of Yogurt Made from Soy Milk and Cow's Milk during Refrigerated Storage

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Introduction: Soy yogurt is preferred by consumers who are allergic to cow's milk. Probiotics can provide gut health.

Purpose: This study's objective was to investigate the physicochemical and microbiological properties of yogurt made from soy milk and cow's milk as affected by probiotics added during refrigerated storage.

Methods: Two types of yogurt were prepared. The first type was prepared with the additions of commercially available yogurt starter containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The second type was prepared with the same yogurt starters plus three probiotics, including *Bifidobacterium lactis* (BB-12), *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus*. Physicochemical characteristics, including pH, color, firmness and syneresis were determined during 4°C storage for 28 d at seven-d intervals. Microstructure and bacterial populations were also determined. Each treatment had five replicates.

Results: No spontaneous syneresis was observed in cow's milk yogurts, but probiotics significantly (P<0.05) reduced syneresis of soy milk yogurt. Probiotics had no effect on the microstructure of cow's milk yogurt, but caused more compact networks in soy milk yogurt. Probiotics shortened the time needed to reach pH 4.5 in both cow's milk and soy milk yogurts. During storage, pH of cow's milk yogurts decreased (4.35 to 4.26 with probiotics and 4.54 to 4.33 without probiotics), but pH of soy milk yogurts remained relatively constant. During storage, cow's milk yogurts showed no color change but soy milk yogurts exhibited decreased L, a, and b values. Probiotics significantly (P<0.05) increased the firmness of cow's milk yogurt. Generally, soy milk yogurt (37.5 to 47.4 g force during compression) was much firmer than cow's milk yogurts (9.8 to 12.8 g force). The soy milk yogurt had significantly higher counts of *Bifidobacterium lactis* BB-12 than cow's milk yogurt. The probiotic populations of all samples were above the level of 10^6 CFU /g.

Significance: The results provide useful information for yogurt production from soy milk and cow's milk for enhancing health.

P2-243 Evaluation of Two Prototypes of Intelligent Packaging with a pH Indicator to Determine Spoiled Cow Milk

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❖ ***Developing Scientist Entrant***

Introduction: Intelligent packaging utilizes chemical sensors to identify the quality and determine the safety of packaged cow milk for consumers. In the future, such intelligent packaging will assist in reducing foodborne illnesses related to spoiled cow milk.

Purpose: To evaluate cow milk packaged in two different prototypes of intelligent packaging during the cow milk's shelf life based on volatile compounds, pH, color change, and sensory characteristics.

Methods: For this evaluation, two intelligent packaging prototypes developed by Braskem (high-density polyethylene with a color indicator based on the pH) were filled with cow milk (Grade A, whole). The whole cow milk samples from the intelligent packages were prepared in triplicates. Measurements and sensory panels were performed during the shelf life of the cow milk samples, over four separate days. For the cow milk samples, gas chromatography-mass spectrometry was used to identify and quantify the volatile compounds (*n*=48) and the pH was determined by a pH meter (*n*=48). The color change of the bottle was measured using the Minolta L*a*b* colorimeter (*n*=48). Thirty-two panelists from Clemson University (18 males and 14 females between 18 and 42 years of age) evaluated the samples.

Results: Color of bottles changed as expected for Trial 1 and Trial 2. Milk from Trial 1 had a pH change that was significantly different due to spoilage reactions. Therefore, Trial 1 is a reliable and accurate intelligent packaging for detecting spoilage of milk. There were no significant differences of sensory attributes (color and appearance; aroma; and overall acceptability) between the milk samples. The milk from Trial 2 was the least liked. Also, we found that hexanal could be a potential biomarker for milk oxidative stress.

Significance: Intelligent packaging systems provide consumers with reliable and accurate information concerning the conditions of the food they are consuming (e.g., food spoilage) and can prevent foodborne illnesses.

Poster

P2-244 Antimicrobial Susceptibility Monitoring of Bacterial Pathogens Isolated from Korean Black Goat

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Introduction: Mastitis is an inflammatory disease of the mammary glands which may cause partial or full damage to the udder. Subclinical mastitis is one of the most important diseases and considered a constant risk of infection for whole herds and their environment. *Staphylococcus* spp. and other environmental pathogens have been proven as the main causative agents of mastitis.

Purpose: The present study was conducted to isolate and identify bacteria that cause mastitis and diarrhea in Korean black goats and evaluate the antimicrobial susceptibility.

Methods: Milk and feces were sampled from 20 Korean black goats (*Capra hircus coreanae*) which were identified subclinical cases in Namwon, Collado, Korea 2018. Subclinical mastitis was confirmed by a somatic cell count (SCC) ≥300,000 cells/ml (Bentley Instruments, Chaska, MN, USA). Feces from 32 goats which showed diarrhea were also sampled. Identification of the isolates was achieved using matrix assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Antibacterial resistance was evaluated for *Staphylococcus* spp. and *Escherichia coli*. MICs were determined by agar dilution for commonly used antibiotics according to Clinical and Laboratory Standards Institute standards.

Results: A total of 118 isolates were recovered (43 from milk and 21 from feces from 20 goats with subclinical mastitis and 54 from 32 goat feces with diarrhea). In milk samples, 11 (26%) *Staphylococcus aureus*, 28 (65%) and one *E. coli* were found while three *E. coli*, nine coagulase-negative staphylococci, and one *Streptococcus pluranimalium* were isolated from feces samples. One *Staphylococcus aureus,* eight coagulase-negative staphylococci from milk and seven coagulase-negative staphylococci from feces showed methicillin resistance. For *Staphylococcus* spp., resistance to ampicillin and penicillin was seen in 58% of isolates and amoxicillin/clavulanate, tetracycline and cephalexin were 21 to 27%. Resistance was absent or very low for enrofloxacin, marbofloxacin, gentamicin, clindamycin and chloramphenicol.

Significance: Even as the size of Korean black goat farming is growing, this type of study is rare; it showed antimicrobial resistance and the need for surveillance.

P2-245 Sporeformer Presence in a Milk Fractionation Process

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✦ ***Undergraduate Student Award Entrant***

Introduction: Because various thermoduric endospores are capable of surviving heat treatment steps common to milk processing, the implementation of spore-reduction technologies is difficult without a proper understanding of the heat classifications, characteristics, and identities of common sporeforming bacteria in dairy processing and powder production.

Purpose: To enumerate thermoduric endospores at multiple points in a commercial powder milk fractionation process using temperature classifications and presence/absence of O_2.

Methods: Product samples were collected at 12 different points in the milk fractionation and drying process and thermally shocked (80°C) to select for thermoduric endospores. The samples were then plated and enumerated using a pour plate method on skim milk plate count agar and incubated at mesophilic (31°C) and thermophilic (55°C) temperatures under both aerobic and anaerobic conditions (n=4, total samples=48).

Results: Spore counts increased throughout the fractionation process (200 CFU/ml in raw milk storage compared to 3070 CFU/ml in post-spray dryer), particularly following pre- to post-ultrafiltration (250 CFU/ml compared to 1000 CFU/ml), and pre- to post-spray drying (630 CFU/ml compared to 3070 CFU/ml), possibly due to decreased water content, increased concentration of the spores, and outgrowth. Mesophilic aerobes were the most prevalent sporeformers identified throughout the process, at 35.8% (7230 CFU/ml) of the total count with mesophilic anaerobes as the next highest proportion at 27.1% (5190 CFU/ml).

Significance: We suspect sporeformer survival during HTST pasteurization and further germination and outgrowth during regeneration sections of unit operations during long production runs since both mesophilic and thermophilic spore counts increased throughout the production process. Because thermoduric bacteria have an adverse impact on powdered milk quality and limit secondary applications of the product, this bacterial growth may be controlled in order to limit concentration and outgrowth during processing, resulting in the production of higher-quality milk powders.

P2-246 Determining the Effect of Individual or Combined Protective Cultures on the Growth of *Listeria monocytogenes* and Shiga Toxin-Producing *Escherichia coli* in Raw Milk

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Introduction: *Listeria monocytogenes* and shiga toxin-producing *Escherichia coli* (STEC), including both O157:H7 and non-O157:H7 serotypes, are pathogens of concern in the production of raw milk cheese. Protective bacterial cultures (PCs) with limited acidification and flavor production represent a potential control strategy.

Purpose: The purpose of this research was to determine the antimicrobial efficacy of commercially produced PCs, used individually or in combinations, against *L. monocytogenes* and STEC when cocultured in raw milk following an incubation time and temperature profile similar to cheesemaking and ripening.

Methods: Six-strain cocktails of *L. monocytogenes*, O157:H7 or non-O157 STEC were inoculated at ~two log CFU/ml in raw milk and stored at 4°C for 24h. Freeze-dried PCs were then added individually or in binary combinations (1:1 ratio) at ~seven log CFU/ml. Coculture samples were incubated at 35°C for four h, followed by 20°C for 20 h, and then held at 12°C for seven days. Experiments were conducted in duplicate and were repeated at least three times.

Results: After seven days, *L. monocytogenes* counts were 5.16, 3.09 and 3.21 log CFU/ml lower than control when cocultured with *Lactococcus lactis* subsp. *lactis* BS-10, *Lactobacillus plantarum* Holdbac, *Listeria* or *Lactobacillus plantarum* LPAL, respectively. Inoculation with LALCULT Protect *Hafnia alvei* B16 inhibited non-O157 STEC and STEC O157:H7 growth with differences of 3.91 and 1.86 log CFU/ml compared to control, respectively. Moreover, potential synergy against *L. monocytogenes* was observed with the combination of BS-10 and *Pediococcus acidilactici* B-LC-20 whereby counts were below the limit of enumeration (less than one log CFU/ml) by day four.

Significance: This study highlights the efficacy of commercial protective cultures for controlling the growth of *L. monocytogenes* and STEC in raw milk. It also identifies a potential synergistic combination as a potential strategy for enhancing the safety of raw milk products. Determining the effectiveness of these cultures and their combinations in the production of raw milk cheese is warranted.

Poster

P2-247 Detection of *Listeria* spp. in Unpasteurized Retail Dairy Products in Maine

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Introduction: ***Listeria*** spp. are ubiquitous in nature and detection at the genus level can be used by food processors as an indicator for the presence of *L. monocytogenes*.

Purpose: This study investigates the occurrence of *Listeria* spp. in unpasteurized retail dairy samples in the state of Maine.

Methods: A total of 104 samples (45 cheese, 59 milk), were collected from the Milk Quality Laboratory in Augusta, ME from June to October of 2018. These products originated from 28 dairy farms in Maine that agreed to participate in this study. Enrichment method was used for all samples to detect the presence or absence of *Listeria* spp. Coliform testing was conducted at the Milk Quality Laboratory and the results were used in this study. Chi-Square test of independence was used, and Pearson's correlation coefficient was used with statistically significance at P<0.01).

Results: The highest rates of *Listeria* spp. positive samples were recorded in July and August, with 83.3% and 25% in unpasteurized milk, and 42.9% and 23.5% in unpasteurized cheese, respectively. Similarly, the highest rates of non-compliant coliform were recorded in July for both products (50% for milk and 33.3% for cheese), however the second highest percentage of violations appeared in October. The prevalence of *Listeria* spp. positive samples was higher (26.3% overall) in mould-ripened cheeses in comparison to soft cheeses (9.5%). The statistic Pearson's correlation showed a strong association (P<0.01) between coliform non-compliance and the presence of *Listeria* spp. in the tested dairy samples, demonstrating the importance and effectiveness of existing testing protocols.

Significance: The findings are beneficial for dairy producers to ensure the safety of their products by enhancing sanitation procedures.

P2-248 A Comparative Evaluation of the GENE-UP *Listeria monocytogenes* Assay for the Detection of *Listeria monocytogenes* in Whey Powder-Unit Dose Format

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Introduction: A method comparison study for the new GENE-UP *Listeria monocytogenes* (LMO 2) unit dose test kit was conducted following the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces.

Purpose: To modify the current AOAC Performance Tested Methods approval for the GENE-UP *L. monocytogenes* assay to include the new unit dose reagent format.

Methods: The candidate method assay was compared to the FDA/BAM Chapter 10 Detection and Enumeration of *Listeria monocytogenes* in Foods (2011). Thirty total replicates were analyzed by both the candidate method and the reference method at three different inoculation levels (zero, 0.2 to two and two to five CFU/test portion). All analytical outcomes were biochemically confirmed by both traditional reference method and by an alternative method, which included an additional streak to bioMérieux's chromogenic Agar *Listeria* Ottavani and Agosti (ALOA) agar. In addition, all candidate method enrichments were evaluated on the original multi dose format for GENE-UP.

Results: The candidate method obtained the following results for the three inoculation levels: zero of five for the uninoculated, six of 20 for the low and five of five for the high. This is compared to zero of five, four of 20 and five of five, respectively, for the reference method. The candidate method demonstrated no statistically significant differences between presumptive and confirmed results ($dPOD_{CP}$) , between candidate and reference method results ($dPOD_C$) or between unit dose and multi dose method results ($dPOD_C$) for the food matrix tested.

Significance: The GENE-UP *Listeria monocytogenes* unit dose assay is considered equivalent to the FDA/BAM Chapter 10 [2] for whey powder. AOAC has approved the new unit dose format for the GENE-UP *Listeria monocytogenes* assay.

P2-249 Occurrence and Antimicrobial Resistance Patterns of *Escherichia coli* O157:H7 and Non-Typhoidal *Salmonella* in Milk and Feces of Lactating Dairy Cows and Camels in Borana, Southern Ethiopia

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Introduction: *Escherichia coli* O157:H7 and non-typhoidal *Salmonella* spp. are common milk-borne pathogens compromising the microbiological safety of raw milk. However, their occurrence in the developing parts of the world like Ethiopia, especially in pastoral livestock production systems, has not been well-investigated.

Purpose: A study was conducted to assess the occurrence and determine antimicrobial resistance profiles of the two pathogens in milk and feces of lactating dairy cows and camels raised under extensive pastoral animal husbandry systems.

Methods: A total of 484 milk and fecal samples (150 samples each from cow milk and feces and 92 samples each from camel milk and feces) were analyzed for the pathogens using bacteriological culture (immunomagnetic separation beads) and further identified using biochemical tests.

Results: In cattle, *E. coli* O157:H7 was detected from 4.7% of both milk and feces and non-typhoidal *Salmonella* from 4.0% and 8.6% of milk and feces, respectively. In camels, 3.3% of fecal samples were positive for *E. coli* O157:H7 and all milk samples were negative. For non-typhoidal *Salmonella* spp., 1.1% and 2.1% of milk and fecal samples from camels were positive, respectively. All isolates of both pathogens were found susceptible to nalidixic acid, gentamicin and ciprofloxacin but resistant for ampicillin. In cattle samples, 92.8% of *E. coli* O157:H7 and 15.8% of *Salmonella* isolates showed resistance to multiple antimicrobials. Inversely, in camels none of *E. coli* O157:H7 isolates were resistant while two of the three (66.7%) *Salmonella* isolates showed multiple antimicrobial resistance patterns.

Significance: Considerable proportions of milk samples, especially from cattle, were positive for drug-resistant pathogens and this could be a significant public health risk. This warrants the setting up of intervention programs to reduce the potential milk-borne transmission (e.g., by promoting boiling milk before consumption) and also targeted education on the prudent use of antimicrobials.

Poster

P2-250 The Evaluation and Implementation of Two Automated Enzyme-linked Fluorescent Assays for the Detection of *Salmonella* and *Listeria monocytogenes* from Large Gram Size Dairy Samples

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Introduction: The use of automated rapid methods have been well established and validated for use in the detection of pathogens in food samples in the traditional 25-gram sample size. However, data is not always available for larger samples. A study was conducted to evaluate the VIDAS UP *Salmonella* and VIDAS *Listeria monocytogenes* II (LMO2) enzyme-linked immunoassay methods for pathogen detection in dairy product samples larger than 25 grams.

Purpose: The purpose was to verify the recovery and detection of *Salmonella* from 375-gram samples and *Listeria* from 125-gram samples for three different dairy product types (whey protein powder, lactose powder and extruded cheddar cheese). In addition to the challenge studies, a blinded study of each sample type was performed for certification of the laboratory.

Methods: Ten each of 125-gram samples of whey powder and cheddar cheese were inoculated with 0.03 CFU/gram *L. monocytogenes* and tested with the AOAC Official Method of analysis OMA (n° 2004.02). Ten each of 375-gram samples of whey powder, lactose powder and cheddar cheese were inoculated with 0.02, 0.02, and 0.01 CFU/gram *Salmonella* Typhimurium, respectively, and evaluated per AOAC Official Method of analysis OMA (n° 2013.01). Five control samples were included in each data set. Six or seven of 10 blinded samples were spiked with a freeze-dried, water-soluble ball containing 30 CFU of either *Salmonella* Typhimurium or *L. monocytogenes* for evaluation by the lab.

Results: The overall agreement was 100% for the 75 *Salmonella* tests and 50 *Listeria* tests in the individual studies

Significance: The different challenge studies demonstrated the efficacy (fit for purpose) of the VIDAS LMO2 and VIDAS SPT for the screening of different dairy products at 125-gram and 375-gram sample sizes. In addition, the blinded studies supported the laboratories' proficiency to perform the assays.

P2-251 Fast (under 19 minutes) Fully-automated or Medium-throughput Semi-automated Multi-contaminant Screening of Milk Samples with the Evidence Series Biochip Analysers

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Introduction: Comprehensive detection of veterinary drugs in milk, including all legislated antibiotics at or below relevant regulatory requirements, is important for consumer protection. The Infiniplex for Milk (IPM) biochip array, based on biochip array technology, allows the simultaneous screening of approximately 130 contaminants from a single undivided milk sample.

Purpose: This study reports the application of IPM to the fast (under 19 minutes) fully automated multi-contaminant screening or the medium throughput semi-automated multi-contaminant screening of milk samples by using Evidence series analysers.

Methods: Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface, were employed and applied to the Evidence MultiSTAT analyser (this system processes a self-contained cartridge containing all the components required for the assays and has the capacity to assess two biochips in under 19 minutes) and to the Evidence Investigator analyser (this system has the capacity to assess 54 biochips in 120 minutes). Raw milk samples (250 or 25 µl respectively) were added directly to the biochips. The results are qualitative.

Results: Up to 130 authorized and unauthorized contaminants were detected (including antimicrobials, anti-inflammatories, antiparasitics, corticosteroids, growth promoters and mycotoxins). The decision levels (concentration of contaminants resulting in >95% of positive results) ranged from 0.04 ppb (aflatoxin M1) to 150 ppb (melamine) in Evidence MultiSTAT and from 0.038 ppb (aflatoxin M1) to 200 ppb (melamine) in Evidence Investigator. Assessment of natural incurred raw milk samples (n=12) showed correlation >90% with LC-MS/MS for both systems.

Significance: IPM solutions are the fast and fully automated or the medium throughput semi-automated multi-contaminant screening of milk samples when applied to the Evidence series analysers. This offers an accurate and reliable analytical application for the comprehensive monitoring of contaminants in raw milk.

P2-252 Microbiological Safety of Pulses-based Fermented Foods Developed and Prepared in the Laboratory

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❖ ***Developing Scientist Entrant***

Introduction: Pulses have several benefits, being powerful superfoods, economically accessible, highly water efficient and helpful against climate change. When used as the main or composite substrate for fermented foods, they give rise to foods containing beneficial probiotics, digestive enzymes and health-boosting nutrients. Like other types of food, they could, however, be contaminated with pathogenic microorganisms during preparation and storage if good hygiene practices are not implemented.

Purpose: This study looks at the incidence of pathogenic microorganisms associated with foodborne illnesses in two laboratory produced pulse-based fermented foods (bambara kunuzaki and pigeon pea yogurt).

Methods: To prepare bambara kunuzaki, bambara groundnut and millet were first steeped in water for 24 hours at 30±2°C, followed by grinding the steeped grains with spices. The mash was then divided into three parts and two parts were gelatinized with hot water. The portions were mixed together at 70 to 75°C and fermented for 24 h. Non-dairy yogurt was also prepared from pigeon pea milk and both products were examined for the presence of moulds, coliforms and aerobic spore formers.

Results: Bambara kunuzaki samples were found to be contaminated with high counts of coliforms and moulds although controlled fermented bambara kunuzaki did not contain moulds at the end of fermentation. Pigeon pea yogurt samples appeared to be of better microbiological quality although aerobic spore formers and moulds were observed in them during the third and fourth week of storage.

Significance: This work suggests that proper hygiene standards of raw materials and processing environment should be a priority during preparation of fermented foods

Poster

P2-253 Comparison of the Bac*gene Listeria* spp. Real-time PCR and BAX System 24E Genus *Listeria* PCR Methods for the Detection of Genus *Listeria* in Food and Environmental Samples from Two Dairy Production Facilities

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Introduction: A large dairy product manufacturer expressed interest in an alternate PCR method for the detection of *Listeria* in their environmental and product testing program due to recurring false-positive detection.

Purpose: The purpose of this study was to compare the performance of two PCR based methods for the detection of *Listeria* in dairy food and environmental samples.

Methods: A total of 244 environmental samples and 33 product samples (ice cream) were collected and tested from two production plants. Environmental samples were collected using a split sampling device that allowed simultaneous collection of two swabs from each sampling site for duplicate testing by each method. Food samples were split into two 25-g aliquots. Enrichment, sample preparation, and PCR testing were conducted exactly as specified by the kit manufacturers. All presumptive positives by either method were taken to culture confirmation.

Results: No presumptive positives for any product samples were seen with either method. For environmental samples BAX gave 50 presumptive positives of which 48 were confirmed by culture (100% sensitivity, 99.1% specificity). The BACGene method gave 40 presumptive positives all of which were confirmed by culture (100% sensitivity, 100% specificity). Out of 56 positive samples, 34 samples agreed between methods. Of the 20 samples that disagreed, 14 were BAX positive/BACGene negative and six were the reverse. By chi-square analysis (0.6645, P=0.35245) the results were not statistically different (P<0.05).

Significance: These results demonstrate equivalence performance of the two methods. Two false positives were observed with the BAX method and none were observed with the BACGene method. Care should be taken when using a split-sampling device as duplicate swabs may not yield the same result despite being taken from the same sampling site.

P2-254 16S rRNA Analysis of Bacterial Genera Present on Wooden Boards at Different Depths from Several Cheese-aging Facilities

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❖ ***Developing Scientist Entrant***

Introduction: Cheese aging often relies on adventitious microorganisms present in the environment to aid in development of cheese flavors, aromas and other organoleptic characteristics. In addition, this microflora might provide a competitive advantage against potential pathogens. A variety of wooden boards are often used for cheese aging. There is little information regarding what microbiota are present on these boards.

Purpose: The purpose of this study is to identify bacterial genera in the microbiota of five different wooden boards collected from three different Wisconsin cheese facilities, used to ripen semi-soft cheese.

Methods: For this study, wooden boards used to ripen semi-soft cheese were obtained from local cheese production facilities. Wood shaving samples were collected from the surface and at a depth of 0.2 cm from the each board using a sanitized drill bit. Sterile PBS was added to the shavings and stored overnight at -20°C. Total genomic DNA was extracted using phenol:chloroform and quantified using a Qubit kit. Primers for the variable regions of 16S rRNA for bacteria (V4) were used to amplify bacterial DNA by PCR. Illumina-based sequencing (MiSeq) was used to obtain maximum sequencing depth and coverage. Data were processed in Mothur software and analyzed using Rstudio.

Results: Our preliminary results suggest that there are at least 235 OTUs (Operational Taxonomic Units) on three boards and 325 OTU on two other boards. *Brevibacterium*, *Brachybacterium* and *Staphylococcus* genera were found in relatively high abundance at the surface and at a depth of 0.2 cm for all five boards. The presence of these genera is not unexpected as they are thought to contribute to the flavor and other characteristics of cheese.

Significance: Wooden boards are commonly used in cheese aging and are thought to contribute to the desired aroma and flavor of the product. Identifying the microbiota associated with wooden boards used for cheese aging might improve our understanding and perhaps allow manipulation of the microbiome to enhance desired characteristics of cheese quality and safety.

P2-255 The Safety of Raw Milk Cheese and Raw Milk Used for Cheesemaking in Ireland

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Introduction: Ireland has an international reputation for the quality, safety and variety of its artisan cheese made from raw milk. It is important for the entire dairy industry that this reputation is not damaged.

Purpose: This work aimed to assess the microbiological and residue (anthelmintic drug residues) risks associated with raw milk used for cheesemaking and raw milk cheese.

Methods: Samples of raw milk, milk filters, curd and cheese from 10 raw milk artisan cheese producers in the south of Ireland were tested. Numbers of presumptive *Bacillus cereus* group, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* were determined. The determination of anthelmintic drug residues, including benzimidazoles, flukicides, macrocyclic lactone (avermectin and milbemycins), levamisole and morantel was also performed.

Results: Neither *L. monocytogenes*, nor *Salmonella* spp. were detected in any of the samples tested and no anthelmintic drug residues were detected. The *E. coli* numbers were similar between dairies with values of < 10 CFU/ml for milk samples, between 10 and 10^2 CFU/g for curd samples and 10^2 and 10^5 CFU/filter for milk filter samples. Presumptive *Bacillus cereus* group were absent in most cases, although numbers were around 50 CFU/ml when they were present in the milk. *S. aureus* numbers were absent in 59%, 52% and 39% of the milk, cheese/curd and milk filter samples respectively. Only one of the remaining samples did not conform with regulatory numbers.

Significance: This survey has shown a good microbiological and residue quality (and low risk) of the raw milk cheese and raw milk used for raw milk cheese produced in Ireland. Moreover it has shown the importance of frequent assessment of raw milk used for cheesemaking and for raw milk cheese, as it allows the identification of potential problems facilitating resolution of these issues before they cause any public health threat.

Poster

P2-256 Source Tracking and Succession of Microbial Communities during the Production of a Farmstead Cheese

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❖ ***Developing Scientist Entrant***

Introduction: Historically, cheese was produced on farms using raw milk and wooden tools, resulting in high taxonomic diversity in indigenous cheese microbial communities. Though modern sanitation practice has improved food safety and consistency, it also diminishes microbial and flavor diversity. The rapid growth of the artisanal cheese industry in the United States has renewed interest in identifying novel and beneficial microorganisms related to food manufacture.

Purpose: The objectives of this study were to characterize the microbial ecosystems of traditional farmstead cheese production and evaluate the role of the environment in microbial transfer to product throughout cheese production and aging.

Methods: A total 108 products (e.g., raw milk, curd, cheese) and environmental (e.g., tools, working surface, air) samples were collected along the cheese-making continuum. DNA was directly extracted and amplified targeting bacterial 16s rRNA and fungal ITS genes. Sequencing was conducted on the Illumina Miseq. Amplicon data were analyzed using DADA2. SourceTracker was applied to estimate the role of the environment in microbial transfer.

Results: Raw milk had the highest microbial diversity among product samples. After overnight ripening in a wooden barrel, the community was dominated by core fermentation- and ripening-related microbes. Dominant bacteria throughout cheese production included *Lactococcus*, *Lactobacillus*, and *Leuconostoc* species, while *Candida*, *Exophiala*, and *Kluyveromyces* were the dominant fungi. During 60 days of aging, microbial community structure in the cheese interior remained similar to fresh cheese, whereas *Brevibacterium*, *Mucor,* and *Trichothecium* species emerged as the dominant microbes on the cheese rind. Among all environmental sources, the wooden barrel used for overnight ripening and cheese production was considered the major source of microbes in cheese with an average relative contribution of 74%.

Significance: These data demonstrated the essential role of the environment, including the use of wooden tools, as sources of dominant microbes that drive the fermentation and ripening process of a traditional farmstead cheese.

P2-257 Assessing Growth and Survival of *Listeria monocytogenes* in Wash Solutions Used in Artisanal Washed Rind Cheese Production

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❖ ***Developing Scientist Entrant***

Introduction: Washed-rind or smear-ripened cheeses are considered high-risk for contamination with *Listeria monocytogenes* due to favorable growth conditions on the cheese paste and rind and multiple points for contamination during post-production cheese care and aging.

Purpose: To evaluate the growth potential of *L. monocytogenes* in washes of differing compositions used in washed-rind cheese production in the United States and complete a risk-assessment for cheesemakers making washed-rind cheese.

Methods: Artisanal cheese producers throughout New England were surveyed on current washed-rind cheese production practices. Five wash solutions were prepared based on survey data, including a beer wash (BW), a cider wash (CW), a wash using freeze-dried ripening culture reconstituted in sterile water (SW), a lactic whey wash (LW), and a Morge wash (M). Washes were inoculated with *L. monocytogenes* and *L. innocua* at a combined concentration of 10^2 CFU/ml and stored at temperature treatments of 5±1°C and 15±1°C. Wash solutions were enumerated using standard methods and plated on CHROMagar *Listeria* at zero, one, two, three, and seven days to assess viable cell counts.

Results: Viable cells were not observed past day one for BW and past day three for CW at both 5±1°C and 15±1 ˚C. An average log reduction of 2.11 was observed for BW at both treatments, and an average log reduction of 2.08 was observed for CW at both treatments. Viable cells were observed past day seven for LW solutions at both 5±1°C and 15±1°C. SW supported significant growth and survival of *L. monocytogenes.* SW at 5±1°C supported a two-log increase over days zero to three, while SW at 15±1°C supported a five-log increase over days zero to three. M results were found to be variable.

Significance: These findings suggest that some wash types used in washed-rind cheese production pose a higher risk for *L. monocytogenes* growth and survival and should be considered as important vectors for transmission in washed-rind cheese production.

P2-258 Differential Growth of *Listeria monocytogenes* in Soft Ripened Cheeses at Refrigerated Temperatures

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Introduction: Illnesses and outbreaks of listeriosis in Canada and the United States have been associated with soft ripened cheese (SRC). Relative to harder cheeses, SRCs, such as Brie, provide a favourable environment for the growth of *Listeria monocytogenes* due to a higher moisture content and lower acidity.

Purpose: The objective of this study was to compare the growth of *L. monocytogenes* in four SRCs of different origin.

Methods: The growth of *L. monocytogenes* BCCDC-A3 (serogroup 4b/4d/4e) in four SRCs stored at 8°C was monitored daily through spread plating on PALCAM agar. Two SRCs each from Canada and France were tested and included cheeses made from both pasteurized (n=2) and non-pasteurized milk (n=2). *Streptococcus*, *Lactobacillus*, and total aerobic bacteria in each cheese were measured on M-17, MRS, and tryptic soy agars, respectively.

Results: Across the four SRCs, *L. monocytogenes* grew at an average rate of 0.29±0.6 log CFU g^{-1} day^{-1} to achieve an average maximum density of 7.2±0.5 log CFU g^{-1} after ten days of incubation at 8°C (n=4). Area under the curve analysis revealed the Canadian pasteurized milk cheese to have significantly higher growth of *L. monocytogenes* than the other three cheeses (Tukey's HSD; P<0.002). Interestingly, this same cheese showed a lack of quantifiable *Lactobacillus* (<10^3 CFU/g) whereas the other three cheeses had concentrations of *Lactobacillus* ranging from 10^6 to 10^7 CFU/g.

Significance: ***L. monocytogenes*** grows readily in SRC at refrigerated temperatures. The growth potential, however, appears to differ between different SRCs and may be related to the density, and possibly the composition, of native microflora.

Poster

P2-259 Understanding of Microbial Communities Potentially Associated with Quality and Safety in Cheddar, Provolone and Swiss Cheeses

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Introduction: Understanding the microbial community of cheese is important in the dairy industry since the microbiota contributes to the physicochemical, sensory aspects, quality and safety of cheese. The microbial composition of cheese varies depending on the types of cheese and cheese making processes.

Purpose: The purpose of this study is to understand and compare the composition of the microbial populations between different types (Cheddar, provolone and Swiss) and locations (core, rind and mixed) of cheeses using microbiome sequencing.

Methods: Three different types of cheese were collected from Oregon State University Creamery and genomic DNA was extracted from core, rind and mixed locations of cheese. Genomic DNA isolated from all the cheese samples were subjected for amplifying and sequencing the V4 region of 16S rRNA gene via an Illumina Miseq platform to assess the bacterial populations among different types and locations of cheese samples.

Results: *Streptococcus* was observed as the most abundant species in two provolone (72 to 85%) and two Swiss (60 to 67%) cheeses while *Lactococcus* was observed one of the most dominant bacteria in five Cheddar cheeses (27 to 76%). Species richness of each cheese was significantly different based on the cheese type (P<0.05). According to the alpha diversity analysis, Cheddar cheese soaked in porter beer exhibited the highest microbial richness while smoked provolone showed the lowest. In addition, a decrease in microbial richness was observed in the core region of the cheese compared to the rind region. Microbial communities of each cheese clustered in a PCoA plot, indicating highly similar microbial composition, based on the types of cheese.

Significance: The results of this study provide better insight into the microbial composition of different types and locations of cheese, contributing to the quality and safety.

P2-260 Modelling Population Dynamics of *Listeria monocytogenes* Strain in Lactic Soft Cheese Following Acid and Osmotic Stress Exposures

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Introduction: Due to its continued exposure to environmental stresses in the food processing environment, *Listeria monocytogenes* cells may develop stress tolerance responses that enhance survival ability, cross-protection to subsequent stress exposures in the food product and cell injury.

Purpose: The aim of the study was to model survival response of *L. monocytogenes* strains individually and as mixed strains in a lactic soft cheese after exposure to acid and osmotic stress and to identify the surviving populations in cheese samples co-inoculated with mixed strains.

Methods: Lactic soft cheese was inoculated with *L. monocytogenes*. The survival responses of *L. monocytogenes* strains in lactic soft cheese were studied following acid and osmotic stress exposures. Survival data of individual and mixed strains were fitted to four inactivation models (log-linear with tail, biphasic, Weibull and Weibull with tail).

Results: Lack of fit (LoF) analysis showed the Weibull and log-linear with tail models provided the best fit of the data and were used to determine kinetic parameters. Analysis of inactivation kinetics showed that the susceptibility of individual strains (P<0.0001) and the form of stress exposure (P<0.05) have a significant effect on survival responses. Although both acid and osmotic stress exposures mainly resulted in enhanced survival of the *L. monocytogenes* strain, in one susceptible strain stress exposure caused inactivation.

Significance: These findings imply that responses of *L. monocytogenes* strains in foods are dependent on the differences in the intrinsic susceptibilities of individual strains. Moreover, when introduced into the food as mixed strains, analysis of the diversity of surviving populations revealed that one strain outlived others and remained as the only survivor after 15 days. Predictions of pathogen responses in foods based on mixed strain cocktails as a way of accommodating strain heterogeneity must be treated with caution.

P2-261 Impact of Use of Natural Whey Starter on the Microbiological Characteristics of Artisanal Brazilian Canastra Cheese during Ripening

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Introduction: Many artisanal cheeses are produced with raw milk and natural whey starter by back-slopping. This practice plays an important role in the sensorial attributes and microbiological characteristics of the cheese.

Purpose: The purpose of this study was to evaluate the influence of natural whey starter on the microbiological characteristics of an artisanal Brazilian cheese during ripening.

Methods: The cheese model was manufactured following the practices adopted by the cheese makers in the Serra da Canastra, Minas Gerais, Brazil. Raw milk (25 l), donated by a dairy industry, was inoculated with 0.3% of three natural whey starters (NWS) obtained from local cheese makers. For ripening, the cheeses were placed on wooden shelves and maintained at 20°C and relative humidity between 60 and 70% for 60 days. Control cheeses (without NWS) were produced with the same raw milk. At seven-d intervals, total mesophilic bacteria, lactic acid bacteria, coliforms, *E. coli*, yeast and molds were determined in cheese samples of 25 g. A factorial design, repeated in three blocks was applied and results were analyzed by ANOVA.

Results: The use of NWS influenced significantly *(P*< 0.05) the counts of coliforms and *E. coli,* but *t*he effect varied according to the type of NWS (P<0.05). After 60 days, reductions in counts of *E. coli* in cheeses manufactured with the three NWS were 1.9, 4.0, and 4.5 log CFU/g, while in control cheeses the reduction was 2.2±0.3 log CFU/g. Results for coliforms were similar to those of *E. coli*. The effect of NWS was not significant *(P*>0.05*)* for the other microorganisms.

Significance: The results suggest that the use of NWS for artisanal cheese production may reduce the counts of coliforms and *E. coli* in the products, however the effect is type of NWS dependent.

P2-262 Diversity of Oxacillin-Resistant *Staphylococcus aureus* Isolated from Cheese

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Introduction: The nosocomial pathogen methicillin-resistant *Staphylococcus aureus* (MRSA) now causes infections in the community and has been cited as a source of foodborne illness. MRSA can be found in cow milk and dairy products, hence fresh cheese, which can be consumed uncooked, may contribute to their dissemination

Poster

Purpose: To elucidate the diversity of oxacillin-resistant isolates recovered from fresh cheese of artisan production in Costa Rica.

Methods: We analyzed 60 cheese samples that were collected in three different days at five retail stores across four provinces. The Vitek system was used to identify the isolates and obtain antibiotic susceptibility profiles. Furthermore, 10 isolates with phenotypic resistance to oxacillin were whole genome sequenced to determine their sequence types, spa types, and SSCmec structures.

Results: A total of 154 isolates were obtained, of which 15 were categorized as resistant to oxacillin with MICs=4 or ≥8 µg/ml. Positive samples were derived from three of the four provinces visited. Three of the 10 sequenced isolates belong to the widespread, human-associated, ST8 t008 type and have a type IVa (2B) SCCMec element. The other seven sequenced isolates did not have *mec* genes. Three of them were classified as ST352 t359, which is of bovine origin, and the rest were typed as ST1 t2207, ST81 t127, and ST352 t1028 strains.

Significance: The finding of human and bovine strains indicates bad hygiene and inadequate pasteurization during production. In addition, our results likely reflect zoonotic MRSA transmission.

P3-01 Genetic Analysis of Natural Microflora in Stored Joraengyi Rice Cakes and Their Capability for Propionic Acid Production

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Introduction: In the Republic of Korea, rice cakes account for the largest portion in the sales of rice processed foods. Propionic acid (PA) is a preservative that control molds, yeast, and bacteria in foods. However, PA can be produced naturally by the fermentation of microorganisms originating from raw materials during storage, although they are not artificially added.

Purpose: The purpose of this study was to verify the natural origin of PA in rice cakes by analyzing the population of natural microflora present in deteriorated Joraengyi rice cakes and investigating microbial growth and capability for PA production.

Methods: Selected PA producing bacteria were cultivated in tryptic soy broth (TSB) supplemented with 10 g/l glucose. Their growth characteristics and PA production were analyzed. The bacteria producing PA were cultured for one, two, four, seven, 10, and 14 d in TSB with 10 g/l glucose and then the PA production was analyzed by a gas chromatography-flame ionization detector.

Results: A total of 98 microbial strains were detected from microflora that grew after the expiration of shelf life of Joraengyi rice cakes. The *Lactobacillus casei* group accounted for 50.48% and *L. buchneri* was 29.60%. PA-producing bacteria were *Propionibacterium thoenii*, *P. cyclohexanicum*, *P. jensenii*, and *P. freudenreichii*. Natural bacterial and *Lactobacillus* spp. did not produce propionic acid for 14 d. On the other hand, *P. cyclohexanicum*, *P. freudenreichii* subsp. *shermanii*, *P. thoenii* and *P. jesenii* produced 2,462.02, 2,904.78, 2,220.64, and 3,519.17 µg/ml during 14 d, respectively.

Significance: This study suggests that the natural microflora of Joraengyi rice cakes during storage can produce propionic acid from natural sources even if a high concentration of propionic acid is not intentionally added.

P3-02 Mass Spectrometry Analysis for Evaluation of Gluten Residues in Wheat Beers

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❖ ***Developing Scientist Entrant***

Introduction: The safety of fermented products derived from gluten-containing grains, including wheat beers, for patients with celiac disease (CD) has remained controversial in recent years.

Purpose: This study comprehensively investigated the wheat beer proteome and its antibody reactivity using SDS-PAGE, western blotting, and mass spectrometry (MS).

Methods: Seven types of commercially available wheat beers were purchased from local stores. Protein concentrations in degassed samples were analyzed with five types of protein quantification methods. Protein size distribution and reactivity with two commercial antibodies were visualized with SDS-PAGE and western blotting. Proteins and peptides in samples were fractionated with a 30 kDa filter. The fraction above 30 kDa was subsequently digested with chymotrypsin, and both fractions were analyzed separately using discovery LC-MS/MS. Endogenous peptide (<30 kDa) data were analyzed independently from chymotryptic peptide data, and all identified peptides were searched against the AllergenOnline Celiac database.

Results: Total protein concentrations in seven beer samples ranged from 0.4 to 6.3 µg/µl, depending on the quantification method used, with no significant differences observed among the beers (P>0.05). Proteins and peptides (five to 50 kDa) were found in all samples, and bands between 25 to 50 kDa showed considerable reactivity with commercial anti-gliadin antibodies. Peptides derived from wheat, barley, and yeast were found in all samples using high-resolution, accurate-mass MS (LC-HRAM). Peptides from gliadin, an important protein fraction for CD, were predominant in peptides identified from wheat, especially in the fraction above 30 kDa. An average of 166 endogenous peptides was found in samples, with up to 40% containing celiac immunogenic motifs.

Significance: Variability exists when evaluating beer proteomes with various analytical methods due to the partial hydrolysis of proteins during the brewing process. Mass spectrometry could serve as a complementary method for the deep coverage of wheat beer proteomes and evaluation of immunogenicity.

P3-03 The Application of Enzymatic Histamine Assay for Fermented Foods

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Introduction: Histamine is a biogenic amine that is produced in spoiled fish and some fermented products, and causes a food-borne disease similar to an allergic reaction. Regulatory limits on histamine in fish have been set in individual countries and/or by international organizations. Therefore, there is a growing demand for a rapid histamine test kit for use in food materials and products. A rapid enzymatic histamine assay kit was recently certified by AOAC Research Institute as a Performance Tested Method for raw and canned tuna, and anchovy fish sauce. A rapid test for histamine may also have applicability for product other than fish.

Purpose: The applications of the enzymatic histamine assay for fermented meat, dairy, vegetables, and wine were evaluated.

Methods: Histamine Test (Kikkoman Biochemifa) and Absorptiometer RGB (two cm optical path length, Kyoritsu Chemical-Check Lab.) were used for the enzymatic histamine assay. Dried salami (Italy), sauerkraut (Germany), cheese (Cheddar, New Zealand and Brie, France) and red wine (France) were assayed with and without spiked histamine. Samples (one g) were in general prepared according to the manufacturer's manual (25-fold dilutions, n=5). The cheese was pretreated using 1 N perchloric acid and 1 N KOH to reduce the product's turbidity. The red wine (two ml) was pretreated with polyvinylpolypyrrolidone to remove pigments and fivefold dilutions were performed. Each sample was assayed at 23 to 37°C as recommended in the manual.

Results: The original histamine levels of the salami, the cheese and the sauerkraut were under the limit of quantification and the recovery rates for 50 mg/kg histamine were 101-105%. Regarding the red wine, 1.4 mg/kg histamine was detected and the recovery rate for 10 mg/kg histamine was 81%.

Significance: The enzymatic histamine assay demonstrated good recovery rates for histamine not only in fishery products but also fermented meat, dairy, vegetable and wine.

P3-04 Mitigation Strategies for Acrylamide in Bread

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Introduction: The Maillard reaction can be responsible in bread for the production of toxic substances such as acrylamide (AA). This contaminant is considered carcinogenic to animals and possibly humans and even neurotoxic and genotoxic.

Purpose: Development of combinations of natural extracts to use as acrylamide reducing agents (ARA) in bread with high consumption by the Portuguese population: rye, wheat and sweet yeast.

Methods: ARA preparations-peel of Rocha pear, oregano, fennel, and lemongrass, had determination of antioxidant activity by UPLC-PDA in production tests (n=320) in nine different combinations of ARA and two baking processes (conventional oven (O1) *vs* convection oven (O2)), as well as sensorial evaluation and determination of AA by UPLC-MS/MS.

Results: For antioxidant activity of the extracts, TPC and DPPH values ranged from 0.7 to 88.5 (mg eq AC/g sample), and 0.2 to nine (mg/ml), respectively. For mitigation effects of the extracts, reduction values were: oregano in rye bread 17.7% (O2), in wheat bread 31.6% (O1) and 21.7% (O2); lemongrass in rye bread 27.5% (O1) and 7.8% (O2); fennel in wheat bread 33.5% (O1) and 41.5% (O2); peel of Rocha pear in rye bread 27.3% (O2), in wheat bread 19.2% (O1) and 12.5% (O2). Sensorial characterization showed an increase when compared to the control sample in the following extracts: oregano in rye bread (O2) from 7.1 to 7.7, oregano in wheat bread (O2) from 7.2 to 7.5, and lemongrass in rye bread (O2) from 7.1 to 7.8. For fennel and peel of Rocha pear extracts, the sensorial characterization showed no differences between samples.

Significance: Using natural extracts proves to be a feasible way to reduce ARA levels in bread, with good sensorial acceptance. Extracts like pear peel represent an advantage, in terms of sustainability and circular economy.

P3-05 Single Kernel Aflatoxin and Fumonisin Levels in Commercial Corn from Texas with Different Bulk Mycotoxin Levels

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❖ ***Developing Scientist Entrant***

Introduction: While the distribution of mycotoxin contamination in bulk corn is known to have skewness there is relatively little data on the distribution of mycotoxins in the single kernels that make up a bulk sample, particularly in commercial samples. Knowledge of single-kernel mycotoxin distribution may explain inaccuracies in bulk-testing and is important for developing single-kernel management strategies such as sorting.

Purpose: Analyze concentration of aflatoxin and fumonisin in single corn kernels from commercial samples with different bulk levels of contamination.

Methods: We obtained 250-g samples of commercial corn from Texas previously tested from bulk aflatoxin and fumonisin by the Office of the Texas State Chemist. Samples were stratified by three levels (Low, Medium, High) of bulk aflatoxin and fumonisin: Low (<20 ppb aflatoxin, <1000 ppb fumonisin), medium (20-50 ppb aflatoxin, 1000-4000 ppb fumonisin), and High (>50 ppb aflatoxin, >4000 ppb fumonisin). This resulted in nine strata for all toxin concentration pairs. From each strata one bulk sample was selected at random and 96 individual kernels picked for a total of 864 kernels. Aflatoxin and fumonisin ELISA kits were used to measure mycotoxins in single kernels. Kernels were considered contaminated at >20 ppb aflatoxin or >1000 ppb fumonisin (FDA regulatory limits).

Results: Among the 864 corn kernels, only seven kernels tested >20 ppb aflatoxin and 39 kernels tested >1000 ppb fumonisin. Comparing contaminated single-kernels to bulk mycotoxin levels, six aflatoxin kernels were from bulk samples with $high_{AF}$/$high_{FM}$ and one from $high_{AF}$/low_{FM}. For fumonisin contaminated kernels, 12 kernels fell in $high_{AF}$/$high_{FM}$, four kernels in $high_{AF}$/$medium_{FM}$, 16 kernels in $high_{AF}$/low_{FM}, four kernels in $medium_{AF}$/$high_{FM}$, three kernels in $medium_{AF}$/$medium_{FM}$, and three kernels in low_{AF}/$medium_{FM}$ bulk level.

Significance: The data shows that the distribution of aflatoxin and fumonisin in single kernels is highly skewed even among contaminated commercial corn with different bulk levels.

P3-06 Significance of Anti-heat Processed Milk Antibody on ELISA-based Detection in a Dark Chocolate Matrix

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Introduction: Undeclared milk is prevalent in chocolate products, so specific analytical methods for use with this difficult matrix are required. The performance of milk ELISAs are compromised in chocolate because of the presence of protein-binding phenolic compounds.

Purpose: Antibody specific for heat processed milk proteins (HPMP-Ab) was generated as a strategy for improved milk detection in processed foods including chocolate commodities.

Methods: Milk incurred cookies (zero, 3.54, and 17.7 ppm), and tempered dark chocolate samples (62% cacao: zero, 0.9, 1.8, 2.9, 7.6, 28,134 ppm; 100% cacao: zero, 0.075, 0.25, 0.75, 2.5, 7.5, 25, 75 ppm) were evaluated using HPMP-Ab by ELISA. The cookies and dark chocolate samples were extracted with high salt buffer (HSB) with 1% Tween 20 and HSB with 1% Tween 20, 5% polyvinylpyrrolidone (PVP), and 1% fish gelatin respectively after removing fat with hexane treatment.

Results: In qualitative immunochemical analysis, modified proteins, including aggregates, were found to react with greater signal intensities when using HPMP-Ab. By sandwich ELISA, HPMP-Ab demonstrated high affinity towards processed purified major milk proteins and proteins of milk-derived ingredients and reference materials subjected to several thermal processing procedures. Acceptable dynamic ranges were observed as evidenced by HPMP-Ab binding curves using incurred cookies and tempered 62% dark chocolate samples, but binding curves were negatively impacted using 100% dark chocolate samples due to an increased baseline absorbance/background. The HPMP-Ab ELISA was highly specific, as no cross reactivity was observed when testing a broad

range of food commodities using incurred cookies as calibrators. The HPMP-Ab ELISA detected declared/undeclared milk proteins in commercial chocolate samples containing a variety of milk-derived ingredients when tempered 62% dark chocolate samples were used as calibrators.

Significance: These results demonstrate the benefits of HPMP-Ab for milk detection in processed foods, including milk in chocolate matrices, and illustrate the necessity for appropriate matrix-specific incurred calibrators of defined composition.

P3-07 Quality Characteristics and Aflatoxin Contents of Homemade Doenjang (Korean Traditional Fermented Soybean PCaste)

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Introduction: Doenjang is a Korean traditional fermented food made with soybean. Depending on the inoculation method, it is divided into traditional doenjang which is inoculated and fermented naturally, and modified doenjang which is fermented by inoculating the selected microorganism. At home, traditional doenjang has been prepared and consumed. However, little is known about the quality and aflatoxin contents of homemade doenjang.

Purpose: To investigate the quality characteristics (moisture, salt content, amino nitrogen, pH, color and fermentation period) and aflatoxin contents (B1, B2, G1 and G2) of homemade doenjang in Korea.

Methods: A total of 529 homemade doenjang samples (300 to 500 g per sample) were collected nationwide in Korea from January to October 2018 using a snowball sampling method. The quality characteristics of doenjang were analyzed with the Korean food code method. The aflatoxin contents in doenjang were determined by a modified Korean food code method that was validated in terms of linearity, limits of detection and quantification (LOD and LOQ), accuracy and precision. The correlations among the investigated factors were also analyzed.

Results: Quality characteristics of the doenjang samples were 57.0±5.5% moisture content, 19.9±4.0% salt content, 19.9±4.0 mg% amino nitrogen, 5.7±0.7 pH, and 32.5±6.7 color (ΔE). In-house validation results obtained for aflatoxins with doenjang matrix were within the acceptable ranges (R^2>0.99, LOD: 0.012 to 0.041 µg/kg, LOQ: 0.036 to 0.125 µg/kg, accuracy: 70.6 to 105.1%, and precision: 1.8 to 8.4%). Among 529 doenjang samples, aflatoxins were detected in 118 (22%), 65 (12%), seven (one percent) and 23 (four percent) samples, respectively. The mean contents of aflatoxins in the aflatoxin positive samples were 5.03, 1.35, 1.14 and 0.10 µg/kg, respectively. Moisture contents of doenjang were negatively correlated with salt contents, and total aflatoxin contents were also negatively correlated with fermentation period.

Significance: These results can be used as basic data to manage the safety and quality of homemade doenjang.

P3-08 Occurrence and Exposure Analysis of Deoxynivalenol, Nivalenol and Their Glucosides in Cereal and Cereal-based Foods in Korea

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Introduction: Deoxynivalenol (DON) and nivalenol (NIV) are the most common contaminants of cereal and cereal-based foods. Deoxynivalenol-3-β-D-glucoside (DON3G) and nivalenol-3-β-D-glucoside (NIV3G) are the modified mycotoxins produced by defense mechanisms of plants which can be converted into free toxin by human or animal intestinal microorganisms.

Purpose: To evaluate the occurrence and exposure of DON, NIV, DON3G and NIV3G in cereal and cereal-based foods in Korea.

Methods: Total 569 samples (0.5 to one kg per sample) were collected in 2017 from Korean markets. Contents of DON, DON3G, NIV and NIV3G were determined by a simultaneous analytical method using immunoaffinity column cleanup and HPLC-UV detection. Daily exposure of these toxins through intake of cereal and cereal-based foods was assessed by a deterministic approach.

Results: Detection rates of DON, DON3G, NIV and NIV3G in 569 samples were 57 (10.0%), 20 (3.5%), 60 (10.5%), and 22 (3.9%), respectively. Mean concentrations for DON, DON3G, NIV and NIV3G in toxin-positive samples was 37.8, 26.3, 77.3, and 90.0 µg/kg respectively. The highest toxin contamination occurred in the breakfast cereal sample. NIV was the most frequently detected mycotoxin with high contamination level among four toxins. Their exposures of DON, DON3G, NIV and NIV3G through intake of cereal and cereal-based foods were 11.2, 10.4, 18.9, and 11.8 ng/kg body weight/day for lower bound (<LOD=zero), and 31.3, 39.8, 42.9, and 43.6 ng/kg body weight/day for upper bound (<LOD=LOD). The estimated dietary intakes were below tolerable daily intake (TDI) levels of one µg/kg and 1.2 µg/kg for DON and NIV, respectively.

Significance: The exposure estimates of DON, NIV and their glucosides based on occurrence data in cereal and cereal-based foods indicated that they might not cause a health concern in Korea.

P3-09 Combined Effects of Temperature and Oxidative Stress on the Growth, Aflatoxin Production, and Gene Expression of *Aspergillus flavus*

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Introduction: Plant-pathogen interaction is affected by climate changes, such as temperature increases and drought, which tend to promote reactive oxygen species production in plants and induce stronger oxidative stress for fungal pathogens. These effects can impact the fungal infectivity and mycotoxin contamination in agricultural crops.

Purpose: To understand the combined effects of temperature increases and oxidative stress on *Aspergillus flavus*.

Methods: Two aflatoxigenic strains (one reference and one wild type) and one atoxigenic strain were incubated under the combination of two different temperatures (28 and 33°C) and three levels of H_2O_2 (zero, one, and five mM) using a "float-culture" method, in which fungi grew on a membrane filter floating on the surface of 25 ml of PDB medium in a nine-cm petri dish. After three days of incubation, fungal biomass production, aflatoxin production (HPLC), and genes expression (qRT-PCR) were tested. All experiments were done with independent triplicates.

Results: Temperature rising from 28 to 33°C caused significant (P<0.05) increases of fungal biomass production and a decrease in aflatoxin production. H_2O_2 level increase from zero to five mM did not affect biomass but significantly (P<0.05) promote aflatoxin production in the reference (53.52±7.56 to 77.10±3.16 µg/g mycelium) and wild type strain (64.29±11.63 to 87.98±6.71 µg/g mycelium).The expression of aflatoxin-biosynthesis genes tended to be elevated by increase of H_2O_2 level. Increased temperature inhibited the expression of structural genes (*aflD*, *aflK,* and *aflQ*) but not regulatory genes (*aflR* and *aflS*).

Significance: This is the first study to examine the combined effects of temperature and H_2O_2 on the growth of *A. flavus* and aflatoxin production. These results can be used to predict the impacts of climate change on aflatoxigenic fungi.

P3-10 Effects of Nzu (Calabash Clay) on Mineral and Aflatoxin Contents in Cows' Milk from Abeokuta, Nigeria

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❖ ***Developing Scientist Entrant***

Introduction: Expensive and non-availability of adsorption materials for the reduction of aflatoxin M_1 (AFM_1) from milk in Nigeria drives the demand for a local readily available adsorbent for the amelioration of the toxin from milk produced by cows.

Purpose: The purpose of this study is to find a suitable, cheap and available adsorbent for the reduction of AFM_1 found in cows milk in Abeokuta Nigeria.

Methods: Adsorption studies of AFM_1 was performed with high-performance liquid chromatography machine (HPLC) using Nzu (calabash clay) (0.5, one and two percent) at aflatoxin contamination rates of 9, 231 and 435 ng/l for five h at 4, 16, 28 and 32° C. The aflatoxin adsorbing capability of the adsorbent depends on the adsorbent concentration, contact time and treatment temperature.

Results: Nzu exhibited very poor adsorbent capacity at three treatment temperatures (4, 16, and 28°C) and also released lead and arsenic into the milk, it decreases the calcium and iron by 25 and 30% respectively, however at 32°C, Nzu demonstrated significant reductions (P<0.05) at all contamination rates but also released lead and arsenic into the milk and reduced the calcium and iron contents by 40 and 60% respectively

Significance: This study indicates the potential of Nzu in reducing aflatoxin AFM_1 at high temperature in developing tropical countries but cannot be used in its amelioration because of its release of lead and Arsenic into milk and reduction of calcium and iron from milk.

P3-11 Effect of Storage Techniques on Aflatoxin Load in Maize Ogi from Uyo Metropolis, Akwa Ibom State, Nigeria

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Introduction: In Nigeria, ogi, an uncooked corn starch, is consumed by an estimated 25% of infants and adults every week

Purpose: To determine the effect of commonly used storage techniques for ogi in Nigeria on its aflatoxin load

Methods: Fifty samples of fresh ogi produced using the traditional spontaneous fermentation technique was randomly purchased from 25 sellers from different markets in Uyo. These were bulked into one sample and divided into nine samples of three treatments of three subsamples each. All samples were screened for aflatoxins (AFTs) B1, B2, G1 and G2. Treatment A was freshly prepared ogi stored at room temperature and screened within 24 hours of preparation. Treatment B (refrigerated at 4°C for seven days before screening) and C, submerged in potable water for seven days before screening (water was decanted and replaced every 24 hours). Determination and quantification of AFTs were done using thin layer chromatography (TLC) with scanning densitometer[a] (Camag TLC Scanner 3, ISO 9001, Reg. No. 11668-01). Recovery of toxin was >85% while the detection limit was below one ppb. A two-way ANOVA of the data was done using GraphPad Prism (version 6.01). Means were separated at P<0.05 using LSD.

Results: AFTs G1 and G2 were below detection in all treatments. For AFT B1, treatments A, B and C had mean values (ppb) of 3.33±3.33, 28.67±8.01 and 54.33±9.53 respectively, while mean value (ppb) of AFT B2 was 0.00±0.00, 3.33±3.33 and 17.00±6.56 in treatments A, B and C respectively. There were significant differences (P<0.05) in the mean values among treatments for AFTs B1 and B2

Significance: This study suggests that ogi is best consumed fresh and that other commonly used storage techniques can increase AFTs B1 and B2 load in ogi.

P3-12 Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus* on Nyjer Seed Cake

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Introduction: Nyjer seeds (40% oil) are pressed for cooking oil and the remaining cake is used as animal feed. Our previous studies indicated that nyjer seed cake supported fungal growth and aflatoxin production. High levels of aflatoxin were detected in milk when dairy cows were fed with-aflatoxin contaminated seed cake.

Purpose: This study investigated the effect of water activity and temperature on the growth and aflatoxin production by *A. flavus and A. parasiticus* on nyjer seed cake (10% oil).

Methods: Nyjer seed cake was adjusted to different water activity a_w levels (0.82, 0.86, 0.90, 0.94 and 0.98) using sterile water. Each plate containing 2.5 g seed sample was spot inoculated with 10 µl spore suspension and incubated at 20, 27 and 35°C in closed glass jars. After five, 10, 15, 20 and 30 days, fungal growth was measured and seed samples were collected for aflatoxin analysis. Aflatoxins (B1, B2, G1 and G2) were determined using high performance liquid chromatography.

Results: Our results show that up to 175 µg/kg AFB1, and 153 µg/kg AFG1 aflatoxins were produced by *A. parasiticus* with 0.94 a_w at 27°C while *A. flavus* produced only up to 13 µg/kg AFB1 with 0.94 a_w at 27°C. There was no fungal growth for both *A. flavus* and *A. parasiticus* at 0.82 a_w. Wilcoxon matched pairs signed-rank test indicated that there was no statistically significant difference in fungal growth on seeds with 10% and 40% oil content (*A. flavus*: P<0.171; *A. parasiticus*: P<0.124) but significant difference in aflatoxin production (*A. flavus*: P<0.001.; *A. parasiticus*: P<0.001).

Significance: Aflatoxin productions were observed at fewer environmental conditions while fungal growth remained similar on nyjer seed cake as compared to ground nyjer seeds. This study can be used to establish storage guidelines for nyjer seed cake to minimize production of aflatoxins.

Poster

P3-13 Reduction of Ochratoxin a in Rice and Oat Porridge by an Indirect Steaming Process with Baking Soda

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Introduction: Ochratoxin A (OTA) is one of the most important mycotoxins owing to its widespread occurrence and toxicity including nephrotoxicity and potential carcinogenicity to humans. OTA has been detected in processed food products due to its heat stability while significant reduction of the toxin may be achieved under higher temperature and alkaline conditions during food processing.

Purpose: In this study, the effects of retort process on the stability of OTA in spiked (20 µg/kg of dry weight basis) rice and oat porridge (10% solid content; w/v) in the presence and absence of baking soda was investigated using a laboratory indirect streaming system.

Methods: The samples were heated in a pot at 85°C central temperature for 10 min to be gelatinized followed by drying in 50°C oven overnight. The entire experiment was replicated three times and samples were analyzed for OTA by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD).

Results: The reduction of OTA in retorted rice and oat porridge were 59% (8.2 ng/g) and 14% (17.2 ng/g), respectively, while greater reduction of OTA was observed with addition of baking soda. The reduction of OTA in the rice porridge with 0.5% and 1% baking soda were 78% (4.4 ng/g) and 68% (6.4 ng/g), respectively. In the oat porridge, reduction of OTA was also evident to result in 58% (8.4 ng/g) and 72% (5.6 ng/g) with 0.5% and 1.0% of added baking soda, respectively.

Significance: These results suggest that OTA in rice and oat may be reduced significantly by indirective steaming process. In addition, added baking soda may positively impact the reduction of OTA.

P3-14 Detection of *Salmonella* Typhimurium in Environmental Sponge Swab Enrichment Cultures Using the bioMérieux VIDAS® SLM, EZ-SLM and the FDA BAM Cultural Assay

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Introduction: Environmental testing for *Salmonella* is vital to a food safety program. Individual sponge swabs are collected from multiple sites in order to take action when screened positive but requires more time and cost. Creating secondary samples post-enrichment could address both while allowing traceback for a positive mixed set. Importantly, savings reinvested in the program would further enhance pathogen surveillance.

Purpose: To assess the impact on *S.* Typhimurium 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 coinoculated at a fractional level with approximately 1.5 CFU S. *Typhimurium* and approximately 15 CFU *Citrobacter freundii* (competitor) (*n*=20). Sponges (*n*=80) were inoculated with 15 CFU *C. freundii* and enrichments from each were mixed with the coinoculated enrichments in a 1:5 ratio. Positive (2-5 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® SLM, EZ-SLM or the FDA BAM reference method. All sample enrichments were streaked to selective/differential isolation media and the plates examined for typical colonies.

Results: Individual sponge enrichments demonstrated identical recovery to the five-sponge mixed enrichments when tested by VIDAS® SLM, EZ-SLM and reference methods. The proportion of fractional positives was 15/20 (POD 0.75) for Lactose Broth enrichments and 16/20 (POD 0.80) for BPW enrichments. The difference in probability (dPOD) between methods was determined. 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%) included 0. All five high inoculated sponges tested positive (POD 1.00) and all 5 negative sponges tested negative (POD 0.00).

Significance: A five-sponge mixed enrichment will provide a comparable recovery to testing individual environmental sponge enrichments.

P3-15 Test for Detection of *Listeria* spp. from Environmental Surfaces without Enrichment

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Introduction: This method is a novel, enrichment-free test for detection of *Listeria* spp. in swab samples taken from environmental surfaces. Results are available in less than one hour. In a previous Performance Tested Method study, the test was validated for stainless steel and sealed concrete surfaces (PTM 081802).

Purpose: A PTM matrix extension study was conducted to validate the method for detection of *Listeria* spp. in swab samples from ceramic tile, plastic, and rubber surfaces.

Methods: Performance of the test method was compared to the U.S. Food and Drug Administration *Bacteriological Analytical Manual* reference culture procedure for detection of *Listeria* spp. in swab samples taken from inoculated ceramic tile, plastic, and rubber surfaces. For each environmental surface, 60 test portions (one by one inch surface areas) were prepared; 40 at a level expected to produce fractional positive results (~ 50 CFU/surface), 10 at a high level expected to produce all positive results (~100 CFU/surface), and 10 uninoculated controls. Data were analyzed using a probability of detection model.

Results: There were no significant differences in performance between the test method and reference culture methods for any of the three surfaces tested, as determined by probability of detection analysis. At the fractional level there were 13, 10, and 15 positive results by LRN assay for ceramic tile, plastic, and rubber respectively. Whereas, FDA/BAM reference culture method has seven positive results for ceramic tile, eight positive results for plastic, and 12 positive results for rubber. All high-level test portions were positive by all methods and uninoculated-control test portions were negative by all methods.

Significance: The method is an effective procedure for detection of *Listeria* spp. from a variety of environmental surfaces. It provides accurate results, without the need for enrichment. This enables food industry personnel to react swiftly to suspected *Listeria* contamination incidents.

P3-16 Evaluation of the Certus Environmental *Listeria* spp. Detection Kit for the Detection of *Listeria* spp. on Environmental Surfaces: AOAC Performance Tested Method 101802

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Introduction: The CERTUS Environmental *Listeria* species Detection Kit (CERTUS E*L* Detection Kit) is a real-time, bio-contained assay designed to accurately detect *Listeria* species (*L. grayi*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*) from environmental surfaces employing an antibody-coupled magnetic microparticle and surface enhanced raman spectroscopy (SERS) nanoparticle technology test system paired with a proprietary selective growth media and detection unit.

Purpose: To evaluate performance of CERTUS E*L* Detection Kit in a AOAC *Performance Tested Methods* design validation study.

Methods: Inclusivity and exclusivity, method comparison studies, product consistency and stability were conducted to evaluate the new method according the AOAC Appendix J guidelines.

Results: In the method comparison studies: stainless steel, ceramic tile, plastic (polystyrene) and sealed concrete environmental surfaces (four by four inch test areas) were tested. No statistically significant differences were found by probability of detection analysis (POD) in any of the surfaces when results were compared to the United States Food and Drug Administration cultural microbiology reference method for *Listeria*. The CERTUS E*L* Detection Kit correctly identified all 50 target *Listeria* isolates and correctly excluded all 30 non-target strains that were analyzed. POD analysis of CERTUS E*L* Detection Kit robustness, product consistency (lot-to-lot) and stability studies demonstrated no statistically significant differences, and no variation was observed between instruments.

Significance: The data collected in these studies demonstrate that the CERTUS E*L* Detection Kit is a reliable method for the rapid and specific detection of *Listeria* from stainless steel, ceramic tile, plastic (polystyrene) and sealed concrete environmental surfaces.

P3-17 Qualitative Comparison of Environmental Swabbing Devices for Recovery of *Listeria monocytogenes* from Stainless Steel

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Introduction: Environmental sampling is an important tool for monitoring pathogens in food production environments. A variety of devices may be used for sampling although their utility for recovery of *Listeria monocytogenes* from food contact surfaces is not understood.

Purpose: To compare qualitative recovery of *L. monocytogenes* from dry stainless steel surfaces using conventional swabs and sponges as well as unconventional wipes, with and without added food residue.

Methods: Stainless steel coupons were spot-inoculated with an *L. monocytogenes* cocktail in BPB or cheese whey at ca. 1.75 log CFU per four by four (small) or eight by eight-in (large) surface and dried overnight. Devices for sampling four by four-in areas included cotton, polyester, foam, and flocked swabs; foam and flocked swabs, cotton sponges, polyester foam sponges and cleanroom and microfiber wipes for eight by eight-in areas. Surfaces were swabbed horizontally, vertically, and diagonally with qualitative detection by enrichment in UVM broth, secondary enrichment in Fraser broth, and confirmation on MOX agar. Fisher's Exact Test was used to determine significance at *P*=0.05.

Results: For the small surfaces without food matrix, the polyester swabs allowed for the highest detection of *L. monocytogenes* (35%; *n*=40), although not statistically different from the other devices. Cotton swabs performed the worst with 12 and 22% positive detection after 24 and 48 h UVM enrichment, respectively. For whey-inoculated small surfaces (*n*=40), the rank by percent positive detection was cotton/polyester > foam > flocked swabs after both enrichment lengths. For the large surfaces (*n*=30 to 40), the foam swabs resulted in more positives regardless of the presence of a food. The flocked swab was statistically worse for both 24 and 48 h enrichment periods (20% and 23% positive, respectively). Overall, the flocked swab was incapable of properly swabbing the large surfaces.

Significance: The foam swabs resulted in more positive detection of *L. monocytogenes* than the other devices regardless of surface size or presence of food residue.

P3-18 Use of 3M Molecular Detection Assays for Detection of *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes* in Fresh Spinach and Environmental Samples

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Introduction: Leafy greens are considered vulnerable to contamination due to usage of sprinkler irrigation water, "triple wash" and their consumption without heating or cooking increase the risk of illness. Pathogen testing can be considered as an effective mean of consumer protection. Use of technologies such the 3M Molecular Detection Assay (3M MDA) combines isothermal DNA amplification and bioluminescence detection providing real-time detection and next day results.

Purpose: To evaluate a LAMP-based method for the detection of *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes* on fresh spinach and environmental samples.

Methods: Fresh spinach was obtained from a local supermarket in Queretaro, México. Forty 25-g samples and forty new-sponges were inoculated with four, seven, and three cells of *Salmonella* Typhimurium, *L. monocytogenes* and *E. coli* O157:H7 respectively. Buffered peptone water-ISO were added for the *Salmonella* and *E. coli* O157 samples and Demmi Fraser broth for *L. monocytogenes* samples (ratio 1:10). Samples were at 35°C for 24 to 30 h for *Salmonella* and *L. monocytogenes*, and at 41.5°C for 24 h for *E. coli O157:H7*. Samples were analyzed by LAMP-based method and confirmed by culture (ISO 11290 and 6579). Three samples of each matrix were used as a negative control. Samples were analyzed by two different technicians.

Results: For *Salmonella* thirty-nine out forty inoculated samples were reported as positive by MDS and confirmed by culture, regardless the technician; one sample was negative by MDS and confirmed as positive by culture. For *E. coli* O157: H7 and *L. monocytogenes* the forty inoculated samples were positive independent the technician. The forty inoculated sponges with *Salmonella* spp. and *L. monocytogenes* were detected by the MDS independent of the technician. All negative control samples yielded a negative result.

Significance: Molecular Detection System is an effective method for detection of *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7 in fresh spinach and environmental samples.

P3-19 In-house Validation of a Loop Mediated Isothermal Amplification (LAMP)-Bioluminescent Technology for the Detection of *Listeria* spp. and *Salmonella* spp. in Three Different Matrices

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Introduction: Food contaminated with pathogens increases the risk of illness. *Listeria* is prevalent in many foods, including produce and ready-to-eat products, and food environment. *Salmonella* has been linked with several food outbreaks including produce and processed food. Loop mediated isothermal amplification (LAMP) is a simple, rapid, specific and cost-effective nucleic acid amplification method.

Purpose: To evaluate the performance of LAMP-bioluminescent method for detection of *Listeria* spp. and *Salmonella* spp. in peppers, deli sandwiches and surface samples.

Methods: Forty-five samples (25 g) of fresh peppers, RTE ham and cheese sandwiches obtained from a local retailer in Pachuca México and surfaces sampled with hydrated NB sponges were evaluated. Samples were contaminated with *Listeria monocytogenes* ATCC 19114, *L. innocua* and *L. ivanovvi* or with *Salmonella* Typhimurium ATCC 14028 at seven different levels (1, 15, 110, 480, 1400, 7500 and 76000 CFU/25 g). Samples were were preenriched with Demi Fraser broth and buffered peptone water-ISO for *Listeria* and *Salmonella* respectively. All the samples were analyzed by LAMP-method and culture confirmed. For each pathogen, product category and environmental sponges, thirty samples were used to evaluate ruggedness, inclusivity and exclusivity. Five samples of each product were used as negative control

Results: All the 153 contaminated samples contaminated with *Listeria monocytogenes*, *Listeria innocua* or *L. ivanovvi* were reported as positive for *Listeria* spp. by LAMP-method and confirmed by culture. All the 153 samples contaminated with *Salmonella* yielded as positive by both detection methods. The eighteen samples inoculated with interferent microorganism were detected as negative for *Listeria* spp. *and Salmonella* respectively. LAMP method had 100% sensitivity for contaminated samples and 100% for inclusivity and exclusivity.

Significance: Use of the LAMP-bioluminescent technology is a suitable method for the recovery of *Listeria* spp. and *Salmonella* spp. from fresh peppers, deli sandwiches and environmental sponges.

P3-20 Independent Validation of a Proprietary Service-Based Method for Detection and Identification of *E. coli* O26, O45, O103, O111, O121, O145 and O157:H7

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Introduction: Neoseek STEC is a multiplex molecular assay for the determination of genotypes of selected Shiga toxin-producing *E. coli*, specifically O157:H7, O26, O45, O103, O111, O121, and O145, from isolates or complex food enrichment samples. The Agena MassARRAY platform, used for high throughput SNP genotyping, has been adapted for this purpose.

Purpose: An independent validation of the method was performed according to the current AOAC validation guidelines in a paired study design as part of the AOAC PTM validation process.

Methods: The service-based method relies on PCR amplification and primer extension to generate allele-specific DNA products which are analyzed by the MALDI-TOF mass spec. 86 independent PCR targets are assayed, including targets for O-group, *stx*, *eae* and other virulence associated genes and subtypes.

Results: Fifty EHEC strains covering the seven O-groups of interest, as well as 34 non-EHEC *E. coli* and closely related organisms were tested. The method correctly identified the inclusivity organisms, with no false positives reported from the exclusivity panel. Enrichments (325 g) of fresh raw beef trim, dual inoculated with O157:H7 and O26:H11 at two different levels, low and high, or uninoculated, were tested by the method and against the appropriate USDA/FSIS-MLG method, 5.09 and 5B.05 respectively. No false positives were observed in the uninoculated samples; nine of 20 and seven of 20 "low" samples were confirmed to have O157:H7 and O26:H11, respectively, and five of five for both in the high inoculum samples by the candidate method and the appropriate reference method.

Significance: Correct O-group identifications and STEC/non-STEC determinations can be made from an isolate or complex enrichment sample in 36 h from time of sample receipt. This approach is a simple and economical method for identification of the seven STEC of current concern by USDA. The method has been found to be equivalent to the USDA/FSIS-MLG reference methods.

P3-21 Evaluation of the of the MC-Media Pad Yeast and Mold Device for the Enumeration of Yeast and Mold: A Collaborative Study

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Introduction: Mold spores can be found in virtually all manufacturing environments, and under certain conditions, can flourish. Spoilage associated with yeast or mold production leads to discoloration, rotting, off-flavors and production of mycotoxins. Molds are found in virtually every manufacturing environment and at high levels, can produce mycotoxins, poisonous substance known to be carcinogenic. The MC-Media Pad Yeast and Mold is a ready-to use culture device combining a test pad coated with medium and water absorption polymers that is designed for the rapid quantification of yeast and mold in food products.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the candidate method to the FDA/BAM Chapter 18 method for orange juice concentrate.

Methods: The candidate method was evaluated in a multi-laboratory collaborative study. A total of 14 technicians from 12 laboratories, representing government and industry, throughout the United States participated. Each laboratory evaluated eight replicates at four levels of inoculation; an uninoculated control level, a low inoculum level, medium inoculum level and a high inoculum level.

Results: Colony counts (CFU/ml or g) were evaluated for repeatability (S_r) and by paired *t*-test for statistical difference, defined as 95% confidences outside >0.5 log the reference method. No statistically significant difference was observed between the candidate and reference method after 48 and 72 h of incubation.

Significance: The data from the study, within the statistical uncertainty, supports the certification of the candidate method as an AOAC Official Method of Analysis, First Action in select food matrices and environmental surfaces after 48 and 72 h of incubation.

Poster

P3-22 Enumeration of Total Aerobic Counts in a Variety of Foods by the MC Media Pad™ Rapid Aerobic Count Device: A Collaborative Study

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Introduction: The aerobic plate count (APC) is used as an indicator of bacterial populations within a sample. An APC can be used to gauge sanitary quality, organoleptic acceptability, adherence to good manufacturing practices and to a lesser extent, as an indicator of safety. The MC-Media Pad RAC is intended to enumerate total aerobic counts using a special medium composition and unique redox indicator dyes for standard and rapid enumeration. The MC-Media Pad pre-sterilized, ready-to-use dry culture media devices simplify testing and minimize the quantity of waste. MC-Media Pad is composed of a unique adhesive sheet, a test pad coated with medium and water absorption polymer, and a transparent cover film.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the candidate method to the USDA FSIS MLG 3.02 for raw ground pork and to SMEDP Chapter 6 for yogurt drink.

Methods: The candidate method was evaluated in a multi-laboratory collaborative study. A total of nine technicians from eight laboratories, representing government and industry, throughout the United States participated. Each laboratory evaluated six replicates at three levels of natural contamination for each matrix; a low inoculum level, medium inoculum level and a high inoculum level.

Results: Colony counts (CFU/mL or g) were evaluated for repeatability (S_r) and by paired-*t*-test for statistical difference, defined as 95% confidences outside >0.5 log the reference method. The MC-Media Pad RAC after 24 and 48 h of incubation were not significantly different at the 95% level from the reference method at all levels of contamination.

Significance: The data from the study, within the statistical uncertainty, supports the claims of the candidate method as being considered equivalent to the reference methods in selected food matrices after 24 and 48 h of incubation.

P3-23 Evaluation of the GENE-UP® SLM for the Detection of *Salmonella* spp. in Various Chocolate Products

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Introduction: Rapid *Salmonella* spp. detection in chocolate products presents unique challenges. The chocolate industry is continually seeking faster time to result, further challenging the limits of available methodologies. There is a significant need for rapid and robust detection methods to decrease the risk of inaccurate results and optimization to combat naturally occurring PCR-inhibitory properties.

Purpose: The purpose of this study was to evaluate GENE-UP (GU), a real-time PCR method, for the detection of *Salmonella* spp. (SLM) in 15 products prevalent in the chocolate industry. Buffered peptone water (BPW) and nonfat dry milk (NFDM) preenrichment methods were evaluated, each with testing of individual and secondary (n=5) samples by PCR analysis.

Methods: Samples (375 g) of each product were inoculated with one to 10 CFU/test portion of a strain of *Salmonella* spp., along with a non-target organism at approximately 10 to 100 times the level of the target strain, tested using two separate enrichments (BPW and NFDM) and incubated at 41.5°C for 20 to 24 hours. For each product, *n*=7 replicates (five inoculated, two uninoculated) were evaluated. Following incubation, samples were analyzed by GENE-UP SLM using two protocols: individual and secondary (N=5) sample PCR analysis. For the secondary samples, five mL of each enrichment were mixed, where a one to five mL portion from a positive sample was mixed with four to five ml portions of negative sample. Additionally, for each product an unpaired 25-g sample was analyzed by the reference method ISO 6579-1:2017. All results were culturally confirmed via extended confirmation methodology.

Results: For all products and iterations, GU provided 100% agreement to the extended culturally confirmed result with acceptable performance compared to the ISO 6579-1:2017 reference method.

Significance: This evaluation demonstrates the suitability of GENE-UP for the detection of *Salmonella* spp. in various products used in the chocolate and confectionery industry.

P3-24 Evaluation of the Biomérieux VIDAS/GENE-UP® Top7 Shiga Toxin-producing *E. coli* Detection System

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Introduction: Testing of beef products for the presence of Shiga toxin-producing *E. coli* (STEC) typically relies on detecting genes that encode Shiga toxin (*stx*), intimin (*eae*) and specific O-antigens. Test systems that utilise an STEC concentration procedure may decrease the number of potential positives (PPs) that require culture confirmation.

Purpose: Evaluate the performance of bioMérieux GENE-UP® in combination with VIDAS® STEC Top7 on Australian manufacturing beef enrichment broths.

Methods: One hundred manufacturing beef enrichment broths previously identified as PP for a Top7 STEC serogroup were included in the study. Seventeen of the 100 PP broths were confirmed positive for O157 (nine), O26 (six), O111 (one) or O103 (one) using a DAWR approved confirmation method. Each broth was subsequently tested using the STEC screening system which involved PCR analysis of the ESPT1 eluate for *stx*, *eae*, O157 and Big6. For comparison, all samples were screened for Top7 STEC using an in-house PCR approach utilizing previously published primers and probes.

Results: A total of 28 PPs were identified using the screening test method. In comparison 77 PPs were identified using the in-house PCR approach. The cohort of 28 PPs identified using the screening test method included 16 (94%) of 17 confirmed STEC samples. One sample (BM52) was not determined to be PP using the screening test method due to a low concentration of *eae*. In comparison, the in-house PCR approach identified all 17 confirmed samples.

Significance: In Australia, the ratio of confirmed STEC positive samples to PPs remains low. This study utilized an STEC concentration step to reduce the number of PPs requiring culture confirmation by 49% whilst identifying 94% of confirmed STEC samples. Such reductions could provide economic benefits to Australian beef processors.

P3-25 Evaluation of the GENE-UP *Cronobacter* spp. Assay for the Detection of *Cronobacter* from Environmental Surfaces (Stainless Steel and Plastic)

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Introduction: *Cronobacter* spp. are neonatal pathogens most commonly associated with powdered infant formula (PIF). With numerous points of potential contamination in PIF and the likelihood of missing its detection in the final product due to low prevalence, a strong quality testing program is paramount.

Poster

Purpose: The objective of this study was to evaluate the performance of a fluorescence resonance energy transfer (FRET)-based real time PCR assay (GENE-UP CRO) for the detection of *Cronobacter* spp. from stainless steel and plastic environmental surfaces according to the current AOAC validation guidelines.

Methods: four by four inch stainless steel samples were inoculated with two levels (two and 20 CFU) of *Cronobacter malonaticus*. Stainless steel samples were also inoculated with high levels (600 CFU) of *Salmonella* Typhimurium ATCC 13311 as competitor organism. One by one inch plastic surface samples were inoculated with two levels (10 and 70 CFU) of *Cronobacter sakazakii*. Following inoculation and stabilization, surfaces were sampled using horizontal and vertical sweeping motions. Replicates from each surface were analyzed by both the candidate method and ISO 22964:2017. All GENE-UP CRO results were culture confirmed by both the traditional ISO Reference Method and by an alternate direct confirmation method using two chromogenic agars [Chromogenic Cronobacter Isolation (CCI) and Enterobacter Sakazakii Isolation Agar (ESIA).

Results: In the method comparison study, the CRO demonstrated no statistically significant differences between presumptive and confirmed results ($dPOD_{CP}$) or between candidate and reference method results ($dPOD_C$) for either stainless steel or plastic samples. All candidate and reference methods for both surfaces provided zero of five uninoculated positives, 14 of 20 positives at the low level and five of five high inoculated positives. The dPODs and the corresponding 95% confidence intervals for both products are 0.0 (-0.43 to 0.43).

Significance: These data support the high sensitivity and use of CRO for the detection of *Cronobacter* spp. from environmental surfaces.

P3-26 Performance Evaluation of 3M Molecular Detection Assay 2 – *Campylobacter* for Detection of *Campylobacter* spp. in Unpasteurised Milk Products and Poultry Matrices

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Introduction: *Campylobacter* is the most prevalent foodborne pathogen causing the highest reported gastrointestinal disease in New Zealand. *C. jejuni* and *C. coli* are most often associated with campylobacteriosis with ~99% of cases due to *C. jejuni*. *Campylobacter* contamination can result in severe public health problems and economic loss. Current culture-based screening method requires a minimum of four days to results, thus an alternative rapid detection method is urgently needed.

Purpose: To assess the performance of the 3M Molecular Detection Assay 2 *Campylobacter* coupled with an advanced ready-to-use *Campylobacter* enrichment media, to detect *Campylobacter* spp. from poultry rinse, raw and processed meat products and unpasteurised milk samples.

Methods: A total of 126 samples were examined using the MDA2 *Campylobacter* detection method, including 36 meat products and 13 poultry rinse samples inoculated at low inoculum level (<10 CFU/sample) or high inoculum level (>1000 CFU/sample), three *Campylobacter* control samples inoculated at less than four CFU/sample and 20 blind samples provided by the NZ Inter-Laboratory Comparison Programme. All unpasteurised milk products were tested according to the new MPI regulations at 125 ml. 54 samples were inoculated at five inoculum levels (less than five CFU/sample, five to 15 CFU/sample, >100 CFU/sample, >1000 CFU/sample and blank), with an incubation time of 22, 24 or 26 h. All samples were confirmed using a cultural reference method MIMM 7.3 (NZ Microbiological Methods for the Meat Industry).

Results: All inoculated samples returned with positive MDA2 detection results and uninoculated samples negative. A 100% agreement between the alternative molecular detection method and the cultural method was achieved, with a 100% relative accuracy, 100% relative sensitivity and 100% relative specificity.

Significance: The alternative molecular detection method coupled with the advanced RTU enrichment media offers a reliable and rapid detection of *Campylobacter* spp. from poultry rinse, raw and processed meat products and unpasteurised milk samples.

P3-27 Characterization of Bacteriophage Targeting *Citrobacter* spp., *Escherichia coli*, and *Klebsiella oxytoca* Used in a Selective *Salmonella* Enrichment Broth by Transmission Electron Microscopy and Whole Genome Sequencing

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Introduction: Closely-related *Enterobacteriaceae* such as *Salmonella* and *Citrobacter* can display common epitopes that may lead to false positive results by antibody-based methods. In order to eliminate this effect, lytic bacteriophages were isolated that recognized bacterial surface receptors present on the competitive bacteria but not the target pathogenic bacteria (e.g., *Salmonella*). These phages are ideal candidates as selective agents for controlling competitive bacteria in a selective food pathogen enrichment broth.

Purpose: The purpose of this study was to characterize the phages used as selective agents in RapidChek SELECT *Salmonella* enrichment broth by transmission electron microscopy (TEM) and whole genome sequencing (WGS).

Methods: Lytic bacteriophages were isolated from environmental and food sources using *Salmonella* immunocrossreactive *Citrobacter* spp., *Escherichia coli*, and *Klebsiella oxytoca* phage hosts. TEM images were obtained with a Zeiss Libra 120 transmission electron microscope and a Gatan Ultrascan 1000 CCD camera. Genomic DNA was isolated from purified phage using a commercially available DNA purification kit. WGS was performed on an Illumina MiSeq instrument platform. BLAST analysis of full length DNA sequences (eight) was performed and closest sequence matches determined from the NCBI nr database.

Results: Four *Citrobacter* spp. phages, three *E. coli* phages, and one *Klebsiella oxytoca* phage were characterized by both TEM and WGS. Results were consistent between methods as they both showed all phages to be dsDNA Caudovirales with the *Klebsiella oxytoca* phage a *Siphonviridae* and the *E. coli* and *Citrobacter* phages *Myoviridae*. Genome sizes ranged from 57.2 kb for the *Klebsiella* phage to 357.1 kb for one of the *E. coli* phages. All of the phage gave high (>97%) DNA identities to phage sequences deposited in the NCBI.

Significance: TEM and WGS characterization of unique phages used in a selective *Salmonella* enrichment broth should provide the foundation for further understanding the molecular basis of their selectivity.

P3-28 Development of a Next Generation Sequencing Workflow for Food DNA Analysis: How to Identify Meat and Fish Species in Complex Food Products

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Introduction: Next generation sequencing (NGS) has been introduced in recent years as a very powerful method for species identification in food products. However, the use of NGS requires the development of the correct workflow to ensure the reliability of the results and to maximize the advantages of this high throughput DNA-based method.

Purpose: Taking advantage of the non-targeted and massive sequencing output obtained by NGS, a workflow was developed and tested to identify meat and fish species in food products.

Methods: The workflow was defined and optimized to meet the following criteria: i) barcoding of several specific DNA regions suitable for species identification (multi-barcoding); ii) definition of consensus primer panels producing very small amplicons (multiplex) to ensure the use in highly processed food where DNA can be highly damaged; iii) optimization of the Ion Torrent technology (Ion Chef System and Ion GeneStudio S5 System, Thermo Fisher Scientific); iv) development of a software for automatic data analysis containing suitable databases with thousands of meat and fish species for species identification.

Results: The workflow was tested on a group of 80 complex samples of fish and meat including 20 artificial DNA mixtures and 60 real food samples. The real food samples were selected to include different processing treatments, namely dry, canned, fresh, frozen and liquid. The NGS workflow was effective at correctly identifying the species present in all the food samples regardless of their processing treatments. Furthermore, the workflow was successfully used simultaneously for meat- and fish-based products analysis in a single NGS run. The workflow from DNA extraction to species identification takes <24 hours and requires limited handling due to automated workflow using the Ion Chef and Ion GeneStudio S5 Systems with automatic software-based analysis for species.

Significance: The rapid time to results and simple workflow make this the first credible solution for in-house screening of samples for multi-species identification in routine food analysis laboratories. SGS partnered with Thermo Fisher Scientific to commercialize the solution.

P3-29 Thermo Scientific Brilliance Campycount Enumeration Method Microval Validation in Comparison to EN ISO 10272-2:2017 in Accordance with ISO 16140-2:2016

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Introduction: The Thermo Scientific Brilliance CampyCount Agar (BCCA) (alternative method) has been validated in accordance with EN ISO 16140-2:2016 for the selective enumeration of thermotolerant *Campylobacter* species in raw poultry products. The alternative method was previously validated by MicroVal certification in comparison to the EN ISO 10272-2:2006 reference method.

Purpose: To renew the validation in line with EN ISO 16140-2:2016, and assess the alternative method performance in comparison to the updated EN ISO 10272-2:2017 reference method.

Methods: Ten grams of raw poultry sample were homogenised with 30 ml of diluent and serially diluted. In duplicate, 100-µl aliquots were spread onto *Brilliance* CampyCount agar plates and incubated at 41.5±1 °C for 48±1 hours in microaerophilic conditions. Presumptive colonies were confirmed using the Thermo Scientific O.B.I.S. Campy Test. The alternative method was compared to the EN ISO 10272-2:2017 reference method.

Results: The relative trueness and accuracy profile study results satisfied requirements of EN ISO 16140-2:2016. The inter-laboratory study results from 14 laboratories showed no statistical bias between the alternative method and the reference method, the average repeatability across all spike levels was 0.18 for the alternative method and 0.21 for the reference method. The average reproducibility across all spike levels was 0.23 for the alternative method and 0.35 for the reference method.

Significance: The *Brilliance* CampyCount Enumeration method is equivalent to the EN ISO 10272-2:2006 reference method for the enumeration of *Campylobacter* species from raw and ready to cook poultry samples.

P3-30 Thermo Scientific *Listeria* Precis Enumeration Method: NF Validation EN ISO 16140-2:2016

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Introduction: The Thermo Scientific *Listeria* Precis Enumeration method (alternative method) has been certified by NF VALIDATION for the enumeration of *Listeria monocytogenes* from a broad range of foods and environmental samples.

Purpose: To renew the validation in line with EN ISO 16140-2:2016, and assess performance in comparison to the ISO 11290-2:2017 reference method.

Methods: Samples were enriched in buffered peptone water (one to ten ratio) and serially diluted as described in ISO 6887:2017. The alternative method was compared to the EN ISO 11290-2:2017 method during the relative trueness, accuracy profile, inclusivity and exclusivity, and inter-laboratory (ILS) studies.

Results: The relative trueness study (74 samples) results satisfied the requirements of EN ISO 16140-2:2016. The accuracy profile tolerance intervals were within the EN ISO 16140-2:2016 acceptability limits for all six matrix/strain combinations. Inclusivity testing (50 *L. monocytogenes*) isolates were all successfully detected and exclusivity testing (31 non-target) isolates showed no cross reactions. The ILS results from 10 laboratories were scatter plotted using reference method vs the alternative method; the data meets the Acceptability Limits (AL) for all levels of contamination, therefore is equivalent to the reference method.

Significance: The Listeria Precis Enumeration method is equivalent to the EN ISO 11290-2:2017 reference method for the enumeration of *Listeria monocytogenes* from a broad range of foods and environmental samples.

P3-31 Thermo Scientific Brilliance Staph 24 Enumeration Method Microval Validation ISO 16140-2:2016

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Introduction: The Thermo Scientific *Brilliance*™ Staph 24 enumeration method (alternative method) has been certified by MicroVal for the enumeration of coagulase positive *Staphylococcus* species from a broad range of foods.

Purpose: To renew the validation in line with EN ISO 16140-2:2016, and assess performance in comparison to the EN ISO 6881-1:1999 DAM2:2017(E) reference method.

Methods: Samples were enriched in buffered peptone water (one to ten ratio) and serially diluted as described in ISO 6887:2017. The alternative method was compared to the EN ISO 6881-1:1999 DAM2:2017(E) method during the relative trueness, accuracy profile, inclusivity and exclusivity, and inter-laboratory (ILS) studies.

Results: The relative trueness study (83 samples) results satisfied the requirements of EN ISO 16140-2:2016. The accuracy profile tolerance intervals were within the EN ISO 16140-2:2016 acceptability limits for all five matrix/strain combinations. Forty-nine out of 51 coagulase positive *Staphylococci* strains were all successfully detected during the inclusivity testing; the remaining two strains were not detected by the alternative or the reference method. Exclusivity testing (35 non-target isolates) showed 2 cross reactions from both the alternative method and the reference method and a further three cross reactions from the reference method only. The ILS results from 11 laboratories were scatter plotted using reference method vs the alternative method; the data meets the Acceptability Limits (AL) for all levels of contamination, therefore is equivalent to the reference method.

Significance: The *Brilliance* Staph 24 Enumeration method is equivalent to the EN ISO 6881-1:1999 DAM2:2017(E) reference method for the enumeration of coagulase positive *Staphylococcus* species from a broad range of foods and environmental samples.

Poster

P3-32 Thermo Scientific *Listeria* Precis Detection Method: NF Validation EN ISO 16140-2:2016

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Introduction: The Thermo Scientific *Listeria* Precis Detection method (alternative method) has been certified by NF VALIDATION for the detection of *Listeria monocytogenes* from a broad range of foods and environmental samples.

Purpose: To renew the validation in line with EN ISO 16140-2:2016 and assess performance in comparison to the EN ISO 11290-1/A1:2005 reference method.

Methods: Samples were enriched in Thermo Scientific ONE Broth *Listeria* (one to ten ratio) and serially diluted as described in ISO 6887:2017. The alternative method was compared to the EN ISO 11290-1/A1:2005 method during the relative level of detection (RLOD), sensitivity study, inclusivity and exclusivity, and inter-laboratory (ILS) studies.

Results: The RLOD study results were below the acceptability limit of 2.5 for an unpaired study design for (RLOD of 0.851 overall for all six matrix/strain combinations). During the sensitivity study, out of 438 samples tested, 21 negative deviations and 24 positive deviations were observed. The number of discordant results is likely due to the fact it is an unpaired study. The observed values for ((ND+PPND)-PD) were below or equal to the acceptability limit for each category individually and for all categories overall, as described in EN ISO 16140-2:2016. Inclusivity testing (50 *Listeria monocytogenes*) isolates were all successfully detected and exclusivity testing (31 non-target isolates) showed no cross reactions. The ILS results from 12 laboratories were analysed and the sensitivity, specificity of the alternative and reference method fulfilled the acceptability limits of EN ISO 16140-2:2016.

Significance: The Listeria Precis Detection method is equivalent to the EN ISO 11290-1/A1:2005 reference method for the detection of *Listeria monocytogenes* from a broad range of foods and environmental samples.

P3-33 Thermo Scientific Suretect *Cronobacter* Species PCR Assay: NF Validation Using the Applied Biosystems Quantstudio 5 PCR Instrument

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Introduction: The Thermo Scientific SureTect *Cronobacter* species PCR assay has previously gained NF Validation by AFNOR Certification for powdered infant formula samples (PIF) (10 g and 300 g) and production environmental samples using the Applied Biosystems 7500 Fast with Applied Biosystems RapidFinder Express 2.0 software according to the EN ISO 16140-2:2016.

Purpose: A study was conducted at ADRIA Développement laboratories to extend the validation claims to include the use of the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument using the RapidFinder Analysis Software version 1.0 for infant formula and environmental samples.

Methods: The following matrices were tested; 10 g PIF with and without probiotics, 300 g PIF with and without probiotics and production environment samples. The method includes a single step enrichment in buffered peptone water, supplemented with six mg/l of vancomycin depending on the sample type and size. Enrichments were incubated for 16 to 24 h (depending on sample type and size) at 37±1°C before the lysates were prepared and 20 µl aliquots of the lysates were transferred to SureTect PCR Tubes containing SureTect Cronobacter species PCR tablets. The tubes were then transferred to the QuantStudio 5 Instrument for processing. The alternative method was tested in comparison to EN ISO 22964:2017.

Results: A total of 208 milk powder and environmental samples were tested using the alternative and EN ISO 22964:2017 methods. Fourteen positive deviations were observed and confirmed with culture methods. The sensitivity of the alternative method was demonstrated to be 91.7% with a relative trueness of 89.4% and a false positive ratio of 6.3%. The alternative method demonstrated equivalent performance for all samples analysed in comparison to the EN ISO 22964:2017 reference method, during NF Validation by AFNOR certification studies.

Significance: The alternative method proved to be a suitable substitute to the EN ISO 22964:2017 reference method for *Cronobacter* species detection.

P3-34 Thermo Scientific Suretect *E. coli* O157:H7 PCR Assay: AOAC-RI PTM Validation Using the Applied Biosystems Quantstudio 5 PCR Instrument

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Introduction: The Thermo Scientific SureTect *E. coli* O157:H7 PCR Assay is a real-time PCR assay for the detection of *Escherichia coli* O157:H7, which has previously gained AOAC validation using the Applied Biosystems 7500 Fast PCR Instrument with Applied Biosystems RapidFinder Express version 2.0 Software.

Purpose: To gain an extension of the AOAC validation to include the use of the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument with the RapidFinder Analysis Software version 1.0. The validation consisted of an inclusivity and exclusivity evaluation and an unpaired method comparison using matrices of raw ground beef (375 g) and fresh spinach (25 g).

Methods: The candidate method was compared to the USDA/FSIS MLG 5.09 for spinach and ISO 16654:2001 for raw ground beef. The raw ground beef was analyzed utilizing both a one in four and a one in five enrichment ratio at both nine and 24 h post-enrichment. The spinach was analyzed utilizing a one in ten enrichment ratio at both eight and 24 h post enrichment. After enrichment the lysates were prepared and run on the QuantStudio 5 PCR Instrument.

Results: For all matrices, statistical analysis using probability of detection at 95% confidence levels demonstrated no statistical difference between the alternative and reference methods during any of the validation studies. All 52 *E. coli* O157:H7 inclusivity isolates were correctly identified and all 30 exclusivity isolates were correctly excluded.

Significance: The candidate method using the QuantStudio 5 PCR Instrument for PCR and RapidFinder analysis software for data analysis proved to be a suitable substitute to the reference methods for E. coli O157:H7 detection in raw ground beef, raw beef trim, fresh spinach and apple juice.

P3-35 Thermo Scientific Suretect *Listeria monocytogenes* Assay: AOAC-RI PTM and NF Validation Using the Quantstudio 5 PCR Instrument

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Introduction: The Thermo Scientific SureTect *Listeria monocytogenes* PCR Assay is a real-time PCR assay intended for the detection of *Listeria monocytogenes* from food products and environmental samples, which has previously gained AOAC-RI PTM and NF Validation by AFNOR Certification using the Applied Biosystems 7500 Fast PCR Instrument with Applied Biosystems RapidFinder Express version 2.0 Software.

Purpose: To conduct AOAC-RI PTM and NF Validation by AFNOR Certification extension studies to validate use of the SureTect *Listeria monocytogenes* PCR Assay with the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument with Thermo Scientific RapidFinder Analysis version 1.0 Software (the alternative method) for a broad range of foods and environmental samples.

Methods: The validation studies were conducted according to the AOAC-RI PTM, NF Validation and ISO 16140-2:2016 guidelines. For the alternative method, all samples underwent an enrichment step followed by direct lysis. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to EN ISO 11290-1:2017.

Results: A total of 150 (AOAC-RI PTM) and 387 (NF Validation) food and environmental samples were tested using the alternative and EN ISO 11290-1:2017 methods. The alternative method demonstrated equivalent performance for all human food and environment samples analysed in comparison to the EN ISO 11290-1:2017 reference method, during the AOAC-RI PTM and NF Validation by AFNOR certification studies.

Significance: The alternative method proved to be a suitable substitute to the EN ISO 11290-1:2017 reference method for *L. monocytogenes* detection.

P3-36 Improved *Salmonella* Detection from Primary Production Samples Using Multiplex PCR Methodology

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Introduction: The primary production environment contains high levels of microflora, leading to challenges in the detection of *Salmonella* from feces and other primary production samples (PPS). Limitations in traditional culture media methods for *Salmonella* detection from PPS mean contamination levels may be often under-reported.

Purpose: The purpose of this study was to evaluate *Salmonella* detection from PPS using the Thermo Scientific RapidFinder *Salmonella* Species, Typhimurium and Enteritidis Multiplex Flex PCR Kit in comparison with ISO 6579-1:2017.

Methods: An unpaired study was prepared by spiking fifty-seven PPS with *Salmonella* at 0.8 to 7.2 CFU/25 g. These were enriched alongside 12 unspiked samples in 225 ml tetrathionate broth followed by a short secondary enrichment in buffered peptone water before immunomagnetic separation, PCR and culture confirmation according to the alternative method. The ISO method was performed in parallel with another 57 spiked and 12 unspiked samples.

Following the same study design, a commercial poultry producer processed 13 PPS, spiked with around 15 CFU/25 g *Salmonella,* and 10 unspiked PPS for the alternative method and a replicate data set for the reference method.

Results: From the 69 PPS tested, the alternative and ISO methods detected *Salmonella* species in 39 versus 34 samples, *Salmonella* Typhimurium in 16 versus 12 samples and *Salmonella* Enteritidis in 14 versus ten samples respectively. The poultry producer identified *S*. species in 16 samples, *S*. Typhimurium in eight samples and *S*. Enteritidis in two samples with the alternative method. The ISO method confirmed 14 *S*. species only and did not differentiate the different serovars present.

Significance: These datasets demonstrate improved *Salmonella* detection, including differentiated serovars, by the alternative method compared to ISO 6579-1:2017, enabling more accurate monitoring of contamination levels in the primary production environment.

P3-37 Superior Detection of Multiple *Salmonella* Serovars from Meat and Environmental Samples Using a Multiplex PCR Method

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Introduction: *Salmonella* is a common contaminant of meat products posing a threat to public health. Foods may be contaminated with more than one serovar of *Salmonella* but limitations in international standard methods may result in only one serovar being confirmed as a present. This leads to poor accuracy in monitoring trends in prevalence.

Purpose: The purpose of this study was to evaluate the detection capabilities for coinfected samples of the Thermo Scientific RapidFinder *Salmonella* Species, Typhimurium and Enteritidis Multiplex PCR Kit in comparison with traditional ISO and FSIS MLG testing methods.

Methods: Fifty-eight 25-g samples, including pork and poultry and production environment samples, were dual-infected with 0.6 to 4.8 CFU *Salmonella* Typhimurium or *Salmonella* Enteritidis and an alternative additional *Salmonella* serovar. Twenty-nine samples were tested according to the alternative PCR method and 29 samples tested according to ISO 6579:2017. A further 58 unspiked samples were tested in the same unpaired manner.

In a second unpaired study, fifty 25-g samples of raw pork sausage meat were triple-infected with 0.8 to 4.0 CFU *Salmonella* Ohio, Typhimurium and Enteritidis. Twenty-five samples were tested according to the alternative PCR method and 25 samples tested according to FSIS MLG 4.09. A further 20 unspiked samples were tested in the same unpaired manner.

Results: From the dual-infection study, the alternative method detected 21 (72.4%) and confirmed 19 (65.5%) coinfected samples while the ISO method confirmed 11 (37.9%) coinfected samples. From the triple infection study, the alternative method detected 12 (48.0%) and confirmed seven (28.0%) triple infected samples while the FSIS method confirmed one (4.0%) triple infected sample.

Significance: These studies demonstrate the superior performance of the *Salmonella* multiplex PCR method for detection of multiple *Salmonella* serovars from a single sample compared to reference methodologies, facilitating improved reporting of serovars *Salmonella* Typhimurium and Enteritidis.

P3-38 Aptamer-based Platform for Optical Detection of *Salmonella* Enteritidis

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Introduction: Current *Salmonella* detection methods focus on detection in laboratory settings, which are often time exhaustive, laborious and require trained personnel.

Purpose: The purpose of this study is to create an aptamer-based platform that can detect *Salmonella* Enteriditis using optical visualization on a microscope.

Methods: Gold coated microscope slides were incubated with *Salmonella* Enteriditis specific aptamer to create a self-assembled monolayer through the thiol-gold bond. To complete the chip 6-mercapto-1-hexanol was incubated with the aptamer coated chip to backfill any uncoated gold area. Completed chips were incubated with *Salmonella* Enteriditis of various concentrations for 30 minutes then measured using a microscope on Raman DXRi. Parameters such as aptamer buffer, aptamer concentration, 6-mercapto-1-hexanol concentration, capture efficiency were all tested. The ability of the aptamer to discriminate from *Escherichia coli* was also tested. Pictures were taken of each chip then, Imagej was used to measure the amount of *Salmonella* bound a chip.

Results: Various immobilization buffers were tested to find the optimal buffer to facilitate gold-thiol interactions, and Dulbecco's phosphate buffer showed significantly stronger interactions. Aptamer concentrations from one to five µM concentrations were tested, with three µM showing the strongest capture efficiency. Aptamer based chips were measured for capture efficiency, they showed capture efficiency of 80% significantly higher than that of *E. coli*

Poster

Poster

(30%) and a non-aptamer chip (20%). The optical limit of detection measured showed statistically significant differences (P<0.0001) down to 10^{-4} CFU/ml from 10^{-7} of *Salmonella* as compared to a chip without *Salmonella (n=5)*.

Significance: Creating simple, and effective detection mechanism for *Salmonella* can reduce cases of foodborne illness in real water matrices and help prevent cross contamination.

P3-39 Validating a Method for Multiplex Screening of *Salmonella* Mutants for Survival on Dry Surfaces

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Introduction: *Salmonella* is an important foodborne pathogen able to survive low-moisture conditions for an extended period of time, although the underlying survival mechanisms remain poorly understood.

Purpose: This study was conducted in order to validate the effectiveness of a nitrocellulose (NC)- based screening method to identify *Salmonella* mutants with impaired desiccation tolerance.

Methods: Multi-gene deletion (MGD) mutant libraries of *Salmonella* Typhimurium strain 14028s were grown in 96-well plates at 37°C for 24 h. Mutants were spotted both on agar plates that were incubated (37°C, 24 h) and on sterile nitrocellulose (six cm by six cm), as model abiotic surface for multiplex screening. NC was dried, stored (25°C, dark), and after seven and 16 d overlaid on agar and incubated (37°C, 24 h). Mutants incapable of surviving on NC were further validated for impaired survival on NC after 24 h and seven d. In-shell pistachios were inoculated with selected mutants, dried to their original A_w and stored (25°C, dark). *Salmonella* populations were enumerated immediately after inoculation, after drying and periodically during storage on agar medium incubated at 37°C for 24 h.

Results: Of the 528 MGD mutants 12 were consistently impaired for survival on NC. Testing four mutants (D01, B03, A09 and D04) on pistachios revealed two mutants (D01 and A09) with significantly (P< 0.05) greater reductions after drying in comparison to the wild type parental strain. Subsequent reductions after seven, 20 and 30 days were also significantly higher for D01 and A09 vs WT. Notable among the genes deleted in the MGD mutants are *csgABCDEFG* and *mdoHGC* operons in D01, *fimACDFHIWYZ* operon in B03 and *recA* in A09.

Significance: Through the NC- based screening method, *Salmonella* mutants defective for survival on abiotic surfaces can be identified, and some of these mutants are also impaired for survival in low-moisture foods.

P3-40 ISO 16140-2 (2016) Validation of Genedisc for the Detection of Shiga Toxin-producing *Escherichia coli* from O157, O111, O26, O103 and O145 Groups

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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 top five serogroups (O157, O111, O26, O103 and O145). Whereas current methods for detection of *E. coli* O157:H7 are well-developed, alternative methods validated against ISO/TS 13136:2012 for top five serogroups are still lacking.

Purpose: For the first time, an independent study compared an alternative method for the detection of STEC from top five serogroups in raw dairy products, vegetables and raw ground beef to the ISO/TS 13136:2012 (reference method) according to the ISO 16140-2:2016 for NF-Validation (Afnor).

Methods: The alternative method is a combination of different real-time PCR assays (GeneDisc, Pall Genedisc Technologies) applicable after enrichment of 25-g test portions diluted (1:10) in buffered peptone water (supplemented with 10 mg/l acriflavin for raw dairy products; prewarmed for ground beef), incubated at 37±1°C for 16 to 20 h or 41.5°C±1°C for eight to 20 h (ground beef) prior to DNA extraction and PCR screening. The alternative method was compared to ISO/TS 13136:2012 in order to evaluate its sensitivity, relative level of detection (RLOD), inclusivity and exclusivity prior to performing the interlaboratory study.

Results: Overall, 231 samples were analysed with both methods, providing 105 to 109 positive results depending on the tested assays combination. Depending on the enrichment protocol and the tested assays combination, the sensitivity of the alternative method ranged between 83.8% and 85.3%, it was between 66.1% and 68.8% for the reference method. Among the five tested matrix/strain pairs, calculated RLOD values (0.761 to 1.249) indicated equivalent levels of detection for both methods. The 50 target strains were detected and no cross-reaction was observed. The interlaboratory study results were satisfactory.

Significance: Equivalent performances but better practicability were observed for the alternative method, for which the present study is the first step towards validation and compliance with European food safety regulation.

P3-41 Prevalence and Characterization of Thermophilic Sporeformers in French Dairy Powders

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Introduction: In the processing of dairy powders, pasteurization is the only heat treatment applied for the destruction of microorganisms. However, bacterial spores present in raw milk remain alive. Biofilm could also be formed in factories, especially in heat exchangers and vacuum concentrators. The temperature conditions in those promote the growth of thermophilic species. These spore-forming bacteria can alter the quality during the process or when the final product is rehydrated for use.

Purpose: The aim of this project is to detect, identify and characterize thermophilic spores in French dairy powders.

Methods: Sixty-two dairy powders samples were treated at 80°C for 10 min and 106°C for 30 min. For each sample, total thermophilic spores population was enumerated (or isolated after enrichment) on MPCA+BCP+PS (ISO/TS 27265:2009) and 313 bacteria were isolated. DNA of all of these bacterial isolates was extracted and amplified with a M13-RAPD PCR method. PCR products were separated by gel electrophoresis. Fingerprints have been analysed using Bionumerics Sofware (version 7.6) and clusters were formed with UPGMA analysis. The 16S RNA gene was sequence for 61 DNA samples regarding their clusters.

Results: Enumeration results show that the higher a powder is processed, the lower the contamination will be. Using UPGMA analysis, the 313 fingerprints obtained were separated into 23 clusters. Species identification of the isolates belonging to these clusters highlighted the spore diversity : 43% were identify as *Geobacillus stearothermophilus* species, 33% from *Bacillus* (29% *B. licheniformis*, one percent each *B. thermoamylovorans*, *B. coagulans*, *B. ginsengihumi*, and *B. smithii*), 20% from *Anoxybacillus flavithermus*, and one percent each *Aneurinibacillus thermoaerophilus*, *Brevibacillus group*, *Paenibacillus naphthalenovorans*, and undetermined bacteria.

Significance: Those results show the prevalence of some genii in milk powder. They are correlated with worldwide studies. Bacterial isolates will next be characterized by a phenotypic approach (enzymatic activities, heat resistance, and ability to form biofilms).

P3-42 Strains Used as Biopesticides from Foodborne Contaminants

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Introduction: *Bacillus thuringiensis* (Bt) is a widespread spore-forming bacteria with a complex life cycle, which is commonly found in soil, water, plants, stored cereals or dead insects. 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. Today it is successfully used against caterpillars, beetles, and flies, including mosquitoes and blackflies. Bt-based products containing crystal proteins and spores are applied to foliage, soil, water environments or even food storage facilities.

Yet from a farm to fork prospective, the major issue is the lack of tools to distinguish and track Bt-based bioinsecticide isolates from other closely related strains also belonging to the *Bacillus cereus* group that may be involved in food poisoning or food spoilage.

Purpose: The aim of this work is to distinguish strains used as biopesticide using pulsed-field gel electrophoresis to track presumptive *B. cereus* contamination.

Methods: A collection of 90 strains, representing the phylogenetic diversity encountered, was characterized for the presence of toxin-encoding genes, Guinebretiere *et al.* phylogenetic classification, as well as the observation of parasporal crystalline inclusions using a phase contrast microscope. The PFGE protocol has been adapted from Liu *et al.* (1997) and from Zhong *et al.* (2006).

Results: For most prevalent Bt strains used in Europe, all tested isolates show huge parasporal crystals and belong to group IV according to phylogenetic classification. The adapted PFGE protocol clearly enable the clustering of strains belonging to the subspecies *Bacillus thuringiensis aizawai*, *kurstaki* and *israelensis.*

Significance: PFGE is a robust and reliable tool used by the Centers for Disease Control and Prevention and European public health laboratory network for foodborne disease surveillance. This study enables the distinction of strains used as bioinsecticide from foodborne contaminants. This study is part of BtID project, supported by the French ministry CASDAR program.

P3-43 ISO 16140-2 (2016) Validation of RAPID'*B. Cereus* Method for the Enumeration of Presumptive *Bacillus cereus* group in Dairy Products, Ready to Eat and Ready to Reheat Products and Cereals, Spices, Dehydrated Fruits and Vegetables

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Introduction: *Bacillus cereus* group includes gram positive spore-forming rod-shaped bacteria, highly dispersed in the food production environment and potentially responsible for food poisoning. The RAPID'*B. cereus* plate allows specific enumeration of *B. cereus* group from food products within one day.

Purpose: An independent study compared this new alternative method to the EN ISO 7932:2005 (reference method) according to the ISO 16140-2:2016 for NF-Validation (Afnor).

Methods: Different matrices were tested: dairy products, ready-to-eat and ready-to-reheat products, cereals, spices, dehydrated fruits and vegetables. Two inoculation procedures were tested: spreading of 0.1 ml of initial suspension or decimal dilutions onto the plate (or one ml onto three plates), or pouring the medium on one ml of initial suspension or decimal dilutions. The plates were incubated 21 h at 30°C±1°C. Characteristic colonies (red colonies most often surrounded by an opaque halo) were enumerated. The possibility to store the plates for 72 h at 5°C ± 1°C was evaluated for spreading inoculation. The relative trueness, accuracy profile, inclusivity and exclusivity were investigated prior to the interlaboratory study.

Results: Overall, 73 and 66 samples were analysed by spreading and pour plate techniques respectively, providing 51 interpretable results each. Among these samples, 23.5% (spreading) and 27.5% (pour plate) were naturally contaminated. The mean difference between alternative method and reference method counts ranged between -0.06 and -0.14 log CFU/g. For spreading procedure, these performances remained unchanged after 72 h storage at 5°C. The lower and upper β-ETI were comprised within the acceptability limits fixed at +/- 0.5 log. The inclusivity/exclusivity study showed satisfying result, highlighting that the alternative plate allows the enumeration of colonies from the seven different phylogenetic groups within *B. cereus* group. Interlaboratory study results were satisfying.

Significance: The alternative method is a reliable method for the enumeration of *B. cereus* group and offers more practicability to the user than the reference method.

P3-44 Development of Standards for Conducting Microbiological Challenge Tests for Food and Feed Products (ISO 20976)

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Introduction: Under the general principles of the Codex Alimentarius on food hygiene, it is the responsibility of Food Business Operators to control microbiological hazards in foods and feed to manage microbial risks. Challenge test is one of the recognized approaches used to validate control measures within the HACCP system, as well as to assess microbiological safety and quality of food, food production processes, food storage conditions and food preparation recommendations for consumers.

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Purpose: The aim of ISO 20976 is to provide general requirements and guidelines for conducting challenge tests on food and feed products, while conforming to existing guidance documents including the one written by NACMCF in 2009. Studies dealing with growth of a specific microorganism in a specific food product relate to ISO20976-1 while ISO20976-2 is for inactivation studies.

Methods: Within the frame of the International Organization for Standardization (ISO), members from all over the world collaborate to create internationally recognized documents providing requirements, specifications, guidelines or characteristics that can be used consistently to ensure that products, processes and services are fit for their purpose.

Results: ISO/TC34/SC09/WG19 working group gathers international experts from food industry, food technology institutes, food testing laboratories, research centers and regulatory bodies. WG19 finalized in 2019 a standardized protocol to conduct challenge tests to study growth potential, lag time and maximum growth rate (ISO 20976-1:2019). This standard has a link with European legislation (Regulation 2073/2005) on microbiological criteria for foodstuffs. This is leading to the development of a new standard on the determination of cardinal values and their use in predictive microbiology (project ISO 23691), in parallel to working towards consensus for ISO 20976-2 describing challenge tests to study inactivation potential and kinetics parameters.

Significance: General and consensus documents on best practice for conducting challenge tests will ensure harmonization and efficiency between all stakeholders.

P3-45 *Salmonella* Typhimurium-specific Signatures as Targets for Detection by Using DNA Aptamers in Foods and the Environment

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* Typhimurium is most frequently associated with foodborne outbreaks. Standard microbial cultivation methods along with PCR are a gold standard for the detection of this pathogen, but these are labor intensive and take about 72 hours for a confirmatory diagnosis. Thus, rapid, specific, and cost-effective assay to detect and differentiate *Salmonella* infection from other foodborne pathogen are direly needed for early recognition to help curtail outbreaks, improve timely clinical interventions and routine surveillance to help prevent outbreaks.

Purpose: This study aimed to develop a highly precise and rapid detection for *Salmonella* Typhimurium using the outermembrane lipopolysaccaride (LPS) and oligopolysaccaride (OPS)-specific aptamers.

Methods: Lipopolisaccharide (LPS) and oligopolysaccaride (OPS) of *Salmonella* Typhimurium were extracted and used for a one step aptamer selection. The selected candidate aptamers were validated for binding to LPS and/or OPS using a dot blot assay. Aptamers bound to LPS and/or OPS dots were eluted and reamplified and cloned for DNA sequencing of individual candidates.

Results: Of thirty OPS-specific aptamer clones, two redundant of aptamer sequences were identifies. There was no redundancy in the amplicon from LPS-specific clones. A second selection against OPS and LPS samples have been performed and amplicons are currently being sequenced. Redundant aptamer sequences aptamers will be synthesized and tested a specificity to the respective targets. Furthermore, these candidate aptamers will be applied to the development of a rapid detection system that can be applied in the field.

Significance: These results suggest that the redundant aptamer sequences are likely capable of capturing *Salmonella* Typhimurium with high affinity and specificity.

P3-46 Amplified Nucleic Acid Single Temperature Reaction for Detection of Genogroup II Human Norovirus

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Introduction: Routine clinical detection assays are available for human norovirus (HNV), but methods are more cumbersome for food and environmental samples. One complication is the absence of detection platforms with potential to yield results on-site and in real-time.

Purpose: Develop and validate of a novel, rapid, and simple isothermal method for direct detection of genogroup II (GII) HNV.

Methods: A region of the RNA dependent RNA polymerase gene of GII.4 HNV was used as target for preliminary design of the amplified nucleic acid single temperature reaction (ANSR) assay. CDC Calicinet data were mined to identify epidemiologically relevant HNV GII genotypes upon which In Silico analysis was done to refine primer/ beacon sequences to assure broad reactivity. GII.4 Sydney and GII.7 strains were tested for best and worst case scenario performance, respectively, using ANSR and a traditional RNA extraction-RT-qPCR method. RNA standards were made to determine ANSR detection limits. Specificity testing was done using a panel of seven genetically similar and/or clinically relevant viruses.

Results: The optimized ANSR was able to detect both GII.4 Sydney and GII.7 with detection limits two to three log higher than traditional RT-qPCR, but without the need for prior RNA extraction and more rapid time-to-result. Detection limits for ANSR using RNA standards were 2.87±0.07 and 2.67±0.18 log copies for GII.4 Sydney and GII.7, respectively. No false positives were observed for any of the non-HNV screened (including hepatitis A, rotavirus, coxsackievirus, and HNV surrogates).

Significance: ANSR is a promising platform for rapid and sensitive detection of GII HNV, responsible for >85% of illness. Further characterization of sensitivity and detection limits is underway using a comprehensive HNV strain collection.

P3-47 Assessment and Comparison of Molecular Subtyping and Characterization Methods for *Salmonella*

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Introduction: The food industry is facing a major transition with regard to methods to be used for confirmation, characterization and subtyping of foodborne pathogens. While whole genome sequencing (WGS) is rapidly becoming both the method of choice and the gold standard for subtyping foodborne pathogens, routine use of WGS by the food industry may often not be feasible, either due to cost constraints or the need for rapid results.

Purpose: In order to facilitate decision making regarding selection of subtyping methods by the food industry, we compared classical serotyping, pulsed-field gel electrophoresis, multilocus sequence typing, ribotyping, and WGS (including WGS-based serotype prediction) for relevant performance characteristics, using *Salmonella a*s a model system.

Methods: Performance characteristics included cost, discriminatory power, expertise required for data collection and interpretation, global access to a given technology and time-to-result.

Results: Our literature-based assessment supports the superior discriminatory power of WGS, however, we also identify circumstances under which use of other subtyping methods may be warranted.

Significance: This presentation provides practical industry guidance and presents a starting point for further comparative evaluation of characterization and subtyping methods to be used by the food industry.

P3-48 Detection of *Salmonella* spp. and *Listeria monocytogenes* in Artificially Contaminated Processed Egg Products Using the Assurance GDS Pathogen Detection System

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Introduction: Both *Salmonella* species and *Listeria monocytogenes* are important foodborne pathogens. Poultry and egg products are among the many food types that may be contaminated with either organism. BioControl Systems, Inc. has developed the Assurance GDS pathogen detection system to allow fast and accurate detection of *Salmonella* and *Listeria monocytogenes* from foods.

Purpose: To demonstrate that the GDS for *Salmonella* and the GDS for *Listeria monocytogenes* assays can detect *Salmonella* spp. and *Listeria monocytogenes* respectively from artificially contaminated processed egg products.

Methods: Thirteen processed egg products were artificially contaminated with 0.03 CFU/g of two *Salmonella* species. One-hundred-g inoculated and uninoculated samples were enriched at a 1:10 ratio in buffered peptone water (BPW) containing ferrous sulfate for 18 to 24 h at 37°C. A total of 130 samples were processed manually and using the automation system PIPETMAX (PPMX), then tested using GDS *Salmonella* and culturally confirmed per the USDA method.

Similarly, eight processed egg products were artificially contaminated with 0.06 CFU/g of two *Listeria monocytogenes* strains. Inoculated and uninoculated 50-g samples were enriched at 1:10 ratio in Demi Fraser Broth (DFB) at 30°C for 30 to 48 h. A total of 80 samples were processed both manually and using PPMX, then tested using GDS *Listeria monocytogenes* and confirmed per the USDA method.

Results: A total of 81 of 104 inoculated and zero of 26 uninoculated processed egg product samples were positive using GDS *Salmonella*, with 100% specificity. Similarly, 54 of 64 inoculated and zero of 16 uninoculated processed egg products were positive using GDS for *Listeria monocytogenes*, with 100% specificity. Both assays yielded statistically equivalent data compared to the paired confirmation results.

Significance: The Assurance GDS pathogen detection system allows rapid and accurate detection of *Salmonella* and *Listeria monocytogenes* from processed egg products.

P3-49 Use of the Assurance® GDS EHEC ID Assay to Genetically Confirm *E. coli* O157:H7-Positive Contaminated Beef and Carcass Cloth Samples According to the Newly Revised USDA FSIS EHEC Definition

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Introduction: Raw meat products, such as beef trim and ground beef, are sources of *E. coli* O157:H7 (EHEC) outbreaks. The beef industry needs a rapid screening method to genetically identify the presence of EHEC, according to the USDA FSIS definition. This study proposed a novel secondary PCR screening method, Assurance GDS EHEC ID for *E. coli* O157:H7 (EHEC ID) assay, to identify EHEC.

Purpose: To validate GDS EHEC ID for *E. coli* O157:H7 as an assay which genetically confirms the presence of EHEC.

Methods: Ground beef, frozen finely textured beef and carcass sampling cloths were inoculated with EHEC and stabilized. Ninety-six samples, of 25 g to 375 g (or one carcass cloth), were inoculated with two different levels (0.003 CFU/g and 0.02 CFU/g) of two EHEC strains and enriched in mEHEC broth for 6.5 to 14 h. Samples were initially analyzed with the GDS for *E. coli* O157:H7 assay, AOAC OMA[S] 2005.04, to presumptively determine samples containing EHEC. All positive samples were next analyzed using the EHEC ID assay and culturally confirmed per USDA method. Inclusivity and exclusivity of the EHEC ID assay was performed by analyzing EHEC and potential cross-reacting organisms, including E. *coli* non-O157:H7 serogroups.

Results: Sixty out of 77 inoculated and none out of 19 uninoculated samples were positive using EHEC ID. The EHEC ID assay yielded 100% specificity compared to the paired USDA confirmation. Further, all 61 inclusivity strains and none of 59 exclusivity strains were identified as *E. coli* O157:H7; the EHEC ID assay demonstrated 100% specificity and sensitivity

Significance: This new secondary screening method, GDS EHEC ID for *E. coli* O157:H7, provides the industry with a more rapid option to genetically identify *E. coli* O157:H7 in beef and carcass cloth samples compared to the USDA culture methods and is compliant with the revised USDA definition of EHEC.

P3-50 Performance Comparison of Shiga Toxin-producing *E. coli* Multiplex Molecular Assays

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Introduction: Thermo Scientific SureTect *E. coli* O157:H7 and Shiga toxin-producing *E. coli* (STEC) Screening and Identification PCR Assays are real-time PCR based assays for the simultaneous detection and differentiation of *E .coli* O157:H7 and the top six non-O157:H7 serogroups in raw beef, produce and dairy samples. The SureTect STEC multiplex assay workflow includes a simple 15-minute sample lysis step followed by an 80 minute PCR run on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument.

Purpose: The purpose of the study was to compare the sensitivity and specificity of the SureTect STEC Multiplex Assays against a leading commercially available real-time PCR assay designed to detect O157:H7 and the top six STEC O serogroups in raw beef samples.

Methods: Sensitivity of *E. coli* O157:H7, O45, and O111 strains were compared during the study. In addition, a total of 90 *E.coli* strains representing various O-serotypes and 120 non-target strains were analyzed. Samples were lysed prior to PCR and analyzed with SureTect STEC Multiplex Assays as detailed in the product's manual. The alternative assay was performed on the same panel of samples according to the manufacturers´ instructions.

Results: SureTect STEC Multiplex Assay workflow proved to be more accurate and sensitive than the alternative assay testing. The sensitivity of the STEC Multiplex ranged between five and 25 CFU per PCR reaction depending on the O serogroup tested, whereas the alternative method returned less sensitivity across all of the strains. Both assays returned a false positive result for *E.coli* O45 with *Enterobacter asburiae*. In addition, the alternative assay returned a false positive result for *E.coli* O45 with *Citrobacter werkmanii* and *Enterobacter ludwigii*.

Significance: The study demonstrated that the SureTect *E. coli* O157:H7 and STEC method is a more reliable workflow for the detection and differentiation of *E. coli* O157:H7 and the top six non-O157:H7 serogroups than the other commercially available real-time PCR assay.

Poster

Poster

P3-51 Validation of a Novel Loop-mediated Isothermal Amplification Method for the Detection of *Salmonella* Enteritidis in Shell Eggs

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Introduction: *Salmonella* Enteritidis is a major public health concern worldwide. Rapid and accurate detection of *Salmonella* Enteritidis in eggs and egg products, which are the major sources of this pathogen, is imperative for surveillance and outbreak investigation. Most molecular detection methods are aimed at the genus *Salmonella*. A *Salmonella* Enteritidis specific LAMP method has been developed in our laboratory.

Purpose: To validate a *prot*6E gene-based LAMP method developed in our laboratory for detecting *Salmonella* Enteritidis in shell egg samples in comparison with the FDA BAM culture method and a real-time PCR method.

Methods: We conducted four separate trials with four *Salmonella* Enteritidis isolates of different phage types. Shell eggs were surface disinfected and cracked aseptically. Each trial consisted of 20 samples (inoculated at ~five cells/l), five positive controls (inoculated at ~50 cells/l), and five negative controls (uninoculated). Each sample/control contained one liter liquid eggs. Preparation of egg samples followed the FDA BAM method. DNA prepared from preenrichment cultures were used for LAMP and real-time PCR assays. LAMP assay was carried out on the Genie III device. PCR (TaqMan *Salmonella* Enteritidis Detection Kit) was performed on an ABI 7500 Fast real-time PCR instrument.

Results: There are 15, 13, 17, and 15 positives for *Salmonella* Enteritidis among 20 samples tested for each trial with FDA BAM culture methods. All 20 positive control samples were positive, and all 20 negative control samples were negative for *Salmonella* Enteritidis. LAMP and PCR results matched the BAM culture results.

Significance: Our newly designed *prot*6E gene-based LAMP method was equally effective in detecting *Salmonella* Enteritidis from shell eggs in comparison with real-time PCR and BAM culture methods. It could be used as another effective tool for the detection of *Salmonella* Enteritidis from shell eggs for FDA in outbreak investigation and enforcing the Egg Rule.

P3-52 Development of a Colorimetric Loop-mediated Isothermal Amplification Assay Using Molecular Beacon HRP-Mimicking for the Rapid Detection of *Listeria* spp. in Mushrooms

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Introduction: In Europe, imported enoki mushrooms were contaminated with *Listeria* species, particularly *Listeria monocytogenes,* and returned or discarded. In European eating style, the enoki mushroom is often consumed in salad without cooking, and contamination with *Listeria* spp. is important for European consumer health. Therefore, a rapid, sensitive, and convenient method for the detection of *Listeria* spp. in mushrooms and their cultivation farms is urgently needed.

Purpose: The objective of this study is to develop a colorimetric loop-mediated isothermal amplification (LAMP) assay using a molecular beacon, horseradish peroxidase-mimicking DNAzyme (HRPzyme), for the rapid and sensitive detection of *Listeria* spp. in mushrooms.

Methods: Two pairs (inner and outer primers) of primers targeting *ssrD* gene and a molecular beacon were designed and synthesized. The optimization of the colorimetric LAMP assay was performed by determining key factors such as concentration of molecular beacon and incubation temperature and time. Sensitive and specificity of the colorimetric LAMP assay were investigated, and the method was validated with artificially contaminated mushroom samples with known amounts of *Listeria* spp. (10^0 to 10^8 CFU/ml) were analyzed by the LAMP assay.

Results: The detection limit of the colorimetric LAMP assay optimized at 60°C was one fg/ml DNA (*ssrD* gene), and the method was confirmed to be specific to *Listeria* spp. The period required to finish the colorimetric LAMP assay was one h. The cutoff value of the method for the artificially inoculated mushroom samples was 1×10^1 CFU/g.

Significance: This study suggests that the LAMP assay for *Listeria* spp. can be used as a point-of-care molecular diagnostic technology because the method does not require any expensive instruments such as a thermocycler and detector.

P3-53 Rapid Detection of *Campylobacter* in Poultry Matrices Using a Loop-mediated Isothermal Amplification (LAMP)-Bioluminescent Assay

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Introduction: *Campylobacter* is one of the most common causes of diarrheal illness in the United States. *Campylobacter* spp. are microaerophilic and require complex media for optimal growth and viability. A combination of ready-to-use (RTU) media allowing growth under aerobic conditions and rapid detection technologies enable easier and faster detection.

Purpose: To evaluate the performance of RTU enrichment media and LAMP-bioluminescent assay for the detection of *Campylobacter* in primary production samples, poultry rinses and raw poultry products as compared to the MLG 41.04 method.

Methods: Primary production samples: Boot swabs from a farm (*n*=30) were enriched under aerobic conditions and analyzed for detection of *Campylobacter* by LAMP assay and culture confirmed with the MLG 41.04 method. **Carcass rinses:** Chicken carcass rinsates collected from rehang and post-chill sites (*n*=30 samples per rinse per method, total *n*=120) were analyzed by an unpaired study using LAMP assay and MLG 41.04 and culture confirmed. **Raw poultry products:** Product mixture rinses from 325 g of three types of raw products (ground turkey, *n*=5 per method; marinated breast, *n*=5 per method; and mechanically separated chicken, *n*=20 per method; total *n*=60) were analyzed with the LAMP assay or MLG 41.04 and culture confirmed.

Results: Primary production samples: Based on the results obtained, direct enrichment of primary production boot swabs in RTU medium under aerobic conditions is compatible with the LAMP assay. The presumptive results were in total agreement with MLG 41.04 culture confirmatio. **Carcass rinses:** For the rehang and post-chill rinsates, sensitivity and specificity was 100%, for both the LAMP assay and MLG 41.04 PCR method. **Raw poultry products:** Sensitivity and specificity was 100% for both LAMP assay and MLG 41.04 PCR method. No statistical comparisons were made as results for both methods (LAMP and PCR) were in total agreement for all matrices tested.

Significance: The RTU medium allowing aerobic incubation and the LAMP assay is comparable to the MLG 41.04 PCR and culture method for detection of *Campylobacter* in a variety of poultry matrices. The LAMP assay is also compatible with traditional blood-free Bolton enrichment broth.

Poster

P3-54 Comparative Evaluation of the Ready-to-Use 3M *Campylobacter* Enrichment Broth and the 3M Molecular Detection Assay 2 – *Campylobacter* for the Detection of *Campylobacter* in a Variety of Poultry Matrices

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Introduction: *Campylobacter* spp. are microaerophilic requiring complex media and incubation under microaerobic conditions for optimal growth and viability. The RTU enrichment broth and the LAMP-bioluminescent assay is designed for rapid and specific detection of *Campylobacter* after 22 to 26 hours of enrichment under aerobic conditions.

Purpose: To compare the CEB and the LAMP assay to the MLG 41.04 for chicken carcass rinse, parts rinse, raw ground poultry, turkey carcass swab and to ISO method for chicken nuggets.

Methods: Four matrices by the LAMP method and the MLG 41.04 and one matrix (chicken nuggets) by the LAMP method and ISO 10272-1:2017 were compared. Naturally and artificially contaminated samples were enriched in CEB for 22 to 26 hours at 41.5±1°C under aerobic conditions (LAMP) and in blood-free Bolton Enrichment broth (BF-BEB) for 46 to 50 hours at 42±1°C under microaerobic conditions (reference). Naturally contaminated samples were carcass rinse, parts rinse and raw ground poultry. Artificially contaminated samples were turkey carcass (100 CFU per carcass, low; 1000 CFU per carcass, high) and chicken nuggets (one CFU/25 g, low); four CFU 25 g, high). Both enrichments were analyzed by the LAMP method and culture confirmed.

Results: The LAMP assay detected all the 50 *Campylobacter* strains tested and did not detect any of the 30 strains from the exclusivity panel. LAMP method detected more confirmed portions (significant difference) than the reference method for poultry parts rinses, raw ground chicken and turkey carcass sponge samples. No significant differences were observed for the chicken carcass rinses and chicken nuggets between the LAMP method and the reference methods. The BF-BEB was compatible with the LAMP assay.

Significance: For all matrices evaluated, the RTU enrichment broth, CEB and the 3M Molecular Detection Assay 2 - *Campylobacter* was equivalent or better than the reference methods for the rapid detection of *Campylobacter*. Hence, LAMP assay is a reliable method for the rapid and specific detection of *C. jejuni, C. lari* and *C. coli* in raw and cooked poultry products.

P3-55 Rapid Detection of *stx1, stx2* and *Eae* from Shiga Toxin-producing *Escherichia coli* in Meat, Produce and Raw Dairy Samples Using Loop Mediated Isothermal Amplification and Bioluminescence Detection

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Introduction: Shiga toxin-producing *E. coli* (STEC) are characterized by the production of Shiga toxins and intimin adhesin. STEC is commonly identified through molecular detection of genes encoding these proteins (*stx1, stx2,* and *eae*). STEC transmission occurs through the consumption of contaminated food and water and can cause human disease such as gastroenteritis, bloody diarrhea and the development of hemolytic uremic syndrome (HUS).

Purpose: To evaluate the performance of a loop mediated isothermal amplification (LAMP)-bioluminescent gene screen method for the detection of STEC in food by comparison to reference PCR gene screen methods USDA MLG 5b.05, FDA BAM Chapter 4A and ISO/TS 13136:2012.

Methods: Inclusivity and exclusivity for 216 bacterial strains with or without *stx1*, *stx2* and *eae* genes were assessed following AOAC guidelines. Method comparison in food was performed with portions of raw meat (*n*=80), raw dairy (*n*=40), and leafy produce and sprouts (*n*=120) inoculated with one to 10 CFU STEC/matrix. Matrices were analyzed as paired and unpaired samples using the LAMP-bioluminescent and reference methods as applicable. Method comparison results were analyzed using Chi-square analysis.

Results: The LAMP-bioluminescent assay provided 100% inclusivity for 116 strains containing combinations of *stx*1, *stx2* and *eae* genes and 100% exclusivity for 100 non-STEC strains. Performance of the method for each matrix showed no significant differences when compared to the reference gene screen methods. The lowest concentration detected by the method was one to five CFU STEC/sample, up to 375 g.

Significance: The LAMP-bioluminescent method offers a rapid and specific approach for the detection of Shiga toxin genes (*stx1/stx2*) and the intimin gene (*eae*) from STEC. This offers food manufacturers and commercial laboratories a next-day result to evaluate the microbiological quality and safety of raw meat, raw dairy, sprouts and leafy greens**.**

P3-56 Performance Evaluation of a Loop-mediated Isothermal Amplification-Bioluminescent Assay for Rapid Detection of *Salmonella* spp. in Boot Swabs and Animal Feed from Brazil

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Introduction: Poultry is recognized as a recurrent vehicle for *Salmonella* and a significant source of salmonellosis. Poultry industry requires control beyond steps of slaughtering and food production, but also control of farm conditions through monitoring of boot swabs and animal feed. Rapid and accurate *Salmonella* detection is critical to increase food safety and protect the consumer and avoid possible negative impact on the brand image.

Purpose: To determine the specificity, sensitivity and accuracy of a loop-mediated isothermal amplification (LAMP)-bioluminescent assay (AOAC 2016.01 and NF 3M 01/16-11/16) for boot swab and animal feed samples compared to ISO 6579-1:2017.

Methods: In a paired study seventy-six (49 boot swab and 27 animal feed) samples (naturally contaminated) from a local farm were preenriched 1:10 in buffered peptone water ISO at 37°C for 20 h, and analyzed with LAMP-bioluminescent assay and ISO 6579. Sensitivity, specificity and accuracy were determined.

Results: Compared to the traditional method, sensitivity, specificity and accuracy of the LAMP bioluminescent assay was 100%, 90% and 96% for animal feed and 100%, 90% and 95% for boot swabs samples, respectively.

Significance: The alternative LAMP-bioluminescent molecular method enabled reliable, rapid and automated detection of *Salmonella* spp. in animal feed and boot swabs.

P3-57 Performance Evaluation of a Loop-Mediated Isothermal Amplification-Bioluminescent Assay for Rapid Detection of *E. coli* O157 in Brazilian Raw Beef and Hamburger Patties.

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Introduction: Raw beef and meat-associated products are a potential source for *E. coli* O157 food poisoning leading to public health problems and economic losses. Rapid and accurate detection of *E. coli* O157 is critical to increase food safety and prevent a negative impact on the brand image.

Poster

Purpose: To determine the specificity, sensitivity and accuracy of a loop-mediated isothermal amplification (LAMP)-bioluminescent assay (NF 3M 01/18-05/17) compared to a PCR method for artificially contaminated raw beef and hamburger patties.

Methods: Samples (12 raw beef and 12 hamburgers, *n*=24), were artificially contaminated with *E. coli* O157:H7 and *E. coli* ATCC 25922 (as an interferent) and compared using an unpaired study design. For the LAMP method, 25-g samples were enriched in 225 ml buffered peptone water ISO for 20 h at 41.5°C for raw beef and 37.0°C for hamburger. For PCR method, 65-g samples were enriched in 195 ml mTSB for 20 h at 35.0°C. Six sets of samples were used with different levels of inocula, where *n*=samples per inoculum per method.

i) Low: 0.66 CFU/25 g (LAMP) and 1.71 CFU/65 g (PCR), *n*=3.
ii) Medium: one CFU/25 g (LAMP) and 2.6 CFU/65 g (PCR), *n*=3.
iii) high: six CFU/25 g (LAMP) and 15.6 CFU/65 g (PCR), *n*=1.
iv) low with interferent: 15 CFU (LAMP) and 39 CFU (PCR) *n*=3.
v) only interferent: 15 CFU (LAMP) and 39 CFU (PCR) per sample, *n*=1.
vi) Uninoculated, *n*=1.Samples were analyzed with both methods and sensitivity, specificity and accuracy were determined.

Results: Sensitivity, specificity and accuracy were 95, 100 and 96% for LAMP and 91, 100 and 92% for PCR, respectively.

Significance: The alternative LAMP-bioluminescent molecular method enabled reliable, rapid and automated detection of *E. coli* O157 in raw beef and hamburger patties.

P3-58 Performance Evaluation of a Fluorescence Resonance Energy Transfer Based Real-time PCR in a Unit Dose Format (SLM2) for the Detection of *Salmonella* spp. in 375 g Dark Chocolate

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Introduction: Chocolate has been sporadically but consistently linked to human Salmonellosis. Increased focus on the preventative measures due to Food Safety Modernization Act implementation has further increased the significance of the role played by the pathogen detection methods such as PCR. Dark chocolates are a complex food matrix containing a range of components that can contribute to *Salmonella* contamination, and can interfere with the PCR. Cacao levels greater than 50% present one such challenge as they contain increased amounts of documented PCR inhibitors like polyphenols.

Purpose: A study was conducted to evaluate the performance of traditional GENE-UP *Salmonella* assay (SLM), and unit dose format, a pelleted lyophilized master mix, (SLM2) for the detection of *Salmonella* spp. from varied sources of dark chocolate at 375 g sample size.

Methods: Dark chocolate matrices with different cacao percent (50 and 100% cacao) were tested. Each dark chocolate matrix was tested per AOAC validation guidelines using 30 unpaired samples, where five and 20 replicates were inoculated with *Salmonella* Typhimurium at a high (~two CFU/test portion) and low levels (0.2 to two CFU/test portion), respectively. Five uninoculated samples were tested . Unpaired test portions were evaluated by SLM/SLM2 and the reference method (FDA-BAM Ch5). All SLM/SLM2 tested samples were enriched in buffered peptone water (1:10) for 22 to 24 h at 42°C. All presumptive SLM/SLM2 results were confirmed with the reference and the alternative culture confirmation method.

Results: As per the {dPOD} evaluation, no significant differences were (95% CI) were observed in the SLM/SLM2 performance as compared to the reference method. For 50% cacao [dPODc: 0.0 to 0.2; LCL: -0.43; UCL: 0.43] ; while for 100% cacao [dPODc: 0.0; LCL: -0.28; UCL: 0.28] data were reported.

Significance: These data indicate that i) the unit dose format (SLM2) performed equally well with the SLM; ii) both SLM/SLM2 had performance equivalent to the reference method when testing dark chocolate at 375 g sample size.

P3-59 WITHDRAWN

P3-60 Surface Plasmon Resonance-based *Salmonella* Typhimurium Detection Using Antibody-linked Magnetic Nanoparticles for Capturing, Purification, and Signal Amplification

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* contaminated leafy vegetables are responsible for 17 outbreaks, 762 illnesses and 26 hospitalizations in United States. Although different methods are available to timely detect *Salmonella* in foods, surface plasmon resonance (SPR) has benefit of real-time, label-free and rapid detection with higher sensitivity and specificity.

Purpose: The purpose of this study was to develop a SPR method in conjugation with the magnetic nanoparticles for rapid detection of low levels of *Salmonella* in leafy vegetables.

Methods: Samples of green leaf lettuce were spiked with *Salmonella* Typhimurium (5.5, 6.0 and 6.5 log CFU/g), and washed with distilled water. Bacteria from washed solution were collected on 0.22 µm pore sized filter membrane after vacuum filtration. The numbers of inoculated bacteria in the samples were determined by XLT-4 agar. Flagellin antigen was captured by the antibody coupled magnetic nanoparticles. The flagellin–antibody–nanoparticles complex was washed and eluted with sample buffer. The whole complex was then injected onto the SPR sensor surface immobilized with a flagellin-specific monoclonal antibody. SPR signals resulting from the binding of complex on sensor surface were recorded and analyzed. Each experiment had six replications.

Results: Detection signals from nanoparticles captured samples were 13 times higher than signals from nanoparticles sandwiched on flagellin samples that were already captured on sensor surface. The biosensor detection sensitivity was 4.9 log CFU with detection signal of 13±9 µRIU. The lowest detection limit in spiked samples was 5.5 log CFU/g with detection signal of 21±13 µRIU in less than four h.

Significance: Our results suggest that SPR coupled with magnetic nanoparticles can be used for rapid detection of *Salmonella* Typhimurium in leafy vegetables with higher specificity and sensitivity. Further study will be conducted to apply this method for detection of other serotypes of *Salmonella* in different foods.

Poster

P3-61 Sensitivity of Petrifilm Staph Express Count Plate for Enumeration of *Staphylococcus aureus* in Various Foods

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❖ ***Developing Scientist Entrant***

Introduction: To enumerate *Staphylococcus aureus* in food, agar media are usually used as a conventional method that needs time for media preparation and plating, and space for the incubation. Therefore, it is necessary to develop a detection method for *S. aureus* that can save time and space.

Purpose: The purpose of this study was to examine the sensitivity of Petrifilm Staph Express Count Plates (STX petrifilm) for detecting *S. aureus* in various foods.

Methods: Twenty-five gram portions of six food samples (beef (chuck tender), marinated pork chop, semi-dried squid, dried filefish, rice cake, and *Japchae* (stir-fried glass noodles)) were inoculated with a mixture of *S. aureus* strains (ATCC29213, ATCC25923, and ATCC13565) at two, three, five, and seven log CFU/g. The samples were left under laminar flow for 15 min to allow the cell attachment and stored at 4°C and 25°C for zero and 24 h. To compare the sensitivity, *S. aureus* cell counts were enumerated on Baird-Parker agar (BPA) and STX Petrifilm, and the results were compared with the *t-test.*

Results: The recovered *S. aureus* cell counts were not generally different between STX petrifilm and BPA. As a result of analyzing the level of significance between the two media, it was confirmed that there was no significant difference between two selective media regardless of the type of food or inoculation concentration (P>0.05). In conclusion, STX Petrifilm is judged to be a replacement for the conventional method of agar culture, as its detection power is similar and convenient compared to BPA.

Significance: The sensitivity of STX is not different than BPA; thus, using STX Petrifilm for *S. aureus* detection in food can save time and space.

P3-62 Characterization and Analysis of *Campylobacter F*lagellin Protein Using a Panel of Monoclonal Antibodies

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter* is one of the most common pathogens responsible for diarrheal related illness globally. Flagella present in the bacteria are not only involved in locomotion but also play an important role in its antigenicity. The flagellar filaments consist of two genes fla A and fla B and are highly homologous to each other.

Purpose: The purpose of this study was to characterize and compare the diversity of flagellin and its related fragments based on the binding patterns of two sets of monoclonal antibodies.

Methods: Ten bacterial strains from three species of *Campylobacter,* namely *C. jejuni, C. coli and C. fetus,* were grown in microaerophilic conditions in *Campylobacter* blood-free agar (CCDA) maintained at 37°C for 96 h. Flagellin proteins from various strains were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot assay. Two groups of monoclonal antibodies (Group I and V) were used to analyze the binding patterns with respect to expressed flagellin.

Results: Both groups of monoclonal antibodies recognized the major band of flagellin protein at molecular mass of 65 kDa and also detected a diverse range of flagellin fragments among the bacterial strains. The Western blot assay showed distinct binding patterns of the two groups of monoclonal antibodies between 45 and 65 kDa among all tested *C. jejuni* strains. The variations in flagellin fragmentation were also observed within the same group of antibodies with respect to their molecular mass and binding intensity in the tested strains. Image tools were used for the comparison and the analysis of diverse flagellin patterns from distinct strains of *Campylobacter*.

Significance: The results showed that variations in binding patterns of monoclonal antibodies to flagellin protein differ considerably among strains of *Campylobacter.* These monoclonal antibodies therefore are valuable tools for strain identification and immunological detection of *Campylobacter*.

P3-63 Quality Indicator Testing of Chocolate and Other Confectionery Products with the TEMPO® Automated Enumeration System

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Introduction: TEMPO is an automated system for the enumeration of quality indicator (QI) organisms in foods. Chocolate and other confectionary products have historically been challenging for plating methods and other quality indicator systems. This can be problematic for chocolate manufacturer's production and quality control.

Purpose: An artificial contamination study was performed with the automated MPN system and various chocolate ingredients to demonstrate the efficacy of this method for the QI testing of chocolate and other confectionary products. For this study, the AC (aerobic count) and the YM (yeast and mold) assays were compared to traditional plating methods.

Methods: Test portions were spiked at three inoculation levels, approximately 200, 400 and 800 CFU/g with Bioball lyophilized cultures. The enumeration values obtained from both methods were converted into log values and an overall bias was determined. There were 144 replicates tested during this investigation.

Results: For AC, the calculated bias is -0.08 with a 95% confidence interval of (-.19 to 0.03) and a paired t-test value of 0.15. There was also an agreement within one log between methods of 96%.

For YM, the calculated bias is 0.10 with a 95% confidence interval of (0.04 to 0.17) and a paired *t*-test value of 0.003. There is a slight bias observed for the YM parameter for these data, however, the agreement rate between methods of 100% indicates a good overall correlation between TEMPO and plating.

A statistical review of the entire data set provided an overall bias of 0.014 with a 95% confidence interval of (-.05 to 0.08) and a paired t-test value of 0.66. The overall agreement between methods for both QI parameters was 98%.

Significance: The investigated system provides an automated method for the enumeration of QI organisms in chocolate and related confectionery products.

Poster

P3-64 A Comparative Evaluation of the GENE-UP *Listeria* spp. Assay for the Detection of *Listeria* Species in Deli Ham and on Stainless Steel Environmental Surfaces Unit Dose Format

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Introduction: A method comparison study for the new GENE-UP *Listeria* spp. (LIS 2) unit dose test kit was conducted following the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces.

Purpose: To modify the current AOAC Performance Tested Methods (PTM) approval for the *Listeria* spp. assay to include the new unit dose reagent format.

Methods: The candidate method assay was compared to the USDA/FSIS-MLG 8.09: Isolation and Identification of *Listeria* from Red Meat, Poultry and Egg Products, and Environmental Samples. Thirty total replicates were analyzed by both the candidate method and the reference method at three different inoculation levels. All analytical outcomes were biochemically confirmed by traditional reference methods. In addition, all candidate method enrichments were evaluated on the original multi dose format.

Results: For the deli ham, the candidate method obtained the following results for the three inoculation levels: zero of five for the uninoculated, six of 20 for the low and five of five for the high. This is compared to zero of five, seven of 20 and five of five, respectively, for the reference method. For the stainless steel, the candidate method obtained the following results for the three inoculation levels: zero of five for the uninoculated, 17 of 20 for the low and five of five for the high. This is compared to zero of five, 13 of 20 and five of five, respectively, for the reference method. For both deli ham and stainless steel, the candidate method demonstrated no statistically significant differences between presumptive and confirmed results ($dPOD_{CP}$) , between candidate and reference method results ($dPOD_C$) or between unit dose and multi dose method results ($dPOD_C$) for the food matrix tested.

Significance: The candidate method *Listeria* spp. unit dose assay is considered equivalent to the USDA/FSIS-MLG 8.09 for both products evaluated. AOAC has approved the new unit dose format for the *Listeria* spp. assay.

P3-65 Performance of 3M Petrifilm Rapid Aerobic Count Plates for Determining Aerobic Counts in Cocoa Products in Comparison to the Traditional Culture Method

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Introduction: The mesophilic aerobic count is used as a compliance requirement to assess the quality of cocoa products in Ecuador. A rapid and reliable method for quantitation of these microorganisms enables timely corrective action in processing plants.

Purpose: To determine the performance of the alternative method, AOAC OMA 2015.13 (3M™ Petrifilm RAC) in comparison to the traditional method, tryptone soy agar (TSA), in cocoa-based matrices, such as chocolate topping and cocoa powder.

Methods: Fifty Samples (N=50) of cocoa-based products (10 g) were diluted in 90 ml of sterile Butterfield's phosphate-buffered diluent (pH 7.2). Forty samples of cocoa powder matrix had natural microbial flora and 10 samples of chocolate topping matrix were inoculated with *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 at three different levels as below:

- Level 1: 0 to 20 CFU/g
- Level 2: 20 to 50 CFU/g
- Level 3: 50 to 100 CFU/g

The samples were tested by the alternative and traditional methods. The alternative method was incubated at 35 ± 1°C for 24 ± 2 h and TSA was incubated at 35 ± 1°C for 48 ± 2 h. The plates were enumerated and the statistical differences were determined using t-test (P=0.05).

Results: The results showed that there were no statistically significant differences (P=0.05) between the alternative method, AOAC OMA 2015.13 and the reference method for chocolate toping (P=0.39) and cocoa Powder (P=1.40). The average recovery (Log CFU/g) for cocoa powder was 2.88 for TSA and 2.84 for 3M Petrifilm RAC and for chocolate topping it was 1.26 for TSA and 1.25 for 3M Petrifilm RAC. The Correlation coefficient (R^2) between the two methods was 0.97.

Significance: 3M Petrifilm Rapid Aerobic Count Plate enabled reliable and rapid detection and counting of mesophilic aerobic bacteria in different cocoa products.

P3-66 Performance of Rapid Enumeration Methods for Lactic Acid Bacteria in Cured Meat Products from Brazil

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Introduction: Sausages and other meat products may include lactic acid bacteria (LAB) in its natural microflora. This group of microorganisms can grow during the meat curing process and reduce shelf life causing possible significant losses in the food industry due to brand damage. Monitoring of LAB using rapid and reliable methods allow for timely action to prevent food loss and reputation.

Purpose: To determine the correlation between two methods, 3M Petrifilm Lactic Acid Bacteria Count Plate and the reference method ISO 15214:2015.

Methods: A total of 26 sausage samples from the Brazilian meat industry were analyzed with 3M Petrifilm LAB Count Plate and ISO 15214:2015. Samples were divided into two groups: inoculated and uninoculated. The first one (n=10) was artificially contaminated with *Leuconostoc* spp. (two log CFU/g) and the second one (n=16) was naturally contaminated (seven log CFU/g). All the samples were paired and analyzed with 3M Petrifilm Lactic Acid Bacteria Count Plate (30°C for 48 h) and ISO 15214:2015 (30°C for 72 h, anaerobic conditions). A paired t-test was used to determine statistical differences (P=0.05).

Results: No statistical differences were observed between the 3M Petrifilm plate method and the ISO method to enumerate LAB in sausages. P-values obtained were 0.619 and 0.439 for inoculated and naturally contaminated samples, respectively.

Significance: The alternative method enabled reliable and rapid enumeration of lactic acid bacteria in sausage samples as compared to the ISO reference method.

P3-67 Performance of a Rapid *E. coli* Enumeration Method in Brazilian Dairy Products

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Introduction: Dairy products are an important item in the Brazilian diet and safety and quality control is a very important matter for the producer and the consumer. Food contamination causes significant losses in the food industry due to public health problems, recalls and negative impact on brand image. Monitoring of indicator organisms such as *E. coli* using rapid and reliable methods allow for timely action to prevent food loss and reputation.

Purpose: To determine the correlation between the two enumeration methods: 3M Petrifilm Rapid *E. coli*/Coliform Count (REC) Plate and ISO 16649-2:2018.

Methods: A total of 44 dairy product samples obtained from Brazilian plants were analyzed: powdered milk (n=6), cured cheese (n=11), lactose powder (n=12), dairy supplement (n=6), and fresh cottage-type cheese (n=9). Samples were contaminated with 0.2 ml of raw milk (approximately 10^4 CFU of *E. coli*). Each sample (25 g) was added to 225 ml of appropriate diluent: buffered peptone water (BPW) (cheese samples) or 0.1% peptone solution (other products) and homogenized. One-ml aliquots from a 1:10 dilution of the homogenate was plated on one 3M Petrifilm REC Plate and three TBX agar plates (0.3 ml, 0.3 ml and 0.4 ml) per sample. 3M Petrifilm REC Plates were incubated at 42°C for 18 h and TBX agar at 44°C for 20 h. Paired t-test was used to determine statistical differences (P=0.05).

Results: The 3M Petrifilm Plate method recovered a mean of 2.60 log CFU/g and TBX agar 2.68 log CFU/g. The alternative method was not statistically different for enumeration of *E. coli* in dairy matrices when compared with TBX agar(P=0.112).

Significance: The alternative method enabled reliable and rapid enumeration of *E. coli* in dairy matrices tested as compared to the respective ISO reference method. Additionally, the desired sensitivity (less than one ml) was obtained using a single REC plate instead of three TBX plates.

P3-68 Evaluation of an Alternative Method for Enumeration of Lactic Acid Bacteria in Brazilian Bacon

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Introduction: Lactic acid bacteria (LAB) are part of the autochthonous microbiota of bacon, necessitating monitoring of shelf life.

Purpose: Assess adequacy of 3M Petrifilm Lactic Acid Bacteria Count plates for enumerating LAB in bacon.

Methods: Bacon (n=40) were purchased from retail outlets in Brazil and diluted using buffered peptone water and Letheen Broth. Dilutions were plated and incubated following four protocols: i) MRS agar, pH 5.7, 30°C, ii) MRS agar, pH 5.7, anaerobiosis, 30°C, iii) APT agar, 25°C, iv) 3M Petrifilm Lactic Acid Bacteria Count Plate, 30°C. Colonies were enumerated after 24, 48 and 72 h, converted to log CFU/g and compared by ANOVA and regression (P<0.05). Colonies were selected and characterized as LAB by Gram stain and catalase.

Results: Mean LAB counts from MRS and the alternative method did not present significant differences independently of incubation time or diluent (P>0.05), while counts on APT after 24 h with buffered peptone water were significantly lower (P<0.05). Considering counts obtained with buffered peptone water, the alternative method presented significant correlation with MRS (r ranging from 0.87 to 0.89; in anaerobiosis, r ranging from 0.94 to 0.95) and APT (r ranging from 0.84 to 0.86) for each incubation time. For samples diluted in Letheen, the alternative method presented significant correlation with MRS (r ranging from 0.92 to 0.94; in anaerobiosis, r ranging from 0.93 to 0.96) and APT (r ranging from 0.77 to 0.79) for each incubation time. Ninety-seven percent (1,032 of 1,063) of colonies were characterized as LAB.

Significance: LAB counts from bacon using the alternative method did not present significant differences when compared to conventional methods, independently of diluent employed. 3M Petrifilm LAB plate results presented a high and significant correlation with MRS and APT, even after 24 h of incubation, indicating its reliability to provide a fast and practical enumeration of LAB.

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P3-69 Elimination of Sampling Error through Comminution of Food

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Introduction: Improving food sampling techniques continues to be a priority in the food safety industry. Microbial contamination of food can be localized in "hot spots", creating the potential for pathogens to go undetected through traditional sampling techniques, such as random sampling.

Purpose: The random sampling technique takes a subsection from various areas in the sample to get a "representative portion," leaving large areas untested. The purpose of this study was to develop a method of comminution of food by blending to improve pathogen detection ability.

Methods: Retail ready-to-eat food commodities deemed high-risk for *Listeria monocytogenes*, *Escherichia coli*, or *Staphylococcus aureus* were targeted for analysis. An analytical portion was removed from the sample using the random sampling method. The remaining sample was then blended along with sterile Butterfields bhosphate Buffer water, as needed to facilitate blending, and a second equivalent analytical portion was then removed, based on the amount of buffer water added. The two analytical portions where tested in parallel using a modified FSIS *Listeria* method, the TEMPO System, and 3M Petrifilm Staph Express Count Plate method.

Results: During this study, 2742 total analyses were performed on 778 unique food samples. Across all commodities and target organisms, there was an increase of 77% in detection of violative contaminants in blended samples versus traditional random sampling. *L. monocytogenes* was detected in 24 of the 729 analyses using blending detected the pathogen with 50% missed via random sampling. *E. coli* was detected in eight of 337 samples using blending with 12.5% missed via random sampling. *S. aureus* was detected in seven of 305 samples using blending with 57% missed via random sampling.

Significance: Comminution by blending allows for more effective mixing of samples leading to an increase in the detection capability of pathogens in food across all tested commodities.

P3-70 One-Step Enrichment Broth for the Simultaneous Recovery of *Salmonella enterica* and *Cronobacter sakazakii* in Powdered Infant Formula

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Introduction: *Salmonella enterica* and *Cronobacter sakazakii* are categorized as dangerous contaminants of powdered infant formula (PIF) that represents a serious health risk for newborn infants. Conventional culture methods are time consuming and not user-friendly for detecting both pathogens. Therefore, the development and implantation of rapid and cost-effective methods allowing for the simultaneous detection of these pathogens remain important.

Purpose: The objective of this study was to develop a new enrichment broth that allows for the one-step simultaneous recovery of *S. enterica* and *C. sakazakii* followed by both RT-PCR and culture detection.

Methods: Response surface methodology was adopted to optimize the broth formula for achieving maximal recovery of both *S. enterica* Agona and *C. sakazakii* co-cultured for 16h at 35°C using the ratios of 1:1, 1:100, 100:1. The PIF samples were inoculated with *S.* Agona and *C. sakazakii* (1:1) and stabilized at room temperature during 14-28 days. One hundred and seventy 25g samples were enriched with the one-step broth for 16h at 35°C and compared to the reference method tested samples. The enrichments were tested using Real-Time PCR and plated directly or after being concentrated using centrifugation onto differential selective agars.

Results: Regardless of the *S.* Agona/*C. sakazakii* ratio tested, both pathogens were able to reach at least 10^8 CFU/mL after a 16h co-culture at 35°C in the one-step broth. Equivalent performance to the reference method (ANOVA and t-test) was observed when the PIF samples that were contaminated with 0.55

Poster

Poster

and 0.41 CFU of sublethally injured *S.* Agona and *C. sakazakii,* respectively, were tested using the one-step broth. No false positive or false negative outcomes were confirmed by transferring to selective enrichment or by a concentration step.

Significance: The one-step enrichment broth allowed for the simultaneous recovery of *S. enterica* and *C. sakazakii* in PIF samples that significantly reduced the time-to-results for RT-PCR or culture-based methods.

P3-71 Evaluation of the Universal Enrichment Broth *Salmonella*, *Staphylococcus*, *Shigella*, *Listeria* and *E. coli* for the Detection of the Main Food Pathogens in Cheeses

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Introduction: Several enrichment broths and different food portion sizes are required for foodborne pathogen analysis depending on the selected method and the food to be analyzed. The use of a single enrichment broth for the growth of the main pathogens would limit the variability of method related peculiarities.

Purpose: The performance of SSSLE enrichment broth, designed for simultaneous growth of *Salmonella*, *Staphylococcus*, *Shigella*, *Listeria* and *E. coli* O157:H7, was evaluated using pasteurized and raw milk cheeses. The limit of detection of the methods using SSSLE broth was determined for *Salmonella*, *Listeria monocytogenes* and *E. coli* O157:H7, and then a comparison of the performance of this broth to those described in Health Canada's reference cultural methods was performed using the probability of detection statistical approach.

Methods: The SSSLE broth did not demonstrate its capacity to support the growth of *L. monocytogenes*, nor that of heat stressed cells (pasteurized cheese). However, the SSSLE limit of detection was determined at one CFU of *Salmonella* and *E .coli* O157:H7 per 25 g of cheese. Comparative trials on 180 unpaired cheese samples unspiked (20), spiked at limit of detection level (80) and ten times higher (80) demonstrated that SSSLE is equivalent to the reference broth for *Salmonella* growth and that its performance is significantly higher when compared with the reference broth for *E. coli* O157:H7 growth.

Results: The results demonstrate that SSSLE broth could be used for the simultaneous growth of *Salmonella* and *E. coli* O157:H7 in raw milk cheeses and other food matrices for which the microorganisms are not exposed to heat stress, such as fresh fruits and vegetables.

Significance: The impact of this project is the demonstration of significant improvements in laboratory methodology for tracking foodborne pathogens for organization testing high volume multi-commodity foods.

P3-72 Selective Supplement for One-Step Enrichment of Low Numbers of Sublethally Stressed *Salmonella* in the Presence of Competitive Flora

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Introduction: Buffered peptone water (BPW) is commonly used as a non-selective preenrichment broth improving the chances for recovering low numbers of sublethally injured *Salmonella* before being transferred to a selective enrichment. A high level of background flora in food can be a serious challenge for the successful detection of *Salmonella* due to an overgrowth of non-target bacteria that might interfere with the performance of the assays using RT-PCR and ELISA-based methods.

Purpose: This study was aimed on the development of a selective supplement enabling an efficacious control of high level of background flora without affecting the recovery and growth of low numbers of sublethally injured *Salmonella*.

Methods: More than 15 different substances including antibiotics, organic and inorganic compounds, detergents, and quaternary ammonium salts were screened for their antimicrobial activity. BPW was used as a basic broth to evaluate the effect of the selected molecules on the growth kinetics parameters of heat-injured *Salmonella* cultured at 35°C alone or in the presence of competing bacteria such as *E. coli*, *Citrobacter freundii*, *Halfnia alvei*, *Enterococcus faecalis*, and *Proteus vulgaris,* using a 1:100 ratio. Response surface methodology was adopted to optimize the selectivity, recovery and growth of *Salmonella*. All essays were carried out three times.

Results: Three inhibitory molecules were selected based on their capacity to control the growth of gram-positive and gram-negative competitive bacteria without affecting the lag phase and growth rate of *Salmonella*. The most efficacious concentrations of the molecules were assessed using factorial experiment that was followed by the central composite design. Using buffered peptone water, supplemented with the optimized selective formula, resulted in an improved recovery of *Salmonella* from meat and poultry samples when compared to the un-supplemented BPW. No interference with RT-PCR detection was observed.

Significance: The new selective supplement can be used with BPW for the one-step enrichment of *Salmonella* in food with high levels of competitive flora.

P3-73 Rapid Quantitative Enumeration of *E. coli* and Coliforms in Foods

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Introduction: Food matrices are routinely monitored for the presence of coliforms and *E. coli*, a part of the fecal coliform group which serve as quality and hygienic indicators. These microorganisms can grow in a wide range of temperatures and can pose health hazards to humans. Traditional methodologies to detect the confirmed presence of these organisms can take two to seven d and poses a burden on food producers. A novel dehydrated thin-film medium was developed to address the need for rapid (18 to 24 h) and simultaneous selective and differential detection of *E. coli*/coliform group of organisms in food matrices. The detection technology was optimized to detect inducible and constitutive *E. coli* in a broad range of temperatures compatible with global methodologies for the detection of these organisms.

Purpose: This study was performed to comparatively enumerate *E. coli*/coliform bacteria using a new dehydrated thin-film medium and reference methodology.

Methods: The method comparison was conducted using a variety of naturally and artificially contaminated food matrices (n>50) with the new dehydrated thin-film test method and routinely used reference methods (FDA/BAM Chapter 4, ISO 16649-2:2017). Each food sample was serially diluted in Butterfield's buffer and one ml was used for the candidate and the reference methods.

Results: ANOVA and mean log difference estimations demonstrated statistically comparable (P>0.05) recoveries of *E. coli* and coliforms across a wide variety of foods between the new dehydrated thin-film method at 18 to 24 h and the reference methods: VRBL- 24 h, 30°C and 35°C, TBX - 24 h, 44.5°C).

Significance: Rapid, quantitative detection of the *E. coli*/coliform group of organisms using a new dehydrated film method was evaluated for a wide variety of foods naturally and artificially contaminated with a variety of *E. coli* and coliforms. The new method was found to provide accurate, actionable results in a shorter time-period with significantly improved interpretation.

Poster

P3-74 Rhamnose-substituted Buffered *Listeria* Enrichment Broth Increases *Listeria monocytogenes* Enrichment Populations in Select Seafood Matrices

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Introduction: Buffered *Listeria* enrichment broth (BLEB) is the preferred liquid enrichment medium of the United States Food and Drug Administration (FDA) for recovery of *L. monocytogenes* from food matrices. Despite the inclusion of selective agents, the enrichment procedure tends to favor the growth of non-target microorganisms at the expense of *L. monocytogenes*. Altering the formulation of BLEB may be a way to improve its functionality and increase the likelihood of recovering *L. monocytogenes* from seafood matrices.

Purpose: This study evaluates the effect of substituting rhamnose (BLEB-rhamnose) for glucose in BLEB on populations of *L. monocytogenes* following selective enrichment of select seafood matrices.

Methods: Portions (25 g) of uncooked crab meat, shrimp, salmon, cod, squid, and scallops were spiked (one to five CFU/g) with *L. monocytogenes* (n=10 strains by three replicates). Selective enrichment was performed using BLEB or BLEB-rhamnose by the FDA-BAM method. *L. monocytogenes* was enumerated using PALCAM agar.

Results: The median *L. monocytogenes* enrichment populations with associated inter-quartile ranges (IQR) were 9.2 (9.2 to 9.4), 5.2 (3.9 to 5.9), 4.4 (3.7 to 4.9), 4.3 (3.3 to 4.6), 4.8 (3.1 to 5.7), 5.8 (5.1 to 7.1), and 6.9 (6.2 to 7.3) log CFU/ml for matrix-free crab meat, shrimp, salmon, cod, squid, and scallops, respectively, when BLEB was formulated with glucose. When BLEB-rhamnose was utilized, the median *L. monocytogenes* enrichment populations were 8.9 (8.4 to 9.0), 8.3 (6.9 to 8.7), 6.2 (5.1 to 6.8), 6.2 (5.4 to 7.0), 6.7 (5.1 to 7.7), 7.1 (5.3 to 7.7), and 7.6 (5.6 to 8.1) log CFU/ml for the same seafood matrices, respectively.

Significance: Rhamnose substituted BLEB increased *L. monocytogenes* median enrichment populations in all seafood matrices tested. For all but one seafood matrix (squid) the improved *L. monocytogenes* enrichment populations were statistically significant (P<0.01). Currently there are no known combinations of selective agents and incubation conditions that allow growth only of *L. monocytogenes*. Medium composition alteration and refinement may be a practicable way to improve the capabilities of regulatory agencies to recover this organism amidst a complex microflora.

P3-75 Evaluation of the 3M Petrifilm Rapid *E. coli*/Coliform Count Plate and 3M Petrifilm Rapid Aerobic Count Plate for Enumeration Microorganisms in Raw Milk Samples in Thailand

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Introduction: Thailand is a producer and exporter of dairy products and has raw milk production capacity around 2,800 tons a day, or just over one million tons per year. Achieving standard microbiological parameters allows milk producers to meet quality standards and compete in the marketplace. Rapid methods allow producers to quickly assess the milk quality and improve their practices.

Purpose: To determine the performance of the 3M Petrifilm Rapid *E. coli*/Coliform Count (REC) Plate and the 3M Petrifilm Rapid Aerobic Count (RAC) Plate in comparison to the FDA-BAM method for the enumeration of total coliforms and aerobic organisms in raw milk samples from Thailand.

Methods: The methods were compared using naturally contaminated (low, medium and high) raw milk samples for total coliforms (n=117) and aerobic (n=126) counts. Each raw milk sample was homogenized and one ml of the homogenate was serially diluted in Butterfield's phosphate buffer. One-ml samples were plated onto each of REC Plate and VRBA plate for coliforms and RAC Plate and Plate Count Agar (PCA) for aerobic organisms and then incubated at 32±1°C. Colonies were counted after 18 to 24 h (REC and VRBA plates), 24±2 h (RAC plates) and 48±3 h (PCA plates). Typical colonies from VRBA plates were further confirmed for gas production using brilliant green lactose bile broth at 24 and 48 h.

Results: Colony counts were used to determine correlation coefficient between the alternative and reference method. Coefficient of determination (R^2) between the two methods was 0.9345 for REC plates and 0.9679 for RAC plates indicating a high degree of correlation between both methods.

Significance: 3M Petrifilm REC and RAC plates provide ease of use and faster time to result (24 h) for enumeration of total coliform and total aerobic microorganisms in raw milk.

P3-76 Development of an Amperometric Biosensor Integrated with Biotinylated Bacteriophages as Novel Sandwich Biorecognition Elements for the Detection of Shiga Toxin-producing *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: Rapid and effective screening of viable Shiga toxin-producing *Escherichia coli* (STEC) cells can prevent STEC-related outbreaks. Current antibody-based biosensors provide specificity and selectivity by direct, indirect or sandwich detection; however, high-cost, instability, and cross-reactivity render it impractical and unreliable.

Purpose: The purpose of this study was to develop a portable STEC-specific amperometric biosensor using a sandwich-type approach, integrated with novel bacteriophages as capture and detection elements.

Methods: Three environmentally-isolated STEC bacteriophages (O26, O157 and O179) were characterized for hosts susceptibility, specificity, ultrastructure and virulence genes. Initially, STEC bacteriophages were chemically modified with biotin. To construct capture elements, biotinylated bacteriophages were immobilized onto the surface of activated streptavidin-coated screen-printed carbon electrode (SPCE) before blocking with 30% casein (overnight, 4°C). Streptavidin-horseradish peroxidase and gold nanoparticles were added to biotinylated bacteriophages solution (overnight, 4°C) to construct the detection elements. Samples (50 µl) were incubated on functionalized SPCE (12 min, room temp) before sequentially adding the detection element, H_2O_2 (40 mM) and 1,1′-ferrocenedicarboxylic acid for amperometric testing. ANOVA with Fisher's LSD was used to compare delta currents of STEC, non-STEC and blank samples.

Results: All bacteriophages were specific to STEC O26, O157 and O179 with icosahedral head and sheathed-tail (*Myoviridae* and *Siphoviridae*). All bacteriophages were also devoid of *stx*1/*stx*2. The optimum biotin concentration (10 mM) showed 94.47% bacteriophage viability. *Listeria monocytogenes* and *Salmonella* Typhimurium had delta currents below the threshold of positive detection (P<0.05). With less than one h turnaround time, the detection limits for O157, O26 and O179 were one, two, and two log CFU/ml with R^2 values of 0.97, 0.99 and 0.87, respectively.

Significance: Bacteriophages circumvent the drawbacks of traditional biorecognition molecules (antibodies). Biotinylated bacteriophages integrated into a portable electrochemical biosensor with a sandwich-type approach provided a rapid detection of viable STEC cells (one to two log CFU/ml) with superior sensitivity and specificity to immune-based assays.

Poster

P3-77 Direct and Rapid Detection of Shiga Toxin-producing

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❖ ***Developing Scientist Entrant***

Introduction: Biosensors combine biological components with physicochemical detectors, which can detect pathogens, such as Shiga toxin-producing *Escherichia coli (*STEC) and other significant analytes. Immunosensors have gained popularity due to bioaffinity and commercial availability but suffer cross-reactivity, interference and high production cost.

Purpose: The purpose of this study was to directly detect viable STEC cells on complex matrices using a portable STEC-specific bacteriophage-based electrochemical biosensor without enrichment and conduct cost analysis against commercially-available STEC screening methods.

Methods: An electrochemical biosensor, comprised of biotinylated bacteriophages, was used to capture and detect viable STEC cells by a sandwich-type recognition approach. Fresh ground beef (FGB) and pasteurized apple juice (PAJ) inoculated with STEC strains, and environmental water samples (25 g or ml) were prepared. Samples (50 µl) were then incubated on bacteriophage-functionalized electrodes (12 min, room temp) before sequentially adding bacteriophage-gold nanoparticles solution (20 ml), H_2O_2 (40 mM) and 1,1′-ferrocenedicarboxylic acid for amperometric test (100 mV/s). Delta currents were analyzed using ANOVA and LSD (P<0.05). A product cost structure was defined to determine the total cost per assay and compare it with current STEC screening methods.

Results: The detection limits (DLs) of FGB were one log CFU/g (O157) and two log CFU/g (O26 and O179) with R^2 values of 0.98, 0.95 and 0.76, respectively. DLs of PAJ were one log CFU/ml (O157) and two log CFU/ml (O26 and O179) with R^2 values of 0.94, 0.95 and 0.83, respectively. Negative controls and natural environmental samples had delta currents below the positive detection threshold. The estimated cost of a single test was $3.28, which is 75% and 77% lower than molecular-based and immunoassay-based methods, respectively.

Significance: The application of the bacteriophage-based biosensor on complex matrices revealed its superior sensitivity and specificity even without enrichment. Low cost and onsite screening capability make it as an excellent alternative to current STEC detection methods.

P3-78 Construction of a GFP-tagged *Listeria innocua* Strain for Use in Detection of Cross-Contamination in Food Testing Laboratories

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Introduction: Many food testing laboratories routinely test for the presence of *Listeria monocytogenes*, the causative agent of the foodborne illness listeriosis, by growing reference strains of the species for comparison. Risks of using these reference strains in the lab include the potential for cross-contamination, leading to false-positive identifications as well as accidental exposure to pathogenic microbes. Tagging the nonpathogenic sister-species *L. innocua* with GFP can lead to a safe and efficient method of confirming cross-contamination.

Purpose: The purpose of this study was to engineer a strain of *L. innocua* with the green fluorescent protein gene (*gfp*) integrated into the chromosome, allowing stable visual fluorescence.

Methods: The integrative plasmid pPL3e containing PSA phage-integrase, erythromycin resistance gene, and Hyper-SPO1 constitutive promoter fused to the *gfp* gene was integrated into the chromosome in a site-directed manner. Electrocompetent *Listeria innocua* NCTC 11288^T cells were electroporated with 1µg plasmid DNA. These cells were recovered for 24 h at 37°C on BHI media containing five µg/ml erythromycin. Transformants were visualized with 440 to 460 nm LED light to confirm fluorescence. Growth curve analysis comparing engineered and parental strains were conducted using a Bioscreen automated analysis system.

Results: Integration of the cassette into the chromosome was confirmed by molecular analysis including PCR and genome sequencing. Stability of the integration was confirmed by 100 generations of growth of the transformed strain in nonselective media, followed by transfer to selective media containing erythromycin. All 108 clones of the transformed strain retained antibiotic resistance and visual fluorescence. One-way ANOVA showed no statistically significant difference in growth between the engineered strain and parental strain (P=0.9997).

Significance: This engineered strain of *L. innocua* emits fluorescence visible by the naked eye, making it a useful tool for food testing laboratories to quickly and safely determine whether samples have been cross-contaminated by the reference strain.

P3-79 Comparing Anaerobic Systems, Culture Vessels and Initial Temperature of Enrichment Broth in the Recovery of *Shigella flexneri* from a High Background Level Food Type

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Introduction: ISO and FDA-BAM methods for *Shigella* recovery from food recommend anaerobic enrichment

Purpose: Determine if equipment used to achieve low or no oxygen during enrichment affects the final yield of *Shigella.*

Methods: ***Shigella*** Broth with 0.5 µg/ml of novobiocin was kept at room temperature or prewarmed to 35°C before inoculation with 10^8 CFU of microbial flora from 25 g spinach and two to four CFU of recombinant *S. flexneri* that fluoresce under UV. Five replicates were incubated at 42°C in three different enrichment vessels (metallic bags, 250 ml and 500 ml flasks) and anaerobic systems: workstation (0% O_2) hypoxic-CO_2 incubators (0.3% O_2) and seven-liter BD GasPak jars. After 22 hrs, serial dilutions were plated onto tryptic soy agar (TSA) and TSA with chloramphenicol to select for the recombinant *S. flexneri*. Total number of bacteria and fluorescent bacteria were recorded and compared to the initial number. In parallel, 10 µl loops of enrichment broths were streaked onto MacConkey plates for incubation at 35 to 37°C. *Shigella*-like colonies were recorded before confirmation under UV light.

Results: Samples that were not in very low O_2 during enrichment showed the least growth for *Shigella,* especially when the broth was not prewarmed, with a difference of four doublings for the *Shigella* population between both groups. Prewarming the broth reduced differences between GasPak, anaerobic chamber and hypoxic CO_2 incubator(P=1.3×10^{-6}) although *Shigella* growth was comparable when 500 ml flasks were used, independent of prewarmed status (P>0.05). Prewarming improved the recovery of *Shigella* on MacConkey. In four of five prewarmed groups, over 50% of the white colonies were *Shigella*, as opposed to roughly 10% in four of five non-prewarmed treatments, despite both groups having similar distributions of bacteria resembling *Shigella.*

Significance: Prewarming media at 35°C prior to enrichment from a high-background level food type improves the visibility of *Shigella* on MAC post-enrichment. Under those conditions, the differences between the three anaerobic systems, and/or the vessel types are minimal, which provides more flexibility to food microbiologists.

P3-80 Optimizing the Recovery of Wild Type *Shigella* from High Background Level Food Matrices

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Introduction: The ISO and FDA-BAM methods for *Shigella* recovery from food recommend anaerobic enrichment at high-temperatures in *Shigella* broth (SB) with novobiocin; however, induction of their type III secretion system (T3SS) at temperatures higher than 35°C may be burdensome.

Purpose: Optimize the competitiveness of wild-type *Shigella* in mixed cultures

Methods: The efficiencies of platings (EOP) of natural surface microflora of spinach and green onion were determined on SB agar plates (SBA), with zero, 0.5 or three µg/mL novobiocin, and incubated at 25, 30, 35 and 42°C. Various *Shigella* isolates, with an active or inactive T3SS, were plated onto TSA, tryptic soy broth agar (TSBA) and SBA alongside other *Enterobacteriacae* typically recovered during food enrichment, and incubated at 30, 35 and 42°C. After 24 h, colony sizes were compared to infer growth differences.

Results: Elevated temperatures were more selective than novobiocin on the initial quantity of bacteria in spinach and green onion rinsates. Compared to 25 and 30°C, EOPs were one to two and three-log lower at 35 and 42°C respectively. Growth of wild-type *S. dysenteriae* was inhibited on TSA at 35°C, compared to the non-virulent variant (P=0.007), but not on SBA [P=1.00] and TSBA [P=0.99]. At 42°C, the virulent isolate was more inhibited than the T3SS-defective isogenic strain on all media. No statistical difference was observed in the apparent growth of the *E. coli* strain competitor and *S. dysenteriae* at 35°C on SBA.

Significance: These results suggest that the current reference methods for *Shigella* favors competitors over virulent *S. dysenteriae* strains. We are currently testing if improved fitness at 35°C can compensate for spinach's higher level of initial competitive flora. The recovery rates of *S. dysenteriae*, *S. flexneri* and *S. sonnei* from artificially contaminated spinach rinsates are being compared at 35, 42 and 42°C after initial incubation at 35°C.

P3-81 Compatibility of Polymorphic Locus Sequence Typing with Commercially Available Environmental Sampling Tests for *Listeria* and *Salmonella*

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Introduction: In response to FSMA and its emphasis on proactive food safety, food processors are increasingly investing significant resources into environmental sampling to detect *Listeria monocytogenes* and *Salmonella enterica* contamination. This is reflected in the recent commercialization of several all-in-one environmental sampling tests (ESTs) for these two highly problematic bacteria.

Purpose: For presumptive-positive ESTs, we hypothesized that a relatively minor additional investment could provide, in addition to species confirmation, valuable strain typing data for tracking pathogen spread through a facility, identifying harborage sites, and distinguishing sporadic from resident contaminants. The basis for this hypothesis is the demonstrated compatibility of polymorphic locus sequence typing (PLST) with crude samples including ground beef and poultry enrichments.

Methods: The InSite (Hygiena) ESTs are representative of a group that conveniently combine disinfectant-neutralizing swab with selective medium that undergoes a color change following pathogen growth. InSite *Listeria* tubes were spiked with dilutions of *L. monocytogenes* strains F2365 (serotype 4b) or 706 (serotype 1/2a) and incubated at 37°C for 48 h; as expected, inoculated tubes underwent a color change from yellow to black. Similarly, InSite *Salmonella* tubes were inoculated with dilutions of *S. enterica* strains Enteritidis 505 and Kentucky 501 and similarly incubated; inoculated tubes changed from purple to yellow. From all inoculated tubes and uninoculated controls, aliquots were heat-treated to inactivate the bacteria, and DNAs purified and used as templates in PCRs targeting the previously described PLST loci LmMT2 or SeMT1.

Results: Gel electrophoresis revealed single clear products of the expected size from all inoculated ESTs; uninoculated controls were negative. PCR products were subjected to dideoxynucleotide sequencing, yielding high quality chromatograms, and clustal and BLASTN analyses demonstrated identity to previously determined sequences for these strains.

Significance: These results demonstrate the feasibility of directly coupling commercially available ESTs with PLST for *Listeria* and *Salmonella*.

P3-82 Culture-independent Typing of Foodborne Pathogens in Poultry Products

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Introduction: Strain typing provides the critical data that food processors need to track down bacterial pathogens, identify harborage sites, and distinguish sporadic from resident contamination. However, established methods for strain typing are costly, technically complex, time consuming, and require pure cultures. Polymorphic locus sequence typing (PLST) is a promising alternative that targets the one or two most phylogenetically informative tandem repeat-containing loci within a bacterial species.

Purpose: As a PCR-based method, PLST has demonstrated compatibility with crude samples including food and environmental enrichments, substantially reducing turnaround time. Here we explore the feasibility of culture-independent PLST (ciPLST), to save additional time and address challenges associated with difficult-to-culture pathogens.

Methods: Retail chicken and turkey samples (16 total) were swabbed, and bacteria eluted in saline. Following centrifugation, DNA was purified from pellets with commercial kits optimized for removal of PCR inhibitors. Nested PCR was used to amplify PLST loci YeMT1 (for *Yersinia enterocolitica*; diversity index=0.98), CjcMT1 (*Campylobacter jejuni/coli*; 0.97), and SeMT1 (*Salmonella enterica*; 0.99). To examine sensitivity, a chicken sample was spiked with dilutions of *Salmonella* Enteritidis.

Results: For YeMT1, six poultry samples yielded PCR products; clustal analysis of their sequences revealed six distinct strains, and BLASTN queries of GenBank genome databases identified two as unique. For CjcMT1, four samples yielded products with distinct sequences corresponding to unique or nearly unique strains of *C. jejuni* or *C. coli*. No sample yielded SeMT1 product, consistent with recent surveys indicating low rates of *S. enterica* contamination in retail poultry. Analysis of *S. enterica*-spiked samples indicated sensitivity on the order of 10^2 CFU.

Significance: ciPLST is a promising, time- and cost-saving alternative to established culture-based typing methods for pathogens that commonly contaminate poultry products including *Yersinia enterocolitica* and, especially in light of their challenging culture, *C. jejuni/coli*.

P3-83 Detection and Antibiotic Resistance Determination of *Campylobacter* in Milk Using Colorimetric-based Microfluidic "Lab-on-a-Chip" Device

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❖ ***Developing Scientist Entrant***

Introduction: Antibiotic-resistant *Campylobacter* is mainly transmitted to humans through the consumption of contaminated foods. Rapid detection of *Campylobacter* and determination of its antibiotic resistance profiles are necessary for clinical treatments and foodborne outbreak investigation.

Purpose: We aimed to develop a rapid, sensitive and portable microfluidic device to identify antibiotic-resistant *Campylobacter* in foods.

Methods: The microfluidic device (35 mm by 45 mm) was made of polydimethylsiloxane using soft lithography. In this device, a 2 by 4 array of chambers for bacteria incubation was connected through a sample introduction channel. *Campylobacter* chromogenic medium and antibiotics were preloaded into each chamber. The presence of *Campylobacter* was confirmed by visualizing color change due to the chromogenic reaction. Whole milk was used as the food model for *Campylobacter* detection. Antimicrobial susceptibility test (AST) was also conducted in the microfluidic device to investigate the minimal inhibitory concentration against selective antibiotics (ampicillin, tetracycline and ciprofloxacin).

Results: Colorimetric-based microfluidic approach showed high specificity to *Campylobacter* (*C. jejuni, C. coli,* and *C. lari*) while other foodborne pathogens (*Salmonella, Listeria monocytogenes,* and *Staphylococcus aureus*) did not generate a color signal. The device had a detection limit of 10^2 CFU/ml *Campylobacter* in milk. Quantification of initial *Campylobacter* concentration was achieved by establishing a mathematical model between turnaround time and initial bacterial concentrations. AST results separately obtained from the microfluidic device and conventional agar dilution method showed the coincidence rates of 11 (100%) of 11 against tetracycline and ciprofloxacin and 10 (90.9%) of 11 against ampicillin. Simultaneous identification and AST of *Campylobacter* in the microfluidic device could be completed within 44 h, whereas standard cultivation method took at least four days.

Significance: The developed microfluidic device provides a one-step identification and AST of *Campylobacter* in foods. This method is promising for on-site, rapid screening of antibiotic-resistant bacteria due to simple operation and low cost.

P3-84 Detection of Botulinum Neurotoxins A, B, E and F in Fifteen Selected Problematic Food Matrices Using the Endopeptidase-Mass Spectrometry Assay

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Introduction: The mouse bioassay remains the gold standard method for confirming the presence of botulinum neurotoxins (BoNTs) in food samples. However, the use of rapid, high-throughput screening strategies (DIG-ELISA and endopeptidase-mass spectrometry) is critical to reduce the use of laboratory animals while achieving sensitive and timely detection of BoNTs.

Purpose: The goal of this study was to determine the limit of detection (LOD) of BoNTs (A, B, E and F) artificially spiked into 15 different food matrices that have previously shown detection issues with the DIG-ELISA, using the Endopep-MS assay.

Methods: The Endopep-MS assay detects the enzymatic activity of each BoNT type on a peptide substrate specific to each BoNT. These peptide cleavage products were analyzed by mass spectrometry using the Bruker MALDI Biotyper. BoNTs/A, B, E and F were individually diluted in phosphate buffered saline with tween 20 (PBST) and added to individual wells of 96-well plates containing samples prepared of 15 different test food matrices.

Results: Lowest LOD of three replicates for Endopep-MS in PBST was 0.5 LD_{50}, 0.1 LD_{50}, 1.0 LD_{50} and 0.1 LD_{50} for BoNT/ A, B, E and F, respectively. Fourteen of the food samples had LOD's at or near the baseline LOD in PBST for all toxin types. The sensitivity of the Endopep-MS assay decreased the most with the black tea sample as observed by the higher LOD (A-2.0 LD_{50}, B-0.5 LD_{50}, E-20.0 LD_{50}, and F-0.25 LD_{50}).

Significance: BoNTs A, B, E and F were detected in 14 of the 15 tested problematic food matrices using the Endopep-MS assay at levels comparable to the baseline control for the assay indicating these foods did not interfere with the sensitivity and selectivity of the assay. In addition, the LOD for BoNT/A, B, E and F were below the typical LOD reported for the mouse bioassay.

P3-85 Detection of Mislabeled Canned Seafood Products Using DNA Barcoding

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Introduction: Recently, the mislabeling of canned seafood products has been identified in the commercial market. The canning process often makes morphological identification impossible and also shears the DNA, making it impossible to barcode the standard 655 base-pair COI fragment with previously published primer sets. While it is possible to use next generation sequencing methods to identify canned species, it is costly and inefficient unless there are a large number of samples to be analyzed. This work will describe the development of a method that uses universally designed primer sets and Sanger sequencing to amplify smaller segments of the COI gene that can be aligned to a reference sequence to create a full-length DNA barcode.

Purpose: This work involves the use of two separate universal primer sets designed to amplify two, 350 base-pair segments of the standard COI gene; which, when aligned to a standard reference sequence, result in a full-length DNA barcode.

Methods: Commercial kits are used to extract the DNA from canned tissue. Optimized PCR conditions are used with both primer sets to amplify the extracted DNA, and traditional Sanger sequencing is used to analyze the samples. The smaller fragments are aligned to a previously published alignment sequence to create a full- length, 655 base-pair consensus sequence that is standard for COI libraries.

Results: The analyzed primer sets have been shown to amplify canned species of squid, octopus, mackerel, sardines, clams, oysters, and mussels. Specifically, when both primer sets are combined, ambiguities in the sequences resulting from degenerate primers can be eliminated.

Significance: This work allows for the species identification of canned seafood products using traditional COI DNA barcoding methods, and without the need to use next generation sequencing.

P3-86 Detection of Staphylococcal Enterotoxins A and B in Chicken Salad with RIDASCREEN and VIDAS Methods

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Introduction: Staphylococcal enterotoxins are among the leading causes of food poisoning worldwide. They are resistant to heat treatment, low pH, and proteolytic enzymes, and remain stable under refrigerated or frozen storage once produced. Only five enterotoxins (SEA, SEB, SEC, SED, and SEE) can be detected using commercial immunoassays or rapid detection methods. Meat and poultry products, mixed foods, and deli salads have been involved in staphylococcal poisoning outbreaks over the past two to three decades.

Purpose: The purpose of this study was to compare the enzyme immunoassay RIDASCREEN SET ELISA (LOD=0.375 ng/g) with the VIDAS SET2 automated enzyme-linked fluorescent assay (ELFA) (LOD=1.0 and 0.5 ng/g for SEA and SEB, respectively) for the detection of SEA and SEB in chicken salad.

Methods: The chicken salad was spiked with SEA (1.0 ng/g), SEB (0.5 ng/g), or both SEA and SEB (1.0 ng/g of each), mixed for 30 min at approximately 3°C, and analyzed qualitatively with the ELISA and automated ELFA kits during refrigerated storage up to 15 days. Quantitative ELISA analyses of samples were also performed at day eight of the storage.

Results: Enterotoxins were detected in the spiked samples using both methods. The quantitative ELISA indicated the presence of SEA and SEB at a concentration of approximately 0.35 and 0.17, respectively. The automated ELFA testing of ten subsamples at day four verified that the spiking and mixing method assured a homogenous distribution of the toxins in the matrix. Matrix spiked with SEA had positive test values ranging from 1.58 to 1.83 on day four and 1.50 to 1.64 on day 15. SEB spiked matrix had positive test values of 0.40 to 0.56 on day four and 0.53 to 0.57 on day 15.

Significance: The findings suggest that both ELISA and automated ELFA methods can detect SEA and SEB in chicken salad at concentrations as low as 1.0 and 0.5 ng/g, respectively.

P3-87 Development of a Molecular Serotyping Assay for *Escherichia coli* Via Targeted Sequencing of the O-Antigen Gene Cluster

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Introduction: Antibody-based serotyping of *Escherichia coli* is laborious have limitations and while whole genome sequencing is becoming a routine method for subtyping, it produces large amounts of data that must be parsed to identify serotype information. A targeted, sequence-based assay and accompanying software for data analysis would be a great improvement over the currently available methods for serotyping.

Purpose: The purpose of this study was to develop a high-throughput, molecular serotyping method for *E. coli* based on O-antigen gene cluster (O-AGC) sequences, as well as software for data analysis and serogroup identification.

Methods: Publicly available *E. coli* O-AGC sequences were analyzed, and those that shared >95% identity were grouped into clusters. Representative sequences from each cluster were selected and analyzed for unique signature regions. Primers were designed and checked for specificity, confirming that there was no off-target mapping to other serogroups or *Enterobacteriaceae* genomes. To validate the assay, we extracted genomic DNA from O-serogroup standard strains, amplified the targeted O-AGC regions, prepared sequencing libraries from the amplified products, and sequenced the libraries on the Ion S5 sequencer. The resulting sequence files were analyzed via the SeroTyper software for confirmation of serogroup.

Results: The initial sequence analysis for primer design revealed a total of 168 clusters of *E. coli* (some serogroups share the same O-AGC sequence and group together) O-AGC sequences with unique signature regions in the *wzm/wzt* or *wzx/wzy* genes. Our primer design method allowed us to pool the 168 primer pairs into a single reaction and test >190 strains per sequencing run. Of the 176 O-serogroup standard strains tested, 173 (98%) were correctly identified by this assay. Three strains, representing serogroups O92, O106, and O126, were misidentified in one replicate.

Significance: The high-throughput, sequence-based method presented here is a reliable alternative to antisera-based serotyping methods for *E. coli*. For Research Use Only. Not for use in diagnostic procedures.

P3-88 Development of an Integrated Detection Platform for the In-Process Surveillance of *Listeria* spp. In Environmental Monitoring Samples

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Introduction: The bacterial foodborne pathogen *Listeria monocytogenes,* has been significantly implicated in high-profile outbreaks linked to fresh produce. The annual economic impact of listeriosis in the United States is estimated at over $2.8 billion.

Purpose: The objective of this study was to develop and validate an integrated detection platform for the in-process surveillance of foodborne pathogens.

Methods: To achieve a high level of sensitivity in environmental samples, the method targeted conserved high copy sequences in the ribosomal RNA of *Listeria* spp. Bacterial cells were subjected to an aptamer-capture step, followed by sample concentration and mechanical lysis. RNA copies were either purified or recovered from crude lysate and were further reverse transcribed. The amplification by reverse transcriptase-qPCR of the ribosomal RNA targeted region was achieved using modified nucleotides to stabilize DNA duplex and promote higher specificity.

Results: Validation experiments indicated that the probe-based assay had an RNA analytical sensitivity limit of less than 10 fg of *Listeria* RNA or less than five CFU/ml by using crude lysate as template (Fisher's exact test, $P<0.0001$). No positive signals were detected when testing non-targeted environmental bacterial strains, such as *Bacillus* spp., *Citrobacter* spp. *Enterobacter* spp., and *Pseudomonas* spp., and preliminary observations indicated low concentrations of *Listeria* were still detected in the presence of 1000 times the amount of non-target RNA. The feasibility of detecting *Listeria* spp. from sponge-swab samples, collected at a leafy greens processing facility, was evaluated. Preliminary results showed that *Listeria* spp. were detected at concentrations ranging from three CFU/ml to 32 CFU/ml (Fisher's exact test, $P<0.001$), recovered from spiked 100 ml-volume samples in the absence of an enrichment culturing step.

Significance: These findings have set the foundation for developing an integrated system to rapidly detect *Listeria* at low cell concentrations from environmental samples in large volume amounts without enrichment steps.

P3-89 Differentiation and Screening of Foodborne Bacterial Pathogen Strains Using Colorimetric Gold Nanoparticles

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Introduction: Source attribution investigation is an important step to prevent further spread of foodborne bacterial pathogens responsible for sporadic infections or outbreaks. Due to the limitation of resources, very few bacterial isolates recovered from samples on an agar plate are commonly used for typing and sequencing. Due to potential presence of multiple strains/genotypes in a single sample, it is sometimes difficult to distinguish colonies of different bacterial strains of the known target species or group with similar morphologies, which may lead to a delay or incapability to identify a causative strain in a food or other source(s) responsible for sporadic infection(s) or outbreak(s).

Purpose: This proof-of-concept study investigated the potential use of a non-specific colorimetric gold nanoparticle biosensor (chemical nose) to rapidly screen the isolates/colonies from agar plates before further expensive and labour-consuming typing and sequencing.

Methods: The reaction is based on the color change of gold nanoparticles in nanoparticle aggregation states when interacting with differently charged bacteria. 10^8 and 10^9 CFU/ml were determined to be the optimal concentrations for the current test. The nanoparticles were incubated with bacteria at 2×10^8 CFU/ml at ambient temperature for under 24 hours depending on experiments. The reaction was observed by color change or absorbance of optical density at wavelengths from 300 to 900 nm with one nm increment after 10 minutes incubation with more consistent results after five hours and the maximum resolution in 24 hours. The absorbance data were analysed using absorbance spectrum graphs and principal component analyses.

Results: This biosensor clearly differentiated between strains representing 11 different bacteria families and genera, and seven *Listeria* species. Furthermore, 18 closely related strains of *Listeria monocytogenes* from various serogroups and sources were also distinguished as different.

Significance: Therefore, this study indicated that the colorimetric gold "chemical nose" nanoparticle biosensor may be potentially used to rapidly screen bacterial isolates of the same known target species for further comprehensive source attribution and epidemiological studies.

P3-90 ID Fungi Plates and Mass Spectrometry Complement Each Other to Facilitate Mold Identification

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Introduction: MALDI-TOF MS is nowadays perceived as one of the most promising alternatives for the identification of spoiler and technological molds, solving the currently encountered issues: lack of robustness of phenotypic procedures while only very few experts are operating in the field. But, in some cases, direct harvesting of molds from agar plates is difficult. Liquid cultivation is then prescribed, postponing the time-to-result.

Purpose: The ID-Fungi Plates contain a membrane filter to facilitate mold harvesting. The workflow combining this new plate format with MALDI-TOF MS was evaluated by two laboratories on respectively 70 and 90 strains from various genera.

Methods: The handling and reliability were compared to direct harvesting from classical Sabouraud plates using the MALDI Biotyper automate. The MALDI Biotyper Reference Library for fungi identification with 58 genera and 151 species, which cover the most important taxa in food mycology, was used.

Results: The required biological material for MALDI-TOF analysis is obtained with only 24 h cultivation when using the ID-Fungi Plates. And these ID-Fungi Plates enable to collect the biomass in less than 10 seconds for almost 90% of the tested strains, while more than one min is usually required for 60% of the strains. More than 80% of the strains were easily identified at the genus or species level using the ID-Fungi Plates, while only 60% were identified using the Sabouraud plates.

Significance: The ID-Fungi Plate with the filter membrane improves the practicability and accuracy of MALDI-TOF MS for mold identification. Results could be easily obtained in one hour starting from isolates on ID- Fungi Plates. The needs for liquid cultivation with longer time-to-result is significantly reduced, and required only for some very specific molds that are usually not encountered in food mycology. Of course, the Reference Library should be constantly implemented with species of relevance in food mycology.

P3-91 Reproducibility of MALDI-TOF MS for Pathogen Confirmation and Identification of Non-pathogenic Bacterial Isolates: Assessment According to the AOAC Guidelines

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Introduction: MALDI-TOF MS is recognized as a valuable method to confirm and identify microbial isolates. Four inter-laboratory studies were recently run to assess the reproducibility of the MALDI Biotyper.

Purpose: Different variable conditions were tested: instruments, operators, types of target plates, culture media.

Methods: Twenty-four to 36 isolates were evaluated per study according to the Appendix J of the AOAC Guidelines (2016). Each set of strains consisted of 16 pathogens including *Salmonella* spp., *Listeria monocytogenes*, *Cronobacter* spp. and *Campylobacter* spp., and eight to 20 relevant non-pathogenic strains. Each study was organized by an independent laboratory: two studies were run in Europe, the two others in North America. Seven to 17 laboratories were involved depending on the study, with 14 to 17 collaborators. Sixteen standard formulations and chromogenics were tested. A non-selective agar was used as a quality control. Reusable and disposable target plates were tested. The appropriate standard procedures (ISO, FDA/BAM, USDA/FSIS) were run in parallel to confirm the pathogen and identify the other isolates.

Results: No impact of the selective culture media was observed. No influence of the tested target plates was noticed. The MALDI Biotyper shows 99.5% to 100% correct confirmation rates of foodborne pathogens depending on the study, and 98.5% to 100% correct identification rates at the group or species level. The correct confirmation rates of the standard procedures vary from 91.3% to 100%, the correct identification rates from 86.5% to 99.6%.

Significance: The MALDI Biotyper provides reliable and reproducible results to confirm the pathogen after a first presumptive screening step, and to identify the non-pathogenic isolates. These collaborative studies demonstrate that the technique could be easily implemented in routine testing.

P3-92 A Rapid, Simultaneous and Simple Method for the Detection of *Salmonella* and *Escherichia coli* in Wheat Flour

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Introduction: Wheat flour and cereal products have been associated with *Salmonella* and *E. coli* outbreaks and recalls. The development of novel methods for their detection in such matrices is critical for implementing strategies for monitoring and control.

Purpose: The purpose of this study was to develop a simple detection method for simultaneous detection of *E. coli* and *Salmonella* in wheat flour by combining short enrichment (E), immunomagnetic separation (IMS) and specific agglutination tests (Agg).

Methods: Growth of *E. coli* strains in A1, EC, lauryl sulfate (LSB), buffered peptone water (PW) and tryptic soy (TSB) broth media were compared for four and eight h at 42°C for selecting the shortest enrichment. A single commercial indirect IMS kit was used for their dual selectivity for *E. coli* and *Salmonella* and exclusivity of non-target strains, and capture efficiencies (CE) were calculated. Broth media and flour were inoculated with 0.1 log CFU/g, enriched for four, eight, and 12 h in TSB, subjected to IMS and tested with commercial agglutination tests.

Results: After four and eight h enrichment in A1, LSB, PW and TSB, the average microbial counts of individual strains were 2.6, 2.7, 3.9, 5.3 and 3.9, 4.5, 7.4, 8.3 log CFU/ml, respectively. The superiority of TSB was confirmed using ANOVA ($P \leq 0.05$) and it was used in the following experiments. The CE values were in the range of 10 to 100% for *E. coli* and 39 to 94% for Salmonella strains. All CE values of non-target bacteria were <15.0%. The optimal E-IMS-Agg approach was able to detect 0.1 log CFU/g *Salmonella* and E. coli in wheat flour after 12 h enrichment.

Significance: This study reports a simple method capable of detecting both *E. coli* and *Salmonella* in wheat flour without advanced laboratory equipment in less than 24 h.

P3-93 Inkjet Printed Nano-patterned Aptamer-based Sensors for Improved Optical Detection of Foodborne Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: The increasing incidence of infectious outbreaks from contaminated food and water supply is still a global burden for food safety. Despite the rapid growth of the biosensor field, more than 80% of the platforms developed at lab scale never will meet the market due to low cost-efficiency, instability of the biomolecules, and lack of large-scale reproducibility of novel biosensing platforms.

Purpose: This work aims to provide a cost-efficient, reliable, and high-throughput approach, for the detection of foodborne pathogens in real samples by chromatographic ink-jet printed paper-based strips.

Methods: We devised an optimized printing procedure for the deposition of DNA aptamers on nitrocellulose substrates. To enhance the signal response for pathogen detection, a carboxyl-modified DNA aptameric sequence highly specific for *E. coli* O157:H7 was covalently conjugated to label particles (PEI-gold decorated polystyrene) via NHS/EDC chemistry. After lateral diffusion testing, an image of colorimetric response was captured with a mobile phone camera, and the image analysis was performed using the image analysis pipeline we designed to quantify the color intensity and correlate it with the amount of target pathogen in the sample. Statistical analysis was conducted by ANOVA, means comparison, and the Shapiro-Wilk test, using the right-tailed normal distribution.

Results: Limit of detection of *E. coli* O157:H7 was found at 25 CFU ml^{-1} in pure culture and 233 CFU ml^{-1} in ground beef with analytical performance r^2=0.988. The as-fabricated platform proved to control the detection response variation within means +/-1 SD for at least 70% of the data collected (n=12), with enough evidence (P<0.05) of its high specificity at genus, species, strain, and serotype level.

Significance: To the best of our knowledge, we introduced for the first time the ink-jet printed patterning of aptamer-based inks, achieving the lowest limit of detection of the state-of-the-art for paper-based optical detection of *E. coli* O157:H7.

P3-94 Colorimetric Detection of *Clostridium perfringens* in a Model Meat System Using Paper-based Microfluidics

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❖ ***Developing Scientist Entrant***

Introduction: *Clostridium perfringens* is linked to approximately one million foodborne illnesses annually. Consistently, meat products serve as vehicle for *C. perfringens* with microbial growth occurring rapidly in food during temperature abuse. To mitigate this problem, a number of diagnostic methods exist, including culture-based, biochemical, or molecular techniques (PCR, LAMP). µPADs (paper-based analytical devices) are useful platforms for microbial diagnostics, since they are portable, require small sample volumes for analysis, and can be adapted to different detection modalities.

Purpose: Our objective was to develop and optimize colorimetric enzymatic assays for µPAD-based rapid detection of *C. perfringens* in roast beef, using a model meat system.

Methods: µPADs were impregnated with an optimized concentration of phenolphthalein diphosphate, indicative of acid phosphatase activity after ammonium hydroxide titration, and testing for optimization also included non-target bacterial isolates (including other *Clostridium* spp.). Roast beef homogenate was prepared with reinforced clostridial media, then inoculated to contain an average of 6.1×10^0 (low), 6.1×10^2 (medium), and 6.1×10^4 (high) CFU/ml of *C. perfringens*. Enrichment was performed anaerobically at 44°C with or without selective supplements, aliquots were removed for analysis at zero, four, eight, and 18 hours and visual confirmation and ImageJ analysis were performed. Real-time PCR targeting 16s rRNA, *cpe*, and *plc* genes was used for confirmation.

Results: Visual confirmation of magenta color and Image J analysis of µPADs indicated discernable acid phosphatase activity. A significant difference in greyscale intensity (P<0.05, n=24) allowed for unambiguous detection of *C. perfringens* in a model meat system at the lowest (6.1×10^0 CFU/ml) and highest (6.1×10^4 CFU/ml) concentrations tested after eight and four hours selective enrichment, respectively.

Significance: We developed and optimized µPAD-based diagnostics for rapid colorimetric detection of *C. perfringens* amenable to application in meats. The method is inexpensive, easy-to-perform, and sensitive, providing a valuable alternative to current detection methods for *C. perfringens* in meat products.

P3-95 Evaluation of *Salmonella* and Shiga Toxin-producing *Escherichia coli* Presence in Various Pet Foods Using Rapid PCR-based Assay as Pre-screening Method

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❖ ***Developing Scientist Entrant***

Introduction: Recent infections and outbreaks have been associated with the handling of contaminated pet food and treats, and there has been an increase in calls for pathogen testing in these products. Although some pet food and treats could be considered safe due to the thermal process they are subjected to, pathogens like *Salmonella* can survive desiccation and in products with low water activity. Moreover, raw pet food can potentially harbor other pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria*.

Poster

Purpose: The objectives of this study were to evaluate the presence of Salmonella and STEC in various brands and types of pet food obtained from retail stores in the West Texas area.

Methods: Pet food samples (*n*=63) were collected from pet stores in Lubbock, Texas. The samples included dog food (*n*=43), cat food (*n*=12), rabbit and hamster food (*n*=4), and other species (*n*=4). Furthermore, samples were classified as i) processed (*n*=48) and raw (*n*=15) or as ii) dry kibble and chewy (30) and moist & semi-moist (33). Twenty-five g of each sample were placed in sterile bags with 225 ml of buffered peptone water (BPW) and stomached for one minute at 230 rpm. Samples were enriched by adding one ml from each bag to nine ml modified tryptic soy broth (TSB) supplemented with eight mg/l novobiocin and acid digest of casein and the samples were incubated at 42°C for 24 h. Enriched samples were subjected to polymerase chain reaction (PCR) screening using the BAX System PCR assay system for both STEC and *Salmonella.*

Results: For both pathogens, prescreening indicated complete absence of STEC and *Salmonella* and no isolates were recovered.

Significance: Although the results of this study indicated absence of pathogens, increasing the safety of pet food processing is warranted to prevent human illness due to direct and indirect handling of potential contaminated products.

P3-96 Enumeration and Pathotyping of *Escherichia coli* in Agricultural Water

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Introduction: Contaminated fresh produce has been implicated in many foodborne outbreaks. Agricultural water may serve as a vehicle for the transmission of pathogens, most of which are intestinal inhabitants of animals or humans. While *Escherichia coli* is commonly used as an indicator of fecal contamination, most strains of this species are harmless. Unfortunately, there are some pathogenic *E. coli* strains that carry virulence genes.

Purpose: The objective of this study was to establish a baseline of water quality on produce farms and identify six pathotypes on *E. coli* isolated from agricultural water in Hawaii.

Methods: Non-municipal water samples (surface, well, catchment) were collected from produce farms in Hawaii. *E. coli* count of the sample was determined by membrane filtration using modified membrane-thermotolerant *E. coli* agar (EPA Method 1603). DNA from each *E. coli* isolate was analyzed via polymerase chain reaction (PCR) with primers targeting twelve genes to identify enteropathogenic, enterohemorrhagic, enterotoxigenic, enteroinvasive, enteroaggregative, and diffusely adherent *E. coli*.

Results: Out of 252 water samples, 34% tested *E. coli*-negative. 83% of water samples were below the geometric mean limit of 126 CFU/100 ml, whereas eight percent of water samples were above the statistical threshold value limit of 410 CFU/100 ml. PCR assay identified three Shiga toxin-producing *E. coli* strains carrying both *stx1* and *stx2* genes, four atypical enteropathogenic *E. coli* strains carrying the *eae* gene but without the *bfp* gene, one hybrid entero-aggregative/uropathogenic *E. coli* strain carrying *pic* gene, one enterotoxigenic *E. coli* strain carrying e*ae* and *astA* genes, and 33 heat-stable enterotoxin 1 encoded *E. coli* strains (EAST1EC) carrying the *astA* gene but no other identifiable pathogenic genes.

Significance: All potentially pathogenic *E. coli* isolates warrant further confirmation. Proper measures must be taken to control pathogens in agricultural water to ensure food safety and protect the public health.

P3-97 Development of an Ultra-Sensitive and Specific Multiplex Single-Tube Nested qPCR Assay for Simultaneous Detection of *Campylobacter jejuni* and *Salmonella* spp.

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter jejuni* and *Salmonella* species are the top two pathogens responsible for human bacterial gastroenteritis worldwide. Poultry and meat-associated products are the principal source of their infections. Traditional culture-dependent methods of microbiological analysis are laborious and time-consuming. Therefore, a sensitive and specific assay is necessary for rapid detection of these pathogenic bacteria in food to protect the public's health and minimize economic losses.

Purpose: This study aimed to develop a multiplex single-tube nested qPCR assay (MSTN-qPCR) for simultaneous detection of *C. jejuni* and *Salmonella*.

Methods: Two nested primer sets were designed based on the *hipO* gene of *C. jejuni* and the *invA* gene of *Salmonella*. The annealing temperatures and concentrations of nested primers were optimized in a range of 50°C to 70°C and 0.05 pmol to 40 pmol, respectively. The specificity of established assay was tested with DNA extracted from three *C. jejuni* strains, three *Salmonella* spp., and nine non-target bacterial species. The sensitivity of MSTN-qPCR was investigated using serial dilutions of *C. jejuni* and *Salmonella* DNA.

Results: Optimal reaction conditions for MSTN-qPCR assay were 0.1 pmol of all four outer primers, 40 pmol of *Salmonella* inner primers, 20 pmol of *C. jejuni* inner primers, 10 pmol of TaqMan probe for *Salmonella*, and 5 pmol of TaqMan probe for *C. jejuni*. The annealing temperature was 65°C for outer amplifications and 55°C for inner amplifications. The established assay could detect as low as 80 fg DNA of both target pathogens at one time without generating amplicons from non-target bacteria.

Significance: The MSTN-qPCR assay provides an ultra-sensitive and specific approach for simultaneous detection of *C. jejuni* and *Salmonella*. It would help regulatory agencies and food manufacturers improve the safety of food supply.

P3-98 Quantification and Discovery of PCR Inhibitors Found in Food Matrices Commonly Associated with Foodborne Viruses

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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, one challenge in its utilization is the presence of compounds in food that can inhibit detection and accurate quantification. Produce and molluscan shellfish are two of the most commonly implicated foods in foodborne norovirus outbreaks. Both are reported to have PCR inhibitors, but recent empirical quantification of the degree to which these compounds inhibit the current generation of RT-qPCR formulations has not been reported.

Purpose: The purpose of this study was to observe and quantify the degree of inhibition that occurs from inhibitory compounds found in produce (pectin) and mollusks (hemocyanin, glycogen).

Methods: RT-qPCR reactions, containing different starting amounts between 10 and 1000000 molecules of genomic RNA from a norovirus surrogate, were spiked with different concentrations of pectin (0.065 to 0.25% w/v), glycogen (1.25 to 10%), and hemocyanin (0.03 to 0.50%). All reactions were performed in triplicate with no inhibitor and no template controls.

Results: Pectin, found in produce, caused complete inhibition at 0.25%, with no significant inhibition observed at 0.065% ($P<0.05$). Past research has implicated glycogen as a main inhibitory compound in oysters; however, even high levels of glycogen (10%) had no significant effect ($P>0.05$) on amplification. Hemocyanin, present in the hemolymph of mollusks and previously untested as a PCR inhibitor, caused complete inhibition at 0.5%, with no significant inhibition at 0.03% ($P<0.05$).

Significance: In sum, this work quantifies the degree of inhibition that occurs from three compounds implicated in foods associated with norovirus transmission, demonstrating that pectin and hemocyanin should be considered when testing produce and mollusks. This information helps inform sample preparation for PCR-based detection of foodborne pathogens from produce and mollusks, as well as identifies a previously unreported specific inhibitory compound found in mollusks.

P3-99 Evaluation of Roka Atlas-based Assay for Major Foodborne Pathogens in Food and Environmental Samples

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Introduction: Foodborne pathogens including *Salmonella*, *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* (STEC) account for nearly 48 million foodborne illnesses -- including 128,000 hospitalizations and 3,000 deaths each year in the United States. Rapid, sensitive and specific detection methods are needed for the identification of major foodborne pathogens at various points in the food supply chain.

Purpose: To evaluate the Roka Atlas assay for the detection of major foodborne pathogens in food and environmental samples.

Methods: A 24 h preenrichment or enrichment for food and environmental samples was used. Samples were added into a modified sample transfer tube for bacterial lysis, template-specific sample extraction, amplification and probe detection per fully automated assay protocol in the instrument. Each assay reagent kit was validated with a set of *Salmonella, E. coli, or Listeria* calibrators (positive and negative) provided by the manufacturer. Results were confirmed with the United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) culture method in tandem.

Results: A total of six food and two environmental matrices were evaluated in this study for *Salmonella,* STEC*, or L. monocytogenes* detection. Three preenrichment broths were also evaluated for *Salmonella* detection using Roka Atlas *Salmonella* assay (SEN). The results from the assay were equivalent in most cases to BAM culture method results for *Salmonella,* STEC*, or L.* monocytogenes, respectively. Universal preenrichment broth was the best preenrichment broth for detecting *Salmonella* in sprout spent irrigation water on the Atlas System.

Significance: The Roka Atlas system provided a more rapid, sensitive, and specific molecular method for detection of major foodborne pathogens from a variety of food and environmental samples.

P3-100 From Stools to Water: Contamination of Irrigation Water Using an Artificial Hand Tool Exposed to Stool Samples Containing Oocysts of *Cyclospora cayetanensis*

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✦ ***Undergraduate Student Award Entrant***

Introduction: Outbreaks of cyclosporiasis are associated with consumption of fresh produce contaminated with oocysts of *Cyclospora cayetanensis*. These oocysts are shed in stools from infected individuals and may eventually contaminate water used to irrigate or wash produce.

Purpose: We conducted experiments to verify the contamination of 10 L of irrigation water with *C. cayetanensis* by artificial "hands" exposed to surfaces contaminated with approximately 0.1 g of positive stool sample.

Methods: A *C. cayetanensis* positive stool sample with a high oocyst load was used for the experiments. Aliquots of 0.1 g of this stool were spiked directly into triplicates of 10 L of irrigation water. In addition, triplicates of 0.1 g of the same stool diluted in 100 µl of PBS were used to contaminate disposable surfaces followed by placing a gloved rubber hand tool into contact with the surfaces for five, 10, and 20 seconds. The "hand" was then submerged in 10 L of irrigation water and swirled for one minute. Samples were processed by dead-end ultrafiltration (DEUF) and *C. cayetanensis* was detected by qPCR. Cycle threshold (Ct) ranges of 22 to 30 were considered strong positives, while Ct higher than 30 were considered moderate to weak positives.

Results: *C. cayetanensis* was detected in the water samples spiked with 0.1 g of stool and the average Ct of 29.96 was obtained. The Ct values of water samples that were contaminated from surfaces that had approximately 0.1 g of stools were 32.65, 32.87, and 35.52 for contact times of five, 10 and 20 seconds, respectively. Non-contaminated water samples used as controls were negative by qPCR.

Significance: We demonstrated that transfer of *C. cayetanensis* to irrigation water is possible by hands exposed to contaminated surfaces with small amounts of positive stools despite short contact times.

P3-101 Isolation and Identification of Three Gram Negative Bacterial Species from Powdered Infant Formula Using MALDI-TOF Mass Spectrometry and rRNA Sequence Analysis

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Introduction: Pathogen detection is a critical factor for the safety of powdered infant formula (PIF) as it effectively facilitates the growth of human-pathogenic microorganisms of public health importance and can be easily contaminated. To date, various pathogenic Gram-negative and Gram-positive bacteria have been identified while examining the PIF contamination related to sporadic cases and outbreaks. This study was carried out with the major intention to find *Cronobacter* species, known PIF-borne opportunistic pathogens, that cause acute meningitis and necrotizing enterocolitis in neonates and are frequently linked to PIF contamination worldwide.

Purpose: The major objective of this study was to rapidly identify the pathogenic bacterial isolates recovered from PIF samples by performing MALDI-TOF MS fingerprinting and rRNA sequencing analysis.

Methods: A total of ten Gram-negative bacterial isolates were recovered from PIF samples and cultured on blood selective agars for DNA isolation and MALDI-TOF MS analysis. For each isolate, one to two colonies were directly spotted on the VITEK MS for species identification. Genomic DNA was extracted

Poster

from the overnight bacterial culture using QIAGEN DNeasy Blood & Tissue DNA Extraction Kit. Subsequently, two-directional Sanger sequencing was performed to confirm species identification characterizing the regions of rRNA gene on an ABI 3500XL Genetic Analyzer.

Results: Three distinct species of Gram-negative bacteria (*Acinetobacter* spp. in one isolate, *Enterobacter cloacae* in six isolates, and *Pseudomonas* spp. in three isolates) were identified which have been implicated as a significant cause of healthcare-associated infections and outbreaks in the past. The samples were found negative for the presence of *Cronobacter* species. Species identification attained by the VITEK MS matched with the rRNA sequencing data analysis.

Significance: MALDI-TOF mass spectrometry and rRNA sequence analysis can be utilized to identify the Gram-negative bacterial isolates recovered from PIF samples.

P3-102 Matrix Extension of a Loop-mediated Isothermal Amplification (LAMP) Assay for Screening *Salmonella* in Raw Pet Food

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Introduction: Raw pet food, comprised of raw meat and vegetables, has become an increasingly popular option for pet owners. Ongoing surveillance programs and frequent product recalls have indicated that this commodity has a high risk of contamination with *Salmonella* and other foodborne pathogens. Improved screening methods are needed to meet the growing testing demands in this field.

Purpose: This matrix extension study aimed to apply a *Salmonella* loop-mediated isothermal amplification (LAMP) assay, previously validated in animal feed and dry pet food, in several raw pet food matrices, and followed guidelines specified in the FDA's Microbiological Methods Validation.

Methods: Five types of raw pet food, including frozen beef, turkey, and pork complete foods and freeze-dried beef and chicken treats, were evaluated in this study. For each matrix, fourteen 25-g portions of raw pet food were inoculated with < 30 cells of *Salmonella* and six 25-g portions were reserved as uninoculated controls. Samples were enriched in lactose broth (LB) or buffered peptone water (BPW) and both enrichment types were processed for testing with the *Salmonella* LAMP assay. Samples were confirmed with conventional culture methods using the FDA's Bacteriological Analytical Manual (BAM) *Salmonella* method.

Results: The LAMP assay obtained positive detection of *Salmonella* in all inoculated raw pet food samples, regardless of the enrichment broth used. Positives were culture confirmed by the BAM *Salmonella* method. The flexibility to use either LB or BPW as the enrichment broth suggests a robust method suitable in a variety of lab workflows.

Significance: The *Salmonella* LAMP assay offers a rapid and reliable approach for routine screening of *Salmonella* in raw pet food matrices. This method offers a valuable tool for ensuring the safety of raw pet food, a product of growing popularity with potential public health impacts due to the presence of *Salmonella* and other foodborne pathogens.

P3-103 Matrix Impact on the Variation of Test Results and Measurement Uncertainty in Proficiency Testing Data from Milk, Infant Formula and Oyster Samples

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Introduction: Reproducibility standard deviation (s_R) is a precision parameter which plays a central role to measure uncertainty. In food microbiology, uncertainty and variability of measurement tend to vary considerably due to the complexity of detecting living organisms. Analyzing this variation is important because it directly relates to laboratories' abilities to detect microorganisms.

Purpose: Mean interlaboratory s_R values from five years of proficiency testing (PT) data for total aerobic counts were computed separately for three matrices: milk, infant formula, and oysters. These data were used to test the hypothesis that mean s_R values differ across matrices. The overall goal was to gain better insight into whether matrix significantly affects variability of total aerobic count results.

Methods: Based on regulatory PT data, s_R values were calculated for studies with the three matrices. All samples were inoculated at similar levels, homogenous and stable, and analyzed for total aerobic counts by trained analysts. The s_R values across matrices were compared using analysis of variance (ANOVA) and followed by Tukey's multiple comparison test. Impact of concentration level and number of participants on s_R was also examined using linear regression.

Results: Mean s_R values for milk, infant formula, and oysters were 0.066, 0.112, and 0.118 log, respectively, and ANOVA showed there was a significant difference across the matrices ($P = 2\times10^{-16}$). Tukey's test showed s_R was significantly different between milk and infant formula and between milk and oysters. Linear regression showed number of analysts was a stronger predictor of s_R (R^2=0.481, $P<2\times10^{-16}$) than concentration level (R^2=0.049, P=0.016).

Significance: This study highlights how interlaboratory data from PT studies can be used to gain an understanding of the variation associated with microbiological testing. These analyses indicate matrix may significantly affect the variation of total aerobic count results. Future studies will build upon these results to explore the impact of other factors including analytical method and analyst experience on overall measurement uncertainty.

P3-104 Method Performance of Two Aerobic Plate Count Methods in the Longstanding Milk Proficiency Testing Program

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Introduction: The United States milk sanitation program is run by the Food and Drug Administration (FDA) and operates under standards outlined in the Grade "A" Pasteurized Milk Ordinance (PMO). According to the PMO, Grade "A" milk samples must be tested using validated and approved total aerobic bacterial count methods. Two of the most commonly used methods are standard plate count (SPC) and Petrifilm aerobic count (PAC).

Purpose: This study compares the performance of total aerobic count methods in milk samples based on FDA proficiency tests (PT) spanning from 2009 to 2016.

Methods: Fourteen milk samples inoculated with various gram-positive and gram-negative bacteria were sent annually for the milk PT. Samples were tested by laboratories using approved total aerobic count methods. Statistical analysis of the resulting 1,848 sets of data was performed to compare performance characteristics of SPC and PAC. For each sample, mean and reproducibility standard deviation (s_R) were calculated for each method. Bias between methods was also calculated per sample. Principal component analysis was applied to identify sources of analyst-specific systematic differences between different types of milk samples.

Results: Mean bias between PAC and SPC across all samples was 0.038 log and mean s_R was 0.052 log and 0.062 log for PAC and SPC, respectively. This suggests the bias between methods is minor considering the population of samples analyzed. In heavy and light cream samples, s_R values were the greatest; 0.072 and 0.062 log for SPC and 0.058 and 0.061 log for PAC. These results indicate fat content of milk samples may impact reproducibility.

Significance: This study demonstrates how PT studies can be used to compare long-term method performance characteristics. Results highlight that even methods using principally different techniques, such as PAC and SPC, can display very similar performance due to well-defined operating procedures provided by regulatory programs.

P3-105 Rapid Detection of *Salmonella* against Other Bacterial Strains Using Hyperspectral Microscope Images

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Introduction: Hyperspectral microscope images (HMI) have shown promise for rapid detection of foodborne bacteria at the cellular level, but a robust classification method is needed prior to field application.

Purpose: Development and validation of a one-class classification model to determine if bacterial cells collected in HMI are *Salmonella* positive or negative.

Methods: Multiple HMI of 27 bacterial strains including *Salmonella*, *Campylobacter*, *E. coli*, *Staphylococcus aureus*, and *Listeria* were collected from broiler chicken rinse isolates over six years at the United States National Poultry Research Center (USNPRC) of USDA, ARS in Athens, GA. Darkfield HMI were collected between 450 to 800 nm from pure bacterial isolates. HMI were processed to extract a mean spectrum that is unique to individual cells found in the images. A one-class soft independent modeling of class analogy (SIMCA) algorithm was developed from 35 HMI of 13 commonly isolated *Salmonella* serotypes including *Salmonella* Enteritidis and *Salmonella* Typhimurium. The SIMCA model was validated on 23 *Salmonella* HMI and 36 non-*Salmonella* HMI.

Results: Utilizing the ImageJ and R platforms resulted in approximately a 30-minute process from sample microscope slide preparation to cellular classification results. SIMCA reported that 93.4% of 3,506 cells were accurately classified, while *Salmonella* sensitivity was 0.969, and the combined specificity of the 14 non-*Salmonella* strains was 0.92. *E. coli* accounted for 39% of the non-*Salmonella* cells tested against the SIMCA algorithm with 98.8% of *E. coli* accurately classified as non-*Salmonella*.

Significance: HMI has the potential for early and rapid detection of *Salmonella* from broiler chicken carcass rinses. Robust validation to confirm the *Salmonella* based SIMCA algorithm maintained efficiency when validated against common foodborne bacteria was necessary prior to field implementation.

P3-106 Detection of *Salmonella* and *Listeria* from Multiple Dairy Products Using the BAX System Real-time PCR Assays

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Introduction: Recent recalls have highlighted dairy products as a reservoir for pathogen contamination. Manufacturers are looking for validated testing methods that allow them to reliably test for pathogens in larger samples and with faster time to result than other methods currently available.

Purpose: The objective of this study was to evaluate the performance of real-time PCR assays for the detection of inoculated *Salmonella*, *Listeria* species and *Listeria monocytogenes* in dairy products including cheese, lactose powder, anhydrous milk fat (AMF) and whey protein concentrate 80 (WPC80) in comparison to the FDA BAM reference methods.

Methods: Bulk samples of each dairy matrix were prepared by inoculating with *Salmonella* or *Listeria* at a low and high level. After stabilization, 375-g test method samples for *Salmonella* were enriched 1:5 (cheese, lactose powder, AMF) or 1:10 (WPC80) in prewarmed (35°C) buffered peptone water and incubated for 22 to 26 hours at 35°C. For the *Listeria* test method, 25-g samples of lactose powder and AMF and 50-g samples of WPC80 were enriched 1:10 in 24 LEB Complete broth and 375-g samples of cheese were enriched 1:5. All sample were incubated at 35°C for 26 to 48 hours. All enrichments were analyzed by real-time PCR and confirmed according to the reference culture method. Reference method samples were enriched according to the procedures in the FDA BAM.

Results: For each sample type inoculated with *Salmonella*, the test method and the reference method demonstrated no significant statistical difference as indicated by POD analysis, except for WPC80 presumptive results tested without the use of a BHI regrowth, and WPC80 reference results. For each sample type inoculated with *Listeria*, the test method and the reference methods demonstrated no significant statistical difference as indicated by POD analysis.

Significance: Overall, the BAX System Real-Time PCR Assays for *Salmonella*, Genus *Listeria* and *L. monocytogenes* demonstrate accuracy and reliability statistically equivalent to the reference methods.

P3-107 Application of Improved Genetically Modified Detection Methods using Screening Multiplex PCR for Authorized Genetically Modified Soybean Processed Food

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Introduction: Given circulation of genetically modified organisms (GMOs) in the world, a multiplex polymerase chain reaction (PCR)-based detection method is one of the high-throughput detection tools for GM-derived material.

Purpose: The purpose of the study was to compare GMO detection methods that conventional PCR and multiplex PCR and determine whether the multiplex PCR technique was applicable to various GM-derived from processed food.

Methods: The triplex PCR was optimized for the transgenic elements, which are P-35S, T-Nos, T-35s, P-RbcS4, T-ORF23, T-E9, T-Pin Ⅱ, T-AHASL, and DP305423. Nine screening elements were tested, individually. The limit of detection (LOD) test was confirmed each multiplex PCR set using certified reference materials (CRM). Evaluation of applicability test of multiplex PCR was used on 10 soybean and 43 commercialized food samples. The detection method in this study was compared with the common GM detection protocol in the Korea food code.

Results: This technique can be a useful tool to simplify confirmation of the presence or absence of GMOs by testing various elements. Four GM events (RRS, A2704-12, A5547-127, FG72) can be confirmed in set 1. Seven events (MON87701, MON89788, MON87705, MON87708, MON87769, DAS44406-6, DAS 68416-4) were detected in set 2 and three events (DP356043-5, DP305423-1, CV127) were detected in set 3. LOD test of each three multiplex sets could be detected until 0.5% of RRS, MON89788, DP305423-1 in genomic DNA of CRM. The applicability of screening method in soybean and soybean-based food products were more rapidly and accurately analyzed one or more GM events than common detection method.

Significance: This screening method is an accurate and rapid tool to detect authorized GM events in processed food. Especially, this study is an effective approach to monitor for low-level presence and adventitious presence of GMOs in internationally traded food.

Poster

P3-108 Development of a Rapid and Accurate Detection Method for *Listeria monocytogenes* in Golden Needle Mushrooms, Using Quantitative Real-time PCR

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* has been detected in mushrooms, and it can cause listeriosis when consumed raw as a salad. Because the pathogen is psychrophilic bacterium, it can grow at low temperatures, even though contamination is at low levels. Therefore, rapid and accurate detection for *L. monocytogenes* in mushrooms is necessary.

Purpose: The objective of this study was to develop a rapid and accurate detection method of *L. monocytogenes* in golden needle mushrooms, using quantitative real-time PCR (qRT-PCR) with *iap2* primers.

Methods: The *iap2* primers for detecting *L. monocytogenes* were developed in broth media in a previous study. A mixture of *L. monocytogenes* strains (100 µl) was inoculated on the mushroom (20 g), and 40 ml of Listeria enrichment broth (LEB) was added, followed by incubation at 30°C for zero, three, six, and nine h. Three-milliliters of the enriched cultures were used for DNA extraction, and the DNA was used to perform the qRT-PCR with the *iap2* primers for melting curve analysis and calculation of cycle threshold (Ct) values.

Results: In melting curve analysis, the annealing temperatures in all samples were in concordance with the positive control (*L. monocytogenes* culture), indicating that application of *iap2* primers to the mushroom is appropriate. In qRT-PCR analysis, the Ct value was observed after three h-enrichment in all samples (four of four), while there were two positive samples at zero h (two of four). Also, this result was in concordance with those from a media-based detection method.

Significance: This result shows that the detection method of *L. monocytogenes* in golden needle mushroom, using LEB and qRT-PCR with *iap2* primers is more efficient than the conventional method.

P3-109 Droplet Digital PCR for Detection of Foodborne Pathogens

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Introduction: The currently validated microbiological methods for the detection of enterohemorrhagic *Escherichia coli* (EHEC) from foods relies on a PCR-based screen for the pathotype-specific genetic markers *stx* and *eae*. In comparison to culture confirmation, this screening method suffers from a very high rate of false positives (up to 92.5%) that is partly due to the inability of current PCR-based methods to determine if both *stx* and *eae* are within the same organism.

Purpose: This study was undertaken to reduce the false positive rate associated with current EHEC screening methods by confirming the presence of *stx* and *eae* from the same cell.

Methods: The ddPCR system used in this study works by partitioning intact cells into emulsion droplets, which subsequently undergo multiplexed end-point PCR. This allows the differentiation of samples where a single organism contains both *stx* and *eae* from samples in which *stx* and *eae* reside in different organisms. A study which compared the response of ddPCR to commercial real-time PCR assays was conducted using over thirty (30) unique simulations of EHEC contamination in ground beef.

Results: In this comparative study the ddPCR assay demonstrated equivalent sensitivity to the established screening techniques. Further the results indicate the ddPCR has the potential to reduce the number of false positives identified in an EHEC screening assay.

Significance: This study demonstrates the ability of the ddPCR system to confirm the co-existence of multiple genes within the same cell in a mixed microbial population; specifically, *stx* and *eae*. Ultimately, this system will result in cost savings by reducing the man-hours and testing expenses associated with the evaluation of false-positive samples. Furthermore, this would enable more samples to be analyzed, which could reduce the probability of contaminated foods reaching consumers.

P3-110 Development of Sensitive DNA Primers to Detect *Listeria monocytogenes* in *Pleurotus eryngii* Directly after Enrichment by Quantitative Real-time PCR

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks by *Listeria monocytogenes* continuously occur. However, conventional methods for identification of *L. monocytogenes* take more than two d. Hence, it is necessary to develop rapid and accurate methods for detecting *L. monocytogenes*.

Purpose: The objective of this study was to detect *L. monocytogenes* in the king trumpet mushroom (*Pleurotus eryngii*), using enrichment broth and quantitative real-time PCR (qRT-PCR).

Methods: The *iap2* primers were designed to target *iap* gene by OligoPerfect. To examine the sensitivity of *iap*2 primers, five log CFU/g of *L. monocytogenes* isolate IL1-1 from a mushroom was inoculated in king trumpet mushrooms, and DNA was extracted to be analyzed by qRT-PCR. The specificity of *iap*2 primers was evaluated with the DNA from *L. monocytogenes,* other *Listeria* spp., *Escherichia coli, Staphylococcus aureus*, and *Salmonella* Typhimurium. A mixture of five *L. monocytogenes* isolates from a mushroom was inoculated in the king trumpet mushroom at one log CFU/g. Forty milliliters of *Listeria* enrichment broth were added into the sample bags and enriched at 30°C for zero to 12 h. Three milliliter aliquots of the enrichment culture (n=4) at every three h were used for DNA extraction, and analyzed by qRT-PCR with the *iap2* primers

Results: The *iap2* primers detected only *L. monocytogenes*, indicating that the specificity was excellent. The detection limit of *iap*2 primers to detect *L. monocytogenes* in the king trumpet mushroom was two log CFU/g without enrichment. The enrichment test results showed that *iap2* primers detected *L. monocytogenes* even at one log CFU/g in the mushroom after nine-h enrichment.

Significance: This result shows that *L. monocytogenes* at one log CFU/g in king trumpet mushrooms can be detected with *iap2* primers developed in this study after nine-h enrichment. Therefore, this method can be used for rapid and sensitive detection for *L. monocytogenes* at low levels in king trumpet mushrooms.

P3-111 A Reduced 90 ml Enrichment to Detect *Salmonella* from Environmental Surfaces Using the BAX System

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Introduction: In food processing environments, bacterial attachment to food contact surfaces can be a source of contamination to foods. Studies has indicated that strains of *Salmonella* can persist for up to 10 years in food processing environments despite successive cleaning and decommissioning of contaminated equipment.

Purpose: The BAX System PCR assay for *Salmonella* and Real-Time PCR assay has been previously validated for environmental surfaces using 225 ml of enrichment media. This study was designed to evaluate the method performance in a reduced enrichment volume.

Methods: Unpaired plastic and stainless steel surfaces (60 each) were inoculated with different *Salmonella* serovars and a competitive microorganism. Once dried, surfaces were sampled by swabbing the test area and then holding sponges at 4°C for 24 h. Test method sponges were enriched in either 90 m; of pre-warmed (35°C) buffered peptone water (BPW) or 90 ml of pre-warmed (35°C) LB. Reference method sponges were enriched in 225 ml of LB. All samples were incubated at 35°C for 20 to 24 h before PCR and culture confirmation following the FDA-BAM Chapter 5.

Results: For plastic surfaces, a $dPOD_c$ value of 0 (-0.25, 0.25) and 0.05 (-0.21, 0.30) was obtained, indicating no statistically significant difference between 90 ml BPW or 90 ml LB, respectively, and the reference method. For stainless steel, a $dPOD_c$ value of -0.35 (-0.57, -0.04) was obtained indicating a significant difference between both 90 ml methods and the reference. Although a higher proportion of positives were obtained by the reference enrichment, samples tested by each method were distinct test portions and when culture is followed to confirm presumptive positives there is 100% agreement.

Significance: This study indicates the ability to successfully reduce the enrichment volume to 90 ml of either BPW or LB to detect *Salmonella* from environmental surfaces using real-time or end-point PCR. Moreover, results were equivalent to the reference culture method.

P3-112 Validation of the BAX System Real-time PCR Assay for *Salmonella* in Fresh Cut Mango

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Introduction: Mangoes are considered a high-risk food capable of causing foodborne illness. In fact, seven *Salmonella* outbreaks have been traced back to mangoes over the last 10 years. Since mangoes are one of the most popular fruits in the world with a high consumption rate, robust pathogen detection methods are needed for consumer safety.

Purpose: This study was designed to evaluate the performance of the BAX System to detect *Salmonella* in fresh cut mango when analyzed as a single test portion and a five-sample pool after enrichment.

Methods: Fresh cut mango portions of 25 g and 375 g (30 each) were inoculated with *Salmonella* Newport at levels expected to create low (0.2 to two CFU/test portion) and high (five to 10 CFU/test portion) spike levels after 48 hours at 4°C. Paired 25-g samples were enriched with 225 ml of buffered peptone water and unpaired 375-g samples were enriched with 1500 ml of prewarmed (35°C) buffered peptone water. All samples were incubated at 35°C for 12 to 20 hours, analyzed by real-time PCR and confirmed according to the FDA BAM reference culture method.

Results: For 375-g samples, 16 low spiked samples were positive by real-time PCR and culture after 12 h of enrichment. For 25-g samples, 12 low spiked were positive by real-time PCR after 14 hours of enrichment whereas 13 samples were confirmed. For both methods, there was no difference in results between individual and pooled samples.

Significance: The results of this study demonstrate that there is no significant statistical difference between the real-time PCR and the reference method to detect *Salmonella* in 375 g or 25 g of fresh cut mango.

P3-113 Validation of Detection of *Listeria monocytogenes* in 125 g Natural Cheese Product by Real-time BAX LM PCR and VIDAS LMO2 Methods

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Introduction: Microbiological methods are generally validated for detection of one cell of *Listeria monocytogenes* in 25-g test portions for specific food matrices. To maximize time and money, many laboratories pool multiple 25-g test portions into one 125-g sample.

Purpose: The objective of this study was to compare the detection of *L. monocytogenes* in 125-g test
portion of Swiss cheese by two candidate methods, the VIDAS LMO2 and the BAX System Real-Time PCR, to 25-g test portions by FDA BAM reference method.

Methods: Individual portions of 25 g and 125 g of Swiss cheese were prepared and inoculated targeting low and high inoculations levels with one strain of *L. monocytogenes* according to FDA's Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds. Inoculation levels were 1.8 and 8.6 CFU/g for low and high target, respectively. Replicate samples (20 low and five high target inoculated samples) for each method were analyzed. Results were statistically analyzed using probability of detection (POD) for unpaired samples.

Results: The presumptive and confirmed results were identical for all samples. Both candidate methods performed as well or better (significant difference at the 0.05 probability level) when detecting *L. monocytogenes* in 125 g of Swiss cheese when compared to the FDA BAM method (25 g).

Significance: Based on the study results, VIDAS LMO2 and BAX System Real-time PCR for *L. monocytogenes* are acceptable methods for use in detecting *L. monocytogenes* in pooled 125 g Swiss
cheese.

P3-114 A Quantitative Approach Utilizing the BAX System Real-time PCR Assay for *Salmonella* to Estimate Log CFU/Sample in Ground Turkey

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Introduction: The United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) does not maintain a zero-tolerance policy for *Salmonella,* despite being a known food safety hazard in poultry. Instead, the prevalence of *Salmonella* is measured under established performance standards. However, many poultry producers are interested in quantifying *Salmonella* to ensure process controls are effectively working.

Purpose: The purpose of this study was to evaluate the efficacy of cycle threshold (C_t) values reported from the BAX System Real-Time PCR assay for *Salmonella* to accurately estimate the log CFU/sample contamination level in ground turkey.

Poster

Methods: In each of two independent experiments, 21 to 325-g samples of ground turkey were inoculated with a five-strain *Salmonella* cocktail at seven one-log increment inoculation levels. Samples were enriched in 975 ml of pre-warmed (45°C) MP media and incubated at 45°C for six and eight h. Triplicate lysates were prepared from each enrichment at each time for a total of 144 PCR tests per experiment. The obtained C_t values at each time point and the inoculation levels were analyzed to create a linear fit equation to estimate log CFU/sample of *Salmonella*. The linear curves at each time point were compared using R^2 and SE to determine the most appropriate enrichment time.

Results: The six h incubation provided the best fit linear curves in both studies (R^2=0.97 and 0.91; SE=0.31 and 0.45) compared to the eight h curves (R^2=0.91 and 0.87; SE=0.60 and 0.68). In the first study, eight h incubation data was truncated at less than three log CFU/sample, the resulting best fit linear curve was more accurate (R^2=0.89; SE=0.37) in enumerating *Salmonella* at a low level (-0.17 to 2.83 log CFU/sample).

Significance: Utilization of CT values for estimating Log_{10}CFU/sample of *Salmonella* prior to enrichment in ground turkey provides an accurate estimation with a wide enumerable range at low concentrations and within-shift time-to-result.

P3-115 Comparison between BAX Cycle Threshold Values and Most Probable Number to Estimate Preenrichment Log CFU/ml of *Salmonella* in Pre-Scald and Re-Hang Chicken Rinsates at a Commercial Processing Facility

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Introduction: The poultry industry currently relies on Most Probable Number (MPN) to estimate the load of *Salmonella* contamination throughout the processing chain. MPN is labor intensive and requires 27 to 72 h total time to results with large variation in estimation.

Purpose: This study was conducted to compare the efficacy of BAX cycle threshold (Ct) values to the industry standard method of MPN when estimating preenriched log CFU/ml of *Salmonella* in pre-scald and re-hang chicken rinsates.

Methods: Pre-scald and re-hang chicken rinsates (30 ml) were combined with 30 ml of 42°C prewarmed BAX MP media, and inoculated with *Salmonella* Typhimurium from zero to six log CFU/ml. Samples were incubated at 42°C and analyzed with real-time PCR at four, six, eight, and 10 h of incubation. The obtained Ct values at each time point and inoculation levels were analyzed to create a linear fit equation to estimate log CFU/ml of *Salmonella* in preenrichment rinsates. MPN was conducted using the three-by-five tube method for all samples, incubated for 24 h at 42°C, screened with real-time PCR, and estimated *Salmonella* log MPN/ml was calculated utilizing the generated linear fit equation. The linear curves at each time point were compared using R^2 and SE to determine the most appropriate enrichment time for both rinsates.

Results: The four h incubation was the best fit linear curve for both pre-scald (R^2=0.99; SE=0.19) and re-hang (R^2=0.94; SE= 0.26) rinsates. There was no difference between linear curves generated from Ct values or MPN at any incubation. Ct values produced a wider estimated enumerable range of *Salmonella* for both pre-scald and re-hang rinsates, compared to MPN estimations.

Significance: Utilization of BAX Ct values for estimating log CFU/ml of *Salmonella*, prior to enrichment in pre-scald and re-hang rinsates provides a wider enumerable range, decreased variation, reduced time to results, and less labor per sample compared to current industry standard MPN methodology.

P3-116 Evaluation of Chlorine Dioxide Gas Treatments against *Salmonella* spp. Artificially Contaminated on Mung Bean Seeds

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Introduction: ***Salmonella*** outbreaks continue to plague sprout growers. Recent foodborne illness outbreaks associated with seeds and sprouts indicate current post-harvest practices have gaps and suggest a need for more effective treatments.

Purpose: The aim of this study was to evaluate the efficacy chlorine dioxide gas (CDG) against four *Salmonella enterica* serovars artificially inoculated on an understudied commodity, mung bean seed and sprouts.

Materials and **Methods:** CDG was generated with a ClorDiSys Minidox-L system. Mung bean seeds (100 g at a time) were immersed (five min) in 400 ml containing either ca. six or nine log CFU/ml of a *Salmonella* serovar cocktail (*Salmonella* Newport, Stanley, Muenchen, and Anatum), which yielded a final cell concentration of 4.63±1.77 and 2.99±0.24 log CFU/g seeds. Samples (400 g each) were treated with six or five mg CDG/L air for eight or 10 h at 22°C and 90% humidity in an environmental incubator on a tray or in a tumbler. Two independent trials of each experiment were performed using four separate sets. Sprout viability was assessed for seeds treated with six mg/l for 10 h. Cells were recovered by blending with 0.1% peptone and enumerated on xylose-lysine-Tergitol-4 agar after CDG challenge. Statistical analysis was completed using JMP Pro 14.0.

Results: CDG applied to seeds under the metal tray and tumbler condition yield the highest reductions (2.13 log CFU/g). However, follow up treatments with a higher CDG concentration (six mg/l CDG for 10 h) yielded highly variable results in the tumbler treatment while the metal tray treatment exhibited up to a 4.68-log CFU/g reduction. Evaluations with the same CDG treatment on sprout viability resulted in up to a 3.30 and 6.39-log CFU/g reduction on seeds and sprouts, respectively, and 94.9 to 98.5% sprout viability.

Significance: These results suggest CDG is efficacious against *Salmonella* spp. artificially contaminated on seeds and sprouts. Furthermore, under these conditions, CDG did not affect the viability of mung bean sprouts. However, treatment conditions appear to be an important factor associated with reproducibility. Ultimately, CDG shows promise as an effective control against sprout contamination and should be further investigated.

P3-117 Efficacy of Propidium monoazide Combined Real-time PCR to Detect Seven Viable Species of Foodborne Pathogens

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Introduction: Real-time polymerase chain reaction (qPCR) is widely used to detect pathogenic bacteria in food samples. This technique represents a reliable method with rapid and selective detection of food-borne pathogen. However, bacterial DNA may persist in the environment for a long time after cell death. Combined propidium monoazide (PMA) with qPCR can be an alternative for enumerating viable bacterial cells in samples.

Purpose: The purpose of this study was to evaluate the efficacy of PMA combined qPCR to differentiate seven species of bacterial DNA between viable and dead cells.

Methods: Seven species each of *Salmonella* Typhimurium, *Escherichia coli, E. coli* O157:H7, *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus* and *Clostridium perfringens* at a final concentration of approximately 10^8 CFU/ml were serially diluted tenfold and used in this study. Cells in one ml aliquots (10^0 to 10^6) were killed by exposure in a heat block at 99°C for 10 min and live and dead cells were treated with 50 µM PMA. Live and dead cells (10^0 to 10^6) treated with PMA or left untreated were also analyzed side by side in the qPCR assay.

Results: PMA treated live cells showed amplification levels similar to unttreated cells, demonstrating that PMA treatment did not affect live cells. Amplification of DNA from PMA treated dead cells was almost inhibited, in contrast to untreated dead cells, which were unaffected.

Significance: This results suggest that PMA combined with qPCR can be used for rapid and efficient routine analysis of bacteria causing foodborne diseases.

P3-118 Evaluation of Alternative Rapid Methods for the Detection of *Salmonella* spp. in Dark Chocolate Using Multiple Incubation Times

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Introduction: Rapid methods for detecting foodborne pathogens are increasingly pushing the boundaries of detection by reducing incubation times to shorten the time from sampling to result, thus increasing the risk of false negative results.

Purpose: The objectives of this study were to challenge the ability of two commercially available real-time *Salmonella* PCR kits to detect a diverse strain collection of *Salmonella* spp. in pure culture and in a dark chocolate matrix using the minimum and maximum incubation times specified.

Methods: Inclusivity was assessed using 70 *Salmonella* strains at one log above the calculated limit of detection (LOD) for each kit. Challenge studies were performed by inoculating *Salmonella* Poona and Salamae onto 20 pieces of dark chocolate per strain, incubating at room temperature for two weeks to achieve fractional positive levels (0.2 to 2.0 CFU/25 g chocolate), and following kit instructions using the minimum and maximum enrichment times. The FDA BAM method was included as a reference method.

Results: Both kits detected all 70 *Salmonella* strains at one log above the LOD. The kits also detected *Salmonella* Poona (10 of 20 confirmed-positive chocolates) using both the minimum (20 and 16 h for kits A and B, respectively) and maximum enrichment times (24 and 20 h, for A and B, respectively). In contrast, for *Salmonella S*alamae-positive samples (19 of 20 confirmed-positive chocolates), kit A had 1 false negative using the minimum enrichment time, whereas kit B was able to detect all positive samples using both enrichment times. There were no significant differences in either kit's ability to detect *Salmonella* at both the minimum and maximum primary enrichment times validated.

Significance: Our data suggests that it is sufficient to validate only the minimum incubation time for *Salmonella* rapid detection kits when the difference between minimum and maximum incubation times is ≤4 h.

P3-119 Independent Validation for the Polyskope 1.0 Multiplex Pathogen Detection Assay for the Detection of Shiga-Toxin-producing *Escherichia coli* Non-O157, *Escherichia coli* O157, *Listeria monocytogenes,* and *Salmonella* Species

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Introduction: The Polyskope 1.0 Multiplex Assay is a novel test to simultaneously detect *Escherichia coli* O157, non- O157 Shiga-Toxin Producing *E. coli* (STEC), *Listeria monocytogenes,* and *Salmonella* species in a single enrichment based on real-time PCR.

Purpose: A *Performance Tested Method℠* study was conducted to validate the Polyskope 1.0 Multiplex Assay for inclusivity and exclusivity as well as a matrix comparison study against industry-standard reference methods.

Methods: This assay was evaluated in an unpaired independent validation study compared to existing reference methods according to the AOAC validation guidelines. The matrix validations comprised twenty (20) replicates inoculated at the lower limit of detection (0.2-2.0 CFU) and five (5) replicates at a higher inoculation level (5 CFU).Polyskope 1.0 evaluated fresh raw ground beef (25 g), deli turkey (25 g), fresh baby spinach (25 g), and stainless steel environmental surface sponges (4" x 4" test area) after inoculation with a suspension of three microorganisms (STEC, *Listeria monocytogenes, Salmonella* species). All matrices were compared to appropriate reference methods from the FDA-BAM, USDA/FSIS-MLG or ISO reference standards.

Results: Polyskope 1.0 demonstrated no statistically significant differences between candidate and reference method results ($dPOD_c$) or between presumptive and confirmed results ($dPOD_{cp}$) for all three food matrices and one environmental surface analyzed. Results from all four inclusivity and exclusivity evaluations indicated the test method can accurately detect the target analytes and correctly excluded all non-target organisms. No differences were observed with the stability (both real-time and accelerated) or lot-to-lot evaluations. Polyskope 1.0 demonstrated robustness by remaining unaffected by small variations in method parameters, which had no statistically significant effect on the results for all eight variations.

Significance: Polyskope 1.0 was shown to be a specific, highly accurate and robust method for the simultaneous enrichment and detection of *Listeria monocytogenes, Salmonella* species, non-O157 STECs, and *E. coli* O157 across four matrices.

P3-120 Rapid Differentiation of Live and Dead Shiga Toxin-producing *E. coli* Using DNA Photo-labeling Combined with PCR

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❖ ***Developing Scientist Entrant***

Introduction: The CDC estimates Shiga toxin-producing *Escherichia coli* (STEC) causes over 265,000 infections and 30 deaths in the United States each year. While only viable STEC can cause illness, most rapid detection methods cannot differentiate between live and dead bacteria. DNA photo-labeling prevents PCR amplification of DNA from dead cells and thus selectively amplifies DNA from live cells.

Purpose: The purpose of this study was to develop and optimize a rapid, PCR-based detection method combined with DNA photo-labeling able to differentiate live and dead STEC.

Methods: Live and dead STEC cells, of various cell mixtures/concentrations and photo-labeling conditions, were treated with or without DNA photo-labeling dye ethidium monoazide (EMA) and then exposed to LED light. Inhibition of PCR amplification of dead cells' DNA was confirmed using end-point PCR.

Results: With photo-labeling, various concentrations of live *E. coli* O157:H7 mixed with dead cells at high cell concentration were selectively detected using end-point PCR by inhibiting amplification of dead cell DNA. Live *E. coli* O121 and O145 (1×10^5 and 1.0×10^8 CFU/ml) were successfully differentiated from dead cells with modified PCR parameters. Limit of detection was 1.0×10^3 CFU/ml. With 12 h enrichment of *E. coli* O157:H7, as low as 1.0×10^2 CFU/ml live cells were detected in presence of 1.0×10^8 CFU/ml dead cells.

Significance: Results suggest that DNA photo-labeling combined with PCR-based detection methods can potentially differentiate live and dead STEC.

Poster

P3-121 Single Lab Validation for the MPN-Real Time PCR Method for Detection of *Vibrio vulnificus* in Oysters

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Introduction: *Vibrio vulnificus* is the leading bacterial cause of morbidity in United States shellfish consumers. The most common method used to detect *V. vulnificus* in oysters, MPN-culture, is time consuming and laborious. Real-time PCR for detection is more rapid and utilizes the AB7500 Fast, the same instrument used in current approved MPN-Real time PCR methods for *Vibrio parahaemolyticus.*

Purpose: To evaluate a MPN-real time PCR method for enumeration of *V. vulnificus* as a more rapid and highly reliable alternative to traditional MPN-culture methods, following the guidelines for single laboratory validation of shellfish methods.

Methods: Inoculated oyster samples were analyzed using the MPN-real time PCR method. Twenty lots of 12 oysters were inoculated with varying levels of *V. vulnificus* ranging from -0.62 to 6.53 log MPN/g and analyzed in replicate.

Results: *V. vulnificus* was inoculated in all oyster samples at levels ranging from -0.62 to 6.53 log MPN/g, demonstrating a linear range of greater than seven log (Pearson's; r=0.978, *P*<0.001). There was no significant difference (two-sided *t*-test) between the inoculum and enumeration by MPN-real time PCR. This data demonstrates the MPN-real time PCR method is sensitive, accurate, and fit-for-purpose of the enumeration of *V. vulnificus* from oysters.

Significance: Up-to-date, rapid, robust, and highly reliable validated methods for the detection of *V. vulnificus* from oysters are needed for more accurate enumeration which can support appropriate risk modeling efforts.

P3-122 *Cronobacter sakazakii* ISO 22964:2017 Testing of Milk Powders Using Commercially Available PCR

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Introduction: The detection of *Cronobacter sakazakii* in milk powder is a major issue, as it is a public health issue and they can survive for long periods in dry conditions.

Purpose: The aim of this study was to use the ISO 22964:2017 method, along with PCR detection, to determine the sensitivity and interference from dead cells.

Methods: The iQ-Check *Cronobacter* spp. kit and the Biotecon Diagnostic Cronobacter Detection LyoKit were used. The Biotecon kit was used with manual and automated DNA extraction methods. Strains ATCC 29004 and 29544 were tested

Results: The numbers of *C. sakazakii* in the resulting enriched samples (from the ISO method) were eight log CFU/ml. These were serially diluted to four, five, and six log CFU/ml, and were tested using both PCR kits and manual DNA extraction. Both kits resulted in positive detection at our, five, and six log CFU/ml. Seven additional different skim milk powders were tested in a similar manner with the Biotecon test kit. Using the manual DNA extraction method at six log CFU/ml, all of the powders resulted in positive detection, while six of the powders resulted in positive detection at five log CFU/ml. Using the automated Roche MagNA Pure Compact System for DNA extraction, all seven powders resulted in positive detection at five log CFU/ml . When four log CFU/ml dead cells were added to the same matrix as used for live cell detection, no cells were detected by PCR in both methods. When five log CFU/ml dead cells were added, there was detection.

Significance: PCR methods are suitable for use with the ISO 2264:2017 method. The automated DNA extraction was more suitable for detection of *C. sakazakii* in skim milk powder samples using the Biotecon kit and there will be no interference from dead cells if the numbers are less than four log CFU/ml.

P3-123 Recovery of *E. coli* O157:H7 by the BAX System in Beef Trim Using Surface Sampling Swabs

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Introduction: The beef industry currently uses sampling techniques such as N60 or N60+ to detect *E. coli* O157:H7 contamination in beef trim. A surface sampling swab provides a simplified sampling approach that is nondestructive and provides labor savings. In combination with post-enrichment pooling, which is commonly practiced, surface sampling has the potential to produce rapid cost-efficient results when screened with PCR and to reduce product waste.

Purpose: Evaluate the performance of a Real-Time PCR assay to detect *E. coli* O157:H7 from beef trim from individual and pooled enriched MicroTally sampling swabs in comparison to the USDA-FSIS reference culture method.

Methods: Thirty swabs were used to sample beef trimmings. Twenty swabs were inoculated with a low level of *E. coli* O157:H7 (one CFU/sample). Similarly, five additional samples were inoculated at a high level (five CFU/sample). All samples were held at 4°C for 30 h. Swabs were combined with 200 ml of pre-warmed (42°C) mTSB and incubated at 42°C for eight to 15 h. Following enrichment, samples were analyzed as a single test portion and as a five-combination enrichment pool by PCR and confirmed according to the USDA-FSIS reference culture method.

Results: PCR and culture returned 12 low level and five high level positives after both eight h and 10 h of enrichment. The positive response from the low-level sample set (POD 0.60) meets the validation criteria POD range (0.25 to 0.75), indicating the expected true absence of cells in some samples. When samples were pooled post-enrichment, there was no significant difference in results at eight h or 10 h.

Significance: This study demonstrates that beef producers can reliably use the BAX System to test for *E. coli* O157:H7 contamination from surface swabs for beef trimmings at eight to 10 h (individual and pooled samples) with equivalent sensitivity to the USDA-FSIS culture methods.

P3-124 Evaluating a High-throughput Targeted Amplicon Sequencing Approach for Simultaneous Detection and Quantitation of Foodborne Bacteria, Viruses and the Parasite *Cyclospora cayetanensis* from Complex Samples

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Introduction: Next-generation sequencing (NGS) methods provide resolution, scalability, and sensitivity for high-throughput surveillance. However, there are still significant challenges in detecting sub-populations of unculturable pathogens (specificity) present in samples at low levels (sensitivity) with the whole genome (WGS) or metagenome (WMS) sequencing approaches.

Purpose: The objective of this study was to demonstrate the utility of a targeted amplicon sequencing method to i) identify microbial content at the species level, ii) quantify intraspecies diversity down to strain level and iii) target low-level contaminants that may be present in complex communities in foods and clinical samples. This approach combines the power of WGS and the versatility of WMS with the robustness of a targeted approach.

Methods: Primer3 software was used to design primers from alignments of multiple sequences of 10 core genes for each of 266 species that included 135 pathogens. The primers were pooled as 1786 (all), 615 (pathogens) and 30 (*Cyclospora cayetanensis* only) pair panels. Microbial DNA community standards and DNA from clinical samples positive for *C. cayetanensis* were used. PCR amplicons were sequenced using Illumina MiSeq Platform. Our in-house bioinformatic pipeline was used for identification and quantification of the targeted organisms from the sequence reads datasets. Briefly, sequenced reads were matched by BLAST to a database of the gene sequences used in primer design.

Results: For the ZymoBiotics standards, the abundance of the reads corresponded to the relative amount of each pathogenic species present in the standard. For the *C. cayetanensis* samples, data from 1786 and 30 pairs were in 100% agreement. The larger panel additionally provided bacterial community stratification.

Significance: Use of a targeted approach for detecting low amount of pathogens provides another efficient and effective tool for the FDA to identify foodborne pathogens such as *C. cayetanensis.* This technique may enhance the detection of such foodborne pathogens in samples implicated in outbreaks.

P3-125 Optimizing a Multiplex qPCR Detection Assay for *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes* on ABI7500

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Introduction: Rapid detection of *Salmonella* spp., *Escherichia coli* O157:H7 (EHEC), and *Listeria monocytogenes* in foods is important for prevention of foodborne illnesses. Currently, there is no existing method approved by the FDA for the simultaneous detection of pathogenic bacteria in food or environmental swab samples; each organism is tested separately. A multiplex qPCR assay would facilitate detection of these three target pathogens after culturing of foods or swabs in a universal enrichment broth.

Purpose: The aim of this study was to optimize a multiplex qPCR assay for the simultaneous detection of *Salmonella* spp., EHEC and *L. monocytogenes* for use in analysis of environmental swabs or food matrices.

Methods: Primer and probe final concentrations ranged from 100 mM to 50 0mM. Reaction efficiency was calculated from the slope of the best fit line of the standard curve at each primer/probe concentration tested using the formula $E=(10^{-1}/\text{slope})^{-1}$. Efficiency is reported as a percentage and an ideal reaction efficiency is considered to be >90%. Reactions were performed in triplicate.

Results: Initial primer/probe concentrations of 200 mM, resulted in efficiencies of 89.88, 79.14, and 89.67% for *Salmonella*, EHEC, and *Listeria,* respectively, in single genomic reactions and 92.26, 81.53, and 103.18% for *Salmonella*, EHEC, and *Listeria,* respectively, when multiple target genomic DNA was combined. When EHEC probe concentration was varied, 150 mM was observed to have the best efficiency (81.8%). EHEC efficiencies of 78.96 and 90.11% were obtained when EHEC primer concentrations were 350 mM and probe concentrations were 150 mM and 200 mM, respectively.

Significance: This study defines the optimal primer and probe concentrations for a multiplex qPCR assay for the simultaneous detection of *Salmonella* spp., *EHEC,* and *Listeria monocytogenes* in foods and swabs allowing for a high-throughput, streamlined analytical approach that will increase the capacity of public health laboratories to rapidly detect multiple pathogens.

P3-126 Performance Validation of the BAX System Free DNA Cleanup Kit to Eliminate PCR False Positives Caused by External Contaminant DNA

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Introduction: PCR allows for exponential amplification of DNA into many copies. However, it does not differentiate between DNA from living cells and, external contaminant DNA resulting from procedures such as bacteriophage interventions. Any contaminating target DNA can be amplified during the PCR reaction and consequently cause false-positive results.

Purpose: To validate the performance of the BAX System Free DNA Cleanup Kit for removal of free, contaminant DNA and eliminate potential PCR false-positive-results.

Methods: First, a bacteriophage solution was diluted to 10^{10} PFU/ml and then treated using the DNA Cleanup procedure. Efficiency of the procedure in removing external contaminating DNA was determined by testing the treatment on BHI broth samples containing up to 400 ng of *Salmonella* or *Listeria* gDNA. To further validate the performance, 16 environmental swabs from surfaces treated with a *Listeria* bacteriophage solution were obtained from a food processing facility. The swabs were enriched and treated using the DNA Cleanup procedure. Effectiveness of treatment in all studies was assayed using PCR.

Results: The procedure was found to be effective in eliminating all external DNA from the bacteriophage solution and was able to eliminate up to 400 ng of target DNA. Thirteen of the 16 environmental swabs were found to be presumptive positive for *Listeria* using PCR. When taken through culture confirmation procedures, 12 were determined to be false positives (unconfirmable) and one true positive was culture-confirmed. The DNA Cleanup treatment was able to eliminate all 12 false-positive results and did not affect the detection of the one true positive.

Significance: The procedure has demonstrated robustness in elimination of contaminant DNA with the ability to remove 400 ng of DNA. Additionally, it has been verified that the treatment will eliminate only free contaminant DNA and will not interfere with DNA within living cells and their subsequent detection by PCR-pathogen-detection assays.

P3-127 Development of a New BAX® System Real-time *E. coli* O157:H7 Single Target PCR Assay

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Introduction: *Escherichia coli* O157:H7 is a pathogenic serotype of Enterohemorrhagic *E. coli* and is associated with several food and waterborne outbreaks worldwide. It is regulated as an adulterant in ground beef by the United States Department of Agriculture (USDA) with a zero-tolerance standard. The development of a single-target real-time *E. coli* O157:H7 PCR assay offers an alternative to the current dual-target assay.

Purpose: To validate the inclusivity, exclusivity and sensitivity performance of the new BAX System Real-Time *E. coli* O157:H7 Single-Target PCR Assay.

Methods: Overnight cultures of *E. coli* O157:H7 (*n*=242) isolated from various sources were prepared at 10^5 CFU/ml for inclusivity testing and non-target *E. coli* O157:H7 strains (n=300 non-O157:H7, *n*=185 non-*E. coli*) were prepared at 10^8 CFU/ml for exclusivity testing. For sensitivity testing, overnight cultures were serial diluted in BHI broth. Cell lysates of the serial dilutions were tested on the PCR assay. To evaluate the performance of the assay in a food matrix, ground beef was enriched for 18 hours and aliquots were inoculated with serial dilutions of an overnight culture before testing by PCR.

Poster

Results: Inclusivity testing demonstrated 100% detection of target organisms and, 100% exclusivity of non-target strains. The sensitivity of the assay was determined to be ≤1×10^4 CFU/ml in both BHI and ground beef studies.

Significance: The new assay provides users an option to choose between a single-target chemistry and the current dual-target chemistry to detect *E. coli* O157:H7 with equally robust performance. The cycling conditions of the new assay have been optimized to be compatible with all other real-time *E. coli* PCR assays in the portfolio. Additionally, the users will be able to test the assay on a new small-footprint instrument platform.

P3-128 Development of a PCR-Typing Method for the Identification of *Salmonella* Serotypes

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Introduction: Genus *Salmonella* is a major foodborne pathogen worldwide and consist of >2,500 serotypes classified by a serological method which has traditionally been a standard method for the classification of *Salmonella*. Serological identification of *Salmonella* is important clinically and epidemiologically to prevent and mitigate salmonellosis outbreaks, however, the serological method is time-consuming, labor-intensive and expensive, and more efficient DNA-based methods for identification of *Salmonella* are needed for public health.

Purpose: To develop and evaluate a set of PCR-serotyping methods for the identification of *Salmonella* serotypes, and as a substitute for inefficient serological methods.

Methods: Primer sets were designed from 64 candidate genes, expected to be unique in the *Salmonella* genus, of *Salmonella* Typhimurium, Typhi and Enteritidis. A total of 34 genes were screened and targeted including *Salmonella* genus-specific and subspecies I-specific genes for the development of a DNA-based PCR serotyping method. Patterns of PCR results of 139 *Salmonella* strains (83 serotypes) were encoded and phylogenetic analysis was performed by BioNumerics program to differentiate between serotypes of *Salmonella*.

Results: Screened target genes revealed various PCR patterns between *Salmonella* serotypes enabling each serotype of *Salmonella* to have identically encoded patterns from PCR results of 34 genes. The analyzed phylogenetic tree from PCR-typing results of 139 strains (83 serotypes) revealed the discrimination of *Salmonella* subspecies I from the other six subspecies and revealed that the patterns of PCR results in this study have enough discriminative power between the serotypes of *Salmonella* subspecies I.

Significance: This study will provide reliable DNA-based means for *Salmonella* serotyping and this method could be applied to the rapid monitoring of *Salmonella* in food industry and food safety clinically and epidemiologically.

P3-129 Development of Multiplex PCR for the Detection of Typhoidal *Salmonella* Serovars

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Introduction: Enteric fever, known as typhoid fever and paratyphoid fever, is caused by typhoidal *Salmonella enterica* serovars (Typhi, Paratyphi A, Paratyphi B and Paratyphi C). Due to these typhoidal *Salmonella* serovars having different epidemiologic characteristics, clinical manifestations, and immune response in human hosts, they are classified apart from non-typhoidal serovars among >2,500 *Salmonella* serovars. Rapid and reliable diagnosis of these typhoidal serovars is needed for mitigating the spread of *Salmonella* during an outbreak.

Purpose: To develop a multiplex PCR method for rapid and reliable identification and separation of typhoidal *Salmonella* serovars from non-typhoidal *Salmonella* serovars.

Methods: Primer sets were designed from 35 candidate genes, expected to be unique within the genus *Salmonella* to *Salmonella enterica* serovar Typhi. A total of four target genes were selected which produce specific PCR results patterns against *Salmonella* Typhi, Paratyphi A, Paratyphi B and Paratyphi C. A sextuplet multiplex PCR was designed including target genes that were *Salmonella*-genus specific, *Salmonella* subspecies I-specific, and typhoidal *Salmonella* serovar-specific, and was evaluated with the genomic DNAs of 139 *Salmonella* strains (83 serotypes).

Results: The PCR results of selected four genes revealed specific PCR product patterns with the genomic DNAs of *Salmonella* Typhi, Paratyphi A, Paratyphi B and Paratyphi C. The performance of the developed sextuplet multiplex PCR for *Salmonella* typhoidal serovars was demonstrated by specifically amplified results, with *Salmonella* serovars discriminated from non-typhoidal *Salmonella* serovars. This result suggested that the multiplex PCR has enough discriminative ability between typhoidal and non-typhoidal serotypes of *Salmonella* subspecies I.

Significance: This study will provide reliable DNA-based means for the diagnosis of typhoidal *Salmonella* serovars and this method could be a useful tool for rapid diagnostics of typhoidal *Salmonella* for the public health of humans, clinically and epidemiologically.

P3-130 Optimization of the Filtering Concentration Method for Rapid Detection of *Escherichia coli* O157:H7 in Lettuce and Cabbage Using Real-time PCR

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Introduction: *Escherichia coli* O157:H7 is one of the most important foodborne pathogens worldwide due to its high incidence and presence of various foods. Although numerous microbiological and molecular methods have been developed for detection, rapid detection of low concentrations in food remains challenging.

Purpose: In the present study, the filtering concentration method for microbes was optimized for rapid detection of *E. coli* O157:H7.

Methods: Performance of the filtering concentration method in terms of time requirements and recovery percentage were compared among seven filters. Tapping with 200 repeats was selected as the most effective detection method. The potential of rapid detection of *E. coli* O157:H7 in lettuce and cabbage using the filtering concentration method was evaluated using real-time PCR.

Results: As an optimal filtration method, MCE filter and detaching method using 200 repetitive tappings were used. The method could detect *E. coli* O157:H7 at a concentration of 10^0 CFU/g within two h without enrichment culture.

Significance: Filtering concentration is an effective concentration method for rapid detection of low levels of foodborne pathogens in lettuce and cabbage.

P3-131 Rapid Detection of *Escherichia coli* O157:H7 in Lettuce and Cabbage by Reducing Homogenization Buffer and DNA Elution Volumes

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Introduction: Foodborne bacteria are typically present at very low concentrations in food. Microbial enrichment is therefore essential to increase microbial cell numbers above the detection limit, a process that is the main hindrance for rapid methods since it generally takes more than 10 hours.

Purpose: This study describes a quick and simple method for concentrating *Escherichia coli* O157:H7 present in lettuce and cabbage, to enable rapid detection without the need for microbial enrichment culture.

Methods: This method involved reducing the homogenization buffer and DNA elution volumes. The homogenization buffer volume was adjusted to 225, 100, 50, 25, and 12.5 ml to isolate *E. coli* O157:H7 from 25 g of lettuce or cabbage. For effective homogenization, filtered air was added until the bags were fully inflated. The bags were sealed, then *E. coli* O157:H7 was obtained from lettuce and cabbage by vigorous hand shaking for 15 s. The extracted DNA was eluted in 200 or seven µl volumes, and cycle threshold values were compared using real-time PCR.

Results: As a result of the modified method, the microbial detection limit was improved 100-fold, so 10^0 CFU/g (three or nine CFU/g) of *E. coli* O157:H7 could be detected in lettuce and cabbage when 12.5 ml of homogenization buffer and seven µl of DNA elution volume was used, without the need for enrichment culture. The total time required for detection, including homogenization, DNA extraction, and real-time PCR, was less than two hours.

Significance: It is suggested that this method could contribute to the prevention of food poisoning incidents in institutional catering settings, such as schools or military facilities, by the rapid detection of foodborne pathogens prior to food consumption or during the food preparation period.

P3-132 Serotype Identification from Metagenomic Sequencing of Flour Inoculated with a Cocktail of *Salmonella enterica*

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Introduction: Flour has been associated with multiple outbreaks due to contamination with *Salmonella enterica*. The ability for *Salmonella* to survive in food commodities of low water activity has been previously studied using a cocktail approach and observing the bacterial counts after storage. By utilizing metagenomics, we can assess which serovars are surviving in these inoculation studies.

Purpose: This study was performed to identify which serovars of *Salmonella enterica* can survive storage at different relative humidities over a six-month time period by metagenomic sequencing.

Methods: Flour was inoculated with a cocktail of *Salmonella enterica* consisting of the following serovars: Agona, Enteritidis, Mbandaka and Newport. The inoculated flour was stored at a constant temperature of 25°C and variable relative humidities (30 and 60%). Samples of two grams were tested in triplicate at different time points. After an overnight incubation at 37°C, DNA was extracted using the Maxwell RSC Cultured Cells Kit, followed by library preparation with the Illumina Nextera DNA Flex kit and sequenced on a MiSeq. Raw fastq files were uploaded into Galaxytrakr and analyzed using the Short-Read Sequence Typer tool (SRST2), using the 7-gene Achtman MLST scheme for *Salmonella enterica*.

Results: By using the SRST2 tool, we were able to predict the sequence types of all four serovars inoculated in all samples up to day 14. At day 112, all four serovars were detected in the flour stored at 30% RH. By day 168, only three serovars at 30% RH and two serovars at 60%RH were detected. The overall coverage of the sequence type suggests that the serovar Mbandaka is not surviving as well.

Significance: By utilizing tools like SRST2 for analysis of metagenomic samples, we are able to identify multiple serovars of *Salmonella* in a contaminated food product. This method could be of high importance in a future outbreak situation where culture-independent methods are used.

P3-133 Comparison of the Molecular Detection Assay 2 *Salmonella* and Korean Standard Method (real-time PCR) for the Detection of *Salmonella* Typhimurium in Mousse and Tiramisu Cakes

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Introduction: The recent *Salmonella* food poisoning in Korea was associated with chocolate mousse cakes served in school meals in September 2018. The incident affected approximately 2000 individuals. Therefore, rapid detection methods are required to prevent food poisoning incidents in institutional catering settings, such as schools or military facilities, to detect foodborne pathogens prior to food consumption or during food preparation.

Purpose: The 3M Molecular Detection Assay 2 *Salmonell*a and Korean Standard Method were compared for the rapid detection performance of *Salmonella* in artificially inoculated mousse and tiramisu cakes.

Methods: Mousse (chocolate and cheese) and tiramisu cakes were artificially inoculated with *Salmonella* Typhimurium. Microbial enrichment culture was conducted using 225 ml buffered peptone water at 37°C for 24 h. After enrichment, the cultures were analyzed using the 3M Molecular Detection Assay 2 *Salmonella* and Korean Standard Method(Real-time PCR). Real-time PCR and 3M Molecular Detection Assay 2 - *Salmonella* amplification reactions were electrophoresed.

Results: Most of the inoculated samples showed similar results except chocolate mousse cakes. In chocolate mousse cakes, the detection ratio of *Salmonella* Typhimurium using the Korean Standard Method was low even after inoculation at concentrations of 10^3 and 10^4 CFU/25 g. However, in cheese mousse cakes, *Salmonella* Typhimurium inoculated at a concentration of 10^1 CFU/25 g was detected using the Korean Standard Method. In chocolate mousse, detection ratio of *Salmonella* Typhimurium using real-time PCR were partial positive to the chocolate mousse with chocolate content of 15% chocolate and negative to the chocolate mousse with chocolate content of 20 to 100% chocolate. Real-time PCR amplification reactions were electrophoresed and confirmed that amplification were not occurred.

Significance: Chocolate could have hindered detection using real-time PCR. The 3M Molecular Detection Assay 2 *Salmonella* showed higher detection ratio than the Korean Standard Method in chocolate mousse cakes.

P3-134 Comparison of the 3M Molecular Detection Assay and Korean Standard Method for Detecting *Salmonella* Typhimurium and *Listeria monocytogenes* in *Yuk-Hwe* and *Yuk-Sashimi*

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Introduction: *Yuk-hwe* and *Yuk-Sashimi* are Korean traditional foods made from raw beef. Therefore, they are potentially hazardous foods. In the present study, the performance of the LAMP bioluminescence assay and Korea Standard Food Codex (KSFC) Method in detecting *Salmonella* Typhimurium and *Listeria monocytogenes* in Korean traditional foods was compared.

Purpose: To compare the performance of 3M Molecular Detection Assay 2 and KSFC Method in detecting *Salmonella* Typhimurium and *L. monocytogenes* in Korean traditional foods.

Methods: *Yuk-hwe* and *Yuk-Sashimi* were artificially inoculated with 10^0 CFU/25 g *of L. monocytogenes* and *Salmonella* Typhimurium. *Citrobacter freundii* and *L. innocua* were used as competitive microflora. All samples were enriched with 225 mL of buffered peptone water for *Salmonella* Typhimurium and demi-Fraser broth for *L. monocytogenes* and incubated at 37°C. After enrichment, samples were analyzed using the 3M Molecular Detection Assay 2 and KSFC Method (real-time PCR, culture method).

Results: All samples inoculated at concentrations of 10^0 to 10^4 CFU/25g without competitive microflora were positive for *Salmonella* Typhimurium and *L. monocytogenes*, as detected by the 3M Molecular Detection Assay 2 and KSFC Method (real-time PCR and culture method). When all samples were inoculated with 10^2 CFU/25 g of competitive microflora, that was partially positive by all methods. The detection efficiency of the 3M Molecular Detection Assay 2 was higher than KSFC Method Method (real-time PCR), but there was no significant difference between KSFC Method Method (culture method). The detection limit of *Salmonella* Typhimurium and *L. monocytogenes* was 10^0 CFU/25 g.

Significance: The 3M Molecular Detection Assay 2, which is a LAMP-based technology, can be used for rapid detection of *Salmonella* Typhimurium and *L. monocytogenes* in raw beef. LAMP bioluminescence assays provide results on a subsequent day and are simple to use compared with the Korean Standard Method.

P3-135 Rapid Detection of Shiga Toxin Producing *Escherichia coli* Using Recombinase Polymerase Amplification

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a diverse subset of *E. coli* consisting of at least four distinct clonal lineages that produce Shiga toxin (Stx) 1 and/or 2. Pathogenic strains cause symptoms ranging from mild to bloody diarrhoea with flu like symptoms, to haemolytic uremic syndrome (HUS), a potentially fatal condition. While the most commonly isolated STEC in Canada is *E. coli* O157:H7, recent outbreaks of non-O157 STEC including *E. coli* O104:H4 in central Europe and *E. coli* O121:H19 in North America highlight the need for accurate and rapid detection methods.

Purpose: The purpose of this project was to develop a multiplexed recombinase polymerase amplification (RPA) assay targeting *Stx* genes for identification of STEC colonies.

Methods: A novel RPA method, which involves isothermal amplification of target genes and real time monitoring of product using a TwistAMP exo probe (TwistDX) was designed for Stx gene detection. A total of 71 STEC and 33 non-STEC bacteria were tested for inclusivity and exclusivity, respectively. A transfer study was performed and method performance was compared to known Stx status, determined by PCR or whole genome sequencing (WGS).

Results: The RPA assay detected 100% (*n*=71) of *Stx* producing bacteria and yielded no false positives. The RPA *Stx* profiles matched those determined by PCR and WGS. A minimum of 10^4 organisms were required for simultaneous detection of *Stx* 1 and 2 targets.

Significance: The RPA method developed herein reduces the time required to confirm *Stx* positive colonies in diagnostic laboratories from 3.5 h for the current PCR method to 20 min for RPA and enhances response time during outbreak investigations.

P3-136 Validation of a Thirty-Second Test for Beta-Lactams at United States Tolerance/Target Levels in Commingled Raw Milk

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Introduction: Testing bulk milk tankers for beta-lactams before unloading to dairy process facilities is a requirement in Appendix N of the Pasteurized Milk Ordinance (PMO) since the 1990s and a common economic practice since the 1970s. Testing time with targeted detection levels has steadily decreased from hours to minutes with technological advances.

Purpose: Validate the thirty-second test for six United States-approved beta-lactams at United States tolerance/target levels according to FDA protocol to evaluate screening tests for bulk milk tankers.

Methods: Rapid one-step assay (ROSA) lateral flow format using EZ-Reader automated incubate and read features (Charm Sciences, Inc., Lawrence MA) were adapted with predictive elements using fast flowing and reacting binding components to deliver a negative result in 30 seconds and a positive result within 30 to 60 seconds. Validation of method followed sensitivity, selectivity, component interference and incurred residue independent lab evaluations (Q-laboratories, Cincinnati OH and Eurofins-DQCI, Mounds View MN) described in FDA protocols for screening methods for raw milk.

Results: Sensitivity in part per billion (ppb=ng/kg) using 90% positive with 95% confidence calculated by probit analysis of percent of detection (POD) curve using seven concentrations met PMO requirements for targeted detection, below but within 50% of target/tolerance levels, for all six United States-approved beta-lactam drugs: penicillin G 2.9 ppb, ampicillin 5.9 ppb, amoxicillin 5.8 ppb, cephapirin 13 ppb, cloxacillin 8.1 ppb, ceftiofur metabolite 73 ppb. No interferences were observed from 33 animal drugs at 100 ppb, somatic cells at 1.2 million/ml, or gram-positive or negative bacterial levels of >300000 CFU/ml. Incurred residues reacted similarly or more sensitive to drug detection levels determined with spiked parent compound.

Significance: Faster and simple milk screening methods at targeted detection levels create opportunities for improved sustainability, automated testing and data collection earlier in the food chain.

Poster

P3-137 Limit of Detection of a ELISA Commercial Kit for the Detection of T-2 Toxin in Foods

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Introduction: T-2 Toxin belongs to the trichothecene group of mycotoxins and is produced by fungi of the genus *Fusarium*. T-2 toxin is often found in agricultural commodities. Due to its cytotoxic and immunosuppressive activity, T-2 toxin is a threat to human and animal health.

Purpose: This study's objective was limit of detection analysis of T-2 toxin in dry cereals, ground beef, hot dogs, infant formula and breaded chicken.

Methods: Matrix limit of detection studies were conducted in dry cereals, ground beef, hot dogs, infant formula and breaded chicken. Each matrix was fortified with T-2 toxin at levels of 800, 400, 200, 100, and blank ng/g and cold stressed overnight. Two different extraction methods used. The first required five g of each food mixed with a 25% solution of 50% methanol/water. The second, meat products and cereal, required five g and extracted with 25 ml of 70% methanol. Infant formula was diluted with 35% methanol/water. A 1000-µl aliquot of the eluate from each sample was analyzed with an automated plate reader at 450-nm wavelength.

Results: First extraction yielded 100% recovery in infant formula and dry cereal. breaded chicken and ground beef did yield some recovery, while hot dogs and luncheon meat did not. The second extraction yielded 93.75% recovery in infant formula. The dry cereal, hot dog, luncheon meat and ground beef had a recovery rate of 0% while breaded chicken did yield some recovery. The lowest limit of detection for this study was 100 ppb/ml.

Significance: The data from this study indicates the second extraction procedure, and commercial detection kit, may offer a suitable screening method for the detection of T-2 toxin in foods. This study has been submitted for review as an official FERN screening method.

P3-138 Validation of an ELISA Detection Method Extension for Abrin in Foods

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Introduction: Abrin is an extremely potent biotoxin produced from the seed of a tropical plant, *Abrus precatorius*, commonly found in the southern United States and the Caribbean. The plant is commonly referred to as the rosary pea or jequirity pea. While all parts of the seed are toxic, the highest concentrations are found in the seeds. The toxin is stable, lasting for long time periods in the environment.

Purpose: This study's objective was to incorporate the validated FERN Abrin method into the FERN Triage method. A parallel comparison was conducted between the current Abrin extraction, using GBS, and the Triage extraction, using a rinse with Bothell/Mod-1/WEAC Broth (BMW).

Methods: A matrix limit of detection and validation studies were conducted in hot dogs, ground beef, and infant formula. The limit of detection (LOD) was measured for hot dog and liquid infant formula samples fortified at levels of 500, 100, 50, 25, and zero ng/g, and ground beef (using both extractions), fortified at 1,000, 500, 100, 50, and zero ng/g and cold stressed overnight. During the validation study, the fortification low level was 50 ng/g and a high level of 500 ng/g. A 150 to 200 µl aliquot was analyzed using a commercially available ELISA assay for result confirmation.

Results: The multi-day validation study consisting of different extraction methods exhibited minor result variations. While the triage extraction method performed well, Abrin toxin degradation was observed between the various matrices.

Significance: The data from this study suggests that the extraction procedure, combined with the ELISA detection assay, may offer a suitable triage screening method for the detection of Abrin in foods. This study has been submitted for review as an official FERN.

P3-139 Detection of Ricin in Foods Utilizing a Handheld Detection Device

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Introduction: Ricin is a potent cytotoxin commonly found in the castor bean plant, *Ricinus communis.* Currently, there is not an antidote available for treatment of ricin toxicity. Due to the commonality of the plant, its ease of dissemination, and historical nefarious use, it is imperative to validate a reliable screening method for the detection of ricin in foods for biodefense purposes.

Purpose: This study's objective was to provide a lateral flow detection (LFD) screening method, combined with a single optimized extraction for preliminary and confirmatory detection of ricin toxin in foods.

Methods: Matrix extension validation studies were conducted in pork rinds, breaded cooked chicken, chili with meat, pork barbeque, RTE meat buckets, and raw sausage by six FERN laboratories. Samples were fortified at levels of 0, 2.5, and 5 µg/g with ricin toxin and cold stressed overnight. Six brands of each matrix were analyzed at each fortification. PBSTM was added in a 1:5 preparation, followed by a period of inversion, rest, and centrifugation. A 150 µl aliquot of the liquid eluate was removed and analyzed using commercially available LFDs and read at 15 and 25-minute intervals. A 100 µl aliquot of eluate was removed and analyzed using a commercially available ELISA assay for result confirmation.

Results: Each matrix analyzed exhibited six (100%) of six specificity. All foods analyzed, other than RTE meat buckets, demonstrated 12 (100%) of 12 sensitivity at all fortifications, with RTE meat buckets demonstrating 11 (91.7%) of 12.

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 ricin 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.

P3-140 Simultaneous Quantification of Aflatoxin, Vomitoxin, and Fumonisin in Corn Using the Envirologix Common Extraction Protocol for Flex Mycotoxin Immunoassays

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Introduction: The immune and gastrointestinal effects of mycotoxins on human health and animal production have been well-characterized. Most mycotoxin research has focused on the prevalence and health impacts of individual mycotoxins, but there is increasing evidence that mycotoxin types frequently co-occur with additive or synergistic cytotoxicity.

Purpose: To enable rapid, on-site testing of multiple mycotoxins simultaneously, the EnviroLogix Common Extraction protocol can be paired with aflatoxin, vomitoxin (DON), and fumonisin quantitative immunoassay lateral flow strips (LFD) from a single sample preparation in under 10 minutes, representing at least a 40% reduction in cumulative test time compared with separate sample extraction protocols.

Methods: Ground corn samples with aflatoxin, DON and fumonisin at low (5.7 ppb aflatoxin/1.9 ppm DON/0.9 ppm fumonisin), medium (19 ppb aflatoxin/5.3 ppm DON/1.9 ppm fumonisin), and high levels (300 ppb aflatoxin/30 ppm DON/5.5 ppm fumonisin) were extracted and tested according to the common extraction kit protocol. Sample extracts were diluted in the provided buffer at 1:1 for DON and aflatoxin, and 1:60 for fumonisin. Test strips were developed in the diluted samples for four min at 22°C. Quantitative results were generated on the QuickScan reader system.

Poster

Results: This rapid test demonstrated CVs of five to 14% across all sample ranges (*n*=12). Sample test means were within 10% of the known mycotoxin level, except for fumonisin in the low-level sample (0.93 ppm) which yielded a mean recovery of 1.2 ppm.

Significance: These results show that this rapid test may be used in robust risk-management mycotoxin testing programs for grain and feed.

P3-141 Detection of Acrylamide in Foods Using Filtration-assisted Optical Detection

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Introduction: Acrylamide is a neurotoxin and carcinogen present in food products and water sources due to high-temperature processing and contamination, and is tightly regulated by regulatory agencies around the world. Acrylamide is quantified using GC and HPLC methods, however, the food industry has been looking for alternative methods with high speed and low cost.

Purpose: Herein, we report a facile optical detection method for acrylamide via a thiolene 'click' reaction with a mercaptan compound, 2,2'-(ethylenedioxy) diethanethiol (EDT).

Methods: Briefly, acrylamide was extracted from food matrices using dichloromethane followed by enrichment by water extraction. The water extract was further purified by solid phase extraction (SPE) and was subjected to reaction with EDT (100°C, 10 min). The resulting reaction mixture was formed grey-colored nanoaggregates with silver nanoparticles (AgNPs), which were quantified by filtration onto filter membranes (0.2 µm pore size), followed by grey scale analysis.

Results: The grey intensity of the nanoaggregates followed a reverse linear regression model (R^2=0.93) against acrylamide concentration (zero to one ppm). The method was also used to detection acrylamide in instant and ground coffee samples with a limit of quantification (LOQ) of 0.1 ppm, low enough to meet the industry requirements.

Significance: The method was also used to detection acrylamide in instant and ground coffee samples. The filtration-assisted method we developed provide a simple, fast and inexpensive approach for optical detection of acrylamide using AgNPs, with potential to be integrated with camera-based device to be performed in low resource and on-site settings.

P3-142 Determination of Endocrine Disruptors and Two Metals in Foods

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Introduction: Perfluoroalkyl substances (PFAS), phthalate esters (PAE), and bisphenols are commonly used in commercial products and could interfere with endocrine systems. Gallium (Ga) and indium (In) are frequently used in photonic industries, and they may result in cell oxidative stress.

Purpose: This study measured 10 PFAS, six PAE, three bisphenols, and two metals in six types of foods and evaluated human exposure through food intake.

Methods: Pork, pork liver, pork kidney, fish, clam, and oysters were investigated (63 samples). One g of homogenized food sample was extracted with QuEChERS and dSPE with EMR-lipid adsorbent. After concentration, the extracts were analyzed with UHPLC-MS/MS and isotope dilution techniques. Regarding the analysis of Ga and In, 0.5-g food samples were prepared with microwave digestion before injecting onto ICP-MS.

Results: Pork liver and pork kidney accumulated more PFAS (total GM: 23.6 ng/g) and PAE (total GM: 784 ng/g) relative to other food items. Bisphenol A was not observed (limit of detection 1.74 to 16.6 ng/g) in all the samples; however, its alternatives Bisphenol F and Bisphenol S were found in seafood (range 0.32 to 2.79 ng/g). Clam and oysters also contained much higher amount of Ga (GM 65.1 ng/g and 32.4 ng/g) then those in other foods (0.18 to 2.02 ng/g) The general population may be exposed to PFAS from the six food items in 13.9 ng/kg per day on average. DEHP and DINP contributed 32.9% (245 ng/kg per day) and 62.1% (462 ng/kg per day) to the total PAE exposure.

Significance: These data indicated that the industry may replace bisphenol A with its substitutes. PFBA and PFPeA accounted for 30 to 50% of the total PFASs in foods, which revealed that the industry may shift the use from long-chain PFASs to short-chain PFASs.

P3-143 Determination of Perfluoroalkyl Substances in Food Packaging in Taiwan

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Introduction: Perfluoroalkyl substances (PFAS) possess oil-resistant and waterproof properties, and are widely used in industrial and consumer products. People are concerned about their persistence and toxicity, and some PFAS have been listed in the Stockholm Convention.

Purpose: This study developed and validated a method for determining 20 PFAS in food packaging with an ultra-performance liquid chromatography/triple-quadrupole tandem mass spectrometer (UPLC-MS/MS), and the method was applied on measuring 32 samples of commercial oil-resistant food packaging in seven categories.

Methods: Samples of 100 cm^2 were cut into pieces and were ultrasonicated in 20 ml methanol at 50℃ for 45 min. After centrifugation, the supernatant was concentrated to one ml. Six types of 20 PFAS were analyzed with UPLC-MS/MS using 15 stable isotope-labeled internal standards in 32 oil-resistant food packaging samples from local markets and restaurants in Taiwan.

Results: The linear range of the analytes was 5.0 to 1,000 ng/dm^2 except for 4:2 FTOH. Most limits of detection were between 0.07 and 11.3 ng/dm^2. The recoveries ranged from 70 to 117% on most analytes at three tested levels, and the precision (% RSD) was lower than 19% (*n*=5). Three of four microwave popcorn packaging contained four- to nine-carbon perfluoroalkyl acids at 8.3-1,960 ng/dm^2 and FTOHs at 121-7,188 ng/dm^2. Perfluoroalkyl acids (5.7 to 48.6 ng/dm^2) and FTOHs (454 to 2,595 ng/dm^2) were also found at one of three oil-proof paper bags. PFBA, PFHxDA, and PFBS were observed at one of three chicken boxes and one of four fry paper bags, ranged from 5.0 to 40.3 ng/dm^2; FTOHs were present in one of the four fry paper bags (22.4 to 167 ng/dm^2).

Significance: PFAS, including eight carboxylic acids, three sulfonic acids, two sulfonamides, three fluorotelomer alcohols, and four polyfluoroalkyl phosphates in food packaging, were analyzed in one assay with optimized sample preparation and instrumental parameters. The detection limits were down to sub-ppb levels.

P3-144 Use of Surface-enhanced Raman Spectroscopy in Determination of Nano-sized Particles in Food Grade TiO_2

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Introduction: Titanium dioxide anatase is an important white food colorant (E171) and used in a variety of food products such as doughnuts, candies, frosting etc. However, numerous recent studies show that around 36% of E171 contains nanosized particles, that could have potential risks to human health and the environment. In spite of this, TiO_2 is widely used in the food industry because of its stability in food systems and affordable cost. The particle size of the commercially produced E171 is not regulated and currently available methods for nanosized particle analysis are very time-consuming and require expertise.

Purpose: The purpose of this research is to develop an innovative and rapid method using surface enhanced raman spectroscopy (SERS) to determine the presence, quantity and particle size of TiO_2 nanoparticles in E171.

Methods: SERS active gallocyanin dye molecules can bind with TiO_2 nanoparticles and can be concentrated on a membrane for analysis under a Raman microscope by the mapping technique.

Results: The concentration of TiO_2 nanoparticles is determined from the percentage of positive spots on the map. The particle size can be estimated from the ratio (R) between the Raman intensity of TiO_2 and the SERS intensity of gallocyanin bound to the nanoparticles. The ratio calculated from 100 nm particles can be used as a cutoff to confirm the presence of nanoparticles. Moreover, innovative SERS imaging is used to develop a visual interpretation of particle size distribution and amount of nanosized particles with a limit of detection of 0.2 ppm. It generates the colored image spectrum from blue to red correlating blue to least intensity spots and red to high-intensity spots. Our results show that the Raman intensity of the TiO_2 nanoparticles increases as the particle size increases. Therefore, blue to red can be correlated with the smallest to largest particle size.

Significance: SERS can be used as an important tool to rapidly analyze the presence of nanoparticles in food grade TiO_2.

P3-145 Particle Size Analysis for Detecting Crystalline Solids in Powder Infant Formula

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Introduction: Economic adulteration, fraud and bioterrorism are concerns in the global infant formula market. Inexpensive food ingredients can fraudulently replace other ingredients to increase margins and weights, which may negatively affect the consuming infants' health.

Purpose: To determine if a crystalline solid (iodized NaCl), added to infant formula powder as low as one percent on a w/w basis, could be routinely detected using a laser diffraction-based particle analyzer.

Methods: Iodized NaCl served as an experimental crystalline solid contaminant in powder infant formula. Four contaminated infant formula samples of equal total weight and varying percentages of iodized NaCl (one, three, five, and seven percent) were created and analyzed using a particle analyzer (Mastersizer 3000 equipped with the Aero S disperser). Using software, a fixed set of parameters was established for repeated determinations. The measurements of the blends and corresponding reference infant formula were performed 25 times each and unaveraged results were reported as Dx 10, Dx 50, and Dx 90. A *t*-test was then performed on the Dx 50 and Dx 90 values analyzed from blends and the respective controls using a 95% confidence interval.

Results: The method and instrumentation proved capable of detecting statistical differences in particle size in all of the blends tested vs. the control (commercial formula). The t-test performed on Dx 50 values from samples of one percent w/w iodized NaCl blend resulted in a *P* value of 0.004 while *P* values for blends greater or equal to three percent were < 0.001. When testing and comparing Dx 90 values, all *P* values were <0.001.

Significance: Laser diffraction particle size analysis can be used as a reliable means of detecting the presence of crystalline solids in amorphous food powders, such as infant formula. This method could be applied as a preliminary rapid screen to localize specific contaminants using confirmatory analysis.

P3-146 Effectiveness of Cleaning Strategies for Removing Milk Chocolate from Pilot-scale Chocolate Processing Equipment

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Introduction: Dark chocolate produced on equipment used to manufacture milk chocolate can contain milk due to cross-contact.

Purpose: The goal of this study was to evaluate the effectiveness of cleaning methods for removing milk chocolate from shared processing equipment.

Methods: Milk chocolate (40°C, 62,000 ppm milk) was processed in a ball mill (~0.35 kg) and a horizontal-shaft conch (2.5 kg), followed by draining the majority of the chocolate. Three cleaning methods were investigated for removal of residual milk chocolate: i) no cleaning, ii) cocoa butter (40°C, five min rinse), and iii) wet cleaning (detergent-rinse-air dry). After cleaning, three batches of milk-free dark chocolate (40°C) were processed in the ball mill (~0.35 kg) and conch (2.5 kg), and each batch was collected. Milk chocolate (1.5 kg) was processed on a three-roller refiner, followed by push-through with dark chocolate (~eight kg), and 0.3-kg samples collected at five-min intervals. Dark chocolate was analyzed for milk by ELISA (Neogen Veratox® for Total Milk). Trials and analyses were completed in triplicate.

Results: Milk levels were below the ELISA limit of quantitation (LOQ; 2.5 ppm) for all three dark chocolate batches produced after wet cleaning the ball mill and conch. The first batch of dark chocolate from the ball mill and conch with no cleaning contained up to 40,300 (1.7% CV) ppm and 18,100 (5.8% CV) ppm milk, respectively. A cocoa butter rinse reduced milk levels in the first batch of dark chocolate to 1,960 (5.0% CV) ppm milk (ball mill) and 2,440 (7.9% CV) ppm milk (conch). Milk levels decreased in subsequent batches of dark chocolate processed in both pieces of equipment. Initial dark chocolate samples from the refiner contained up to 2,140 (3.2% CV) ppm milk. After ~three kg of dark chocolate was processed on the refiner, measured milk levels were below the ELISA LOQ.

Significance: Cleaning strategies were identified for minimizing allergen cross-contact from use of shared chocolate processing equipment.

P3-147 Transfer of Shrimp Allergens to Oil and French Fries Using Shared Fryers

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Introduction: Minute amounts of shrimp can cause anaphylactic reactions in individuals with shellfish allergy. Many restaurants use shared fryers to cook various types of foods including seafood and vegetables, resulting in cross-contact.

Purpose: The objective of this study was to investigate transfer of shrimp proteins into frying oil and to French fries cooked in the same oil.

Methods: Ten batches of black tiger shrimp (100 g/batch) were fried in 4.7 l soybean oil (176°C, three min) and samples of frying oil (150 ml) were collected. Four batches of French fries (100 g/batch) were cooked in shrimp-contaminated oil (176°C, three min). Oil and French fry samples were analyzed using two crustacean ELISA assays and a total protein (Pierce bicinchoninic acid– reducing agent compatible) test. To evaluate the effects of frying time on detection of crustacean protein, shrimp were fried at 176°C for up to 70 min, and samples obtained were analyzed by ELISA and total protein tests. Experiments were completed at least three times.

Results: Maruha Nichiro ELISA and ELISA Systems tests detected up to 29.0 ppm (38% CV) crustacean protein and 0.91 ppm (53% CV) tropomyosin, respectively, in oil used to fry shrimp. An average of 144 ppm (13% CV) total protein was measured in oil after frying 10 batches of shrimp. Tropomyosin levels in French fries cooked in the shrimp-contaminated oil were below the limit of detection (0.05 ppm) of the ELISA System kit. However, the Maruha Nichiro kit detected crustacean protein in the French fries. A dramatic reduction in ELISA detection of shrimp proteins was demonstrated as a function of frying time.

Significance: This study provides evidence of transfer of shrimp protein to frying oil and to a secondary food. Levels of shrimp proteins transferred to frying oil and a secondary food may be underestimated by ELISA due to changes in shrimp protein that occur during frying.

P3-148 Sandwich ELISA Targeting Ara h 2 and Ara h 3 for Improved Detection and Quantitation of Peanut in Foods

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Introduction: Failure to detect undeclared peanut, especially in thermally processed foods, can affect food allergen labeling compliance.

Purpose: To develop a sandwich ELISA targeting Ara h 2 and Ara h 3 for reliable peanut detection and quantitation in processed foods.

Methods: Polyclonal antibodies against Ara h 2 and Ara h 3 from light-roasted peanut flour were raised in rabbits. The immunoglobulin G (IgG) antibodies were affinity purified using protein G columns. The specificity of antibodies against polypeptides from raw and roasted peanuts was confirmed by Western blot. A sandwich ELISA was developed using equal amounts of anti-Ara h 2 and anti-Ara h 3 IgG as capture antibodies and using their HRP conjugates as detector antibodies. Proteins extracted from light-roasted peanut flour were used to generate standard curves and samples were extracted in carbonate buffer for peanut quantitation. Cross-reactivity to plant- and animal-based foods was assessed. Peanut flour (0, 2.5, 5, 10, 25, 100 and 500 ppm)-incurred cookies baked at 190°C for 25 and 30 min were analyzed in triplicate to evaluate method performance.

Results: The Ara h 2 antibody exhibited similar immunoreactivity toward raw, light-roasted and dark-roasted peanut, whereas Ara h 3 antibody demonstrated reduced immunoreactivity toward dark-roasted peanut. The developed ELISA using both antibodies was sensitive, with a limit of quantitation of at least 0.78 ppm peanut protein in foods. Negligible or no cross-reactivity was observed with 40 different foods including select legumes. The ELISA detected peanut flour at incurred levels ≥2.5 ppm in dough and ≥5 ppm in baked cookies. Defatting and sonication improved peanut protein recovery from cookie matrices compared to vortex extraction. Higher recoveries were observed at low incurred levels of peanut flour.

Significance: Improved peanut quantitation methods can reduce false-negative results protecting peanut allergic consumers from inadvertent exposure to peanut in processed foods.

P3-149 Development and Validation of a Quantitative Monoclonal Antibody-based ELISA for the Detection of Sesame in Common Food Products

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Introduction: Sesame seed is currently regulated for labeling purposes as an allergen in Europe, Canada, New Zealand and Australia. While not considered one of the 'Big 8' food allergens in the United States, it is believed that the prevalence of sesame allergies is on par with allergies to soy and fish and the FDA recently announced that it is "advancing a new effort for the consideration of labeling for sesame to help protect people with sesame allergies."

Purpose: The objectives of our study were to i) develop a quantitative monoclonal antibody (MAb)-based ELISA for the detection of sesame and ii) validate the assay for sensitivity, recovery and cross-reactivity (CR) against a large panel of seeds, cereals, tree nuts, legumes, and spices.

Methods: MAbs were generated and screened for reactivity to black and white sesame seeds as well as flours generated from processed sesame seeds. A sandwich ELISA (sELISA) was developed using a pair of MAbs that efficiently detected the sesame allergen Ses i 6, a legumin-like seed storage protein which constitutes 60-70% of total sesame protein. Validations were performed using external proficiency test and reference materials as well as in-house and commercial samples to determine key assay parameters.

Results: The limit of detection for the assay was calculated to be 0.22 ppm, while the range of quantitation was determined to be one to 40 ppm sesame seed flour. Recovery from spiked samples and proficiency test materials was satisfactory and the assay was able to successfully detect sesame seed after common commercial processing such as toasting and roasting.

Significance: The MonoTrace Sesame ELISA is a novel MAb-based assay that is sensitive and highly specific to sesame, which is an allergen of rising concern within the US. The assay represents an attractive alternate to polyclonal antibody-based methods for detecting sesame contamination within raw and processed foods and ingredients.

P3-150 Development of a Monoclonal Antibody-based ELISA for the Specific Detection of Fish Tropomyosin

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Introduction: It is estimated that up to 0.4% of the overall United States population is fish-allergic. Allergies to seafood (fish and crustacean shellfish), peanut, and tree nuts are generally lifelong and account for the majority of allergic reactions in teenagers and adults. Labeling of foods that contain major food allergens, such as fish, is currently required according to the United States Food Allergen Labeling and Consumer Protection Act.

Purpose: The objectives of our study were to i) generate monoclonal antibodies (MAbs) to the known fish allergen tropomyosin, and ii) develop a MAb-based sandwich ELISA (sELISA) for the detection of a wide variety of fish species with limited cross-reactivity (CR) to shellfish, including crustacea and mollusk.

Methods: Tropomyosin purified from a mixture of raw and cooked fish samples was used to immunize female BALB/c mice from which MAb-producing hybridoma cells were generated. MAbs were screened for reactivity to a wide variety of oily (e.g., salmon) and white fin fish (e.g., cod). A sELISA was developed using a pair of MAbs in conjunction with optimized extraction conditions (buffer and temperature) that allowed for sensitive and specific detection of tropomyosin from 10 fish species.

Results: The MAb-based sELISA was shown to detect fish tropomyosin in both raw and cooked samples. In a series of proficiency test samples prepared with fish, crustacea, and/or mollusk contamination, the assay was able to specifically detect fish tropomyosin without demonstrating CR to crustacea or

mollusk. Testing of in-house spiked samples demonstrated that the assay was also able to successfully detect fish in a variety of raw and processed foods and ingredients.

Significance: The MonoTrace Fish ELISA targets the known allergenic fish protein, tropomyosin. The assay is highly specific to fish, recognizing various species of forage, oily, and white fish without demonstrating cross-reactivity to other seafood (*n*=11) or meat (*n*=6).

P3-151 WITHDRAWN

P3-152 Making Sulfur-free White Wine through the Use of α-Pinene

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Introduction: Because sulfur dioxide causes human health risks, including dermatitis, urticaria, angioedema, diarrhea, abdominal pain, which also widely used preservative in winemaking due to its well-known antioxidant and antimicrobial properties. Today's consumers demand high-quality foods that are free from additives, fresh testing, microbiologically safe.

Purpose: With this in mind, this study uses the α-pinene to alternative the sulfur dioxide in the white grape wine fermentation model, and the parameter of white grape wine also analyzed. α-pinene is a fragrance monomeric compound derived from fruit aroma substances including grapes. Wine treated with α-pinene (0.3125%, 0.625%, 0.125% group) were compared with wines treated with sulfur dioxide.

Methods: The parameter (include specific gravity, pH value, total soluble solids, ethanol, percent transmittance, titrate acid, Lab, total pectin) between SO2 group and SO2 free red wine (α-pinene treat group) was studied.

Results: The transmittance of five groups fell between 70 and 90, the α-pinene group (0.03125% & 0.0625%) is superior to the control group and the sulfur dioxide group. The percent transmittance and L (82-85), *a* (3.3-3.49), *b* (3.01-3.03) value show the significant difference was observed in the α-pinene group (n=9, p<0.05). Addition of α-pinene or sulfur dioxide can effectively inhibit the browning of white wine storage at 180th days, but the controlled increase about 0.25 absorption wavelength. Other parameters of white grape wine no differences were found in all groups.

Significance: The conclusions showed the α-pinene have the excellent antibacterial, antioxidant ability and also extending the browning reaction of white grape wine, demonstrated a good alternative material for winemaking.

P3-153 Factors That Impact Survival of *Salmonella* during Storage of Beans and Batch Production of Cold Brew Coffee

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❖ ***Developing Scientist Entrant***

Introduction: Cold brew coffee, which eliminates the final thermal treatment step from the traditional brewing process, has increased in popularity in recent years. The ability of *Salmonella* to survive on coffee beans or during production of cold brew coffee beverages is unknown.

Purpose: To evaluate the survival of *Salmonella* on roasted coffee beans and in cold brews under typical storage conditions.

Methods: Green *Coffea arabica* beans (fully wet-washed and sun dried, grown in Siguatepeque, Honduras) were roasted to light, medium, or dark specifications using a one-kg Probatino Roaster, and then inoculated with a five-strain *Salmonella* cocktail collected from agar lawns in 0.1% peptone (12.5 m; to 100 g). Inoculated beans were dried at 23°C for one d, followed by 55°C for 17 h, and then stored at four, 10 or 23°C for one month. Ground inoculated beans (2.5 g) were mixed with 25 ml of spring water (low brew ratio); an additional 2.5 g of uninoculated ground beans was added for the high brew ratio. Cold brews were stored at 4 or 23°C, sampled over 24 h and plated onto tryptic soy agar and ChromSal. All experiments were replicated twice with triplicate samples within each replicate (*n*=6).

Results: No significant declines (*P*>0.05; 4 and 10°C) or small but significant (*P*<0.05; 23°C) declines of *Salmonella* were observed on medium roast coffee beans during three months of storage. Populations of *Salmonella* decreased by 0.3 to 1.4 log and two to five log over 24 h in brews held at 4 and 23°C, respectively. At most time points, reductions of *Salmonella* in the brewed coffee were not significantly different among light, medium and dark roasts; significantly greater (*P*<0.05) reductions were observed at the high brew ratio.

Significance: *Salmonella* can survive on coffee beans contaminated after roasting; brewing temperature and brew ratio significantly influence *Salmonella* survival in cold brews.

P3-154 Evaluation of a CO_2 Monitoring System for the Early Quality Testing for a Variety (Chocolate, Vanilla, Coffee) of High Protein Beverage Drinks

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Introduction: Consumers today have a passion for a wide variety of ready-to-drink (RTD) cold and hot beverages. The consumer request for high protein and unique flavors can present different microbial challenges. New flavor additions should be evaluated for compatibility and challenged with organisms of interest to ensure the detection method is fit-for-purpose.

Purpose: Four different flavors (vanilla, vanilla chai, chocolate and coffee) and two protein levels(16 g and 30 g) of RTD beverages were challenged with <10 CFU per 15.2 oz. container with either *Bacillus*, *Staphylococcus*, or *Pseudomonas* and evaluated for growth and detection.

Methods: Individual beverages were inoculated in quadruplicate and incubated for 22 to 26 h at 35°C and then tested individually or mixed by three (one inoculated sample with two uninoculated samples) to determine if early contamination could be determined based on microorganism CO_2 production. Quadruplicates were prepared from each of inoculated bottle to establish reproducibility and any limitations of the product on organism recovery or the assay for detection of pooled samples.

Results: Dairy protein beverages were compatible (≤ 10 ml) in the BacT/ALERT system with no false positives in 60 replicates. Each of the challenge organisms grew to high enough levels after only 24 h hold time to be detected in the BacT/ALERT system in < 24 hours, even when mixed (three by two ml). Organism rate of growth was unique to the flavor and class of organism. *Bacillus* grew and were detected between 6 and 15 h, *Pseudomonas* between six and 18 h and *Staphylococcus* between four and 13 h. Media containing neutralizers was required for *Staphylococcus* recovery from chocolate and coffee flavored drinks.

Significance: The different challenge studies demonstrated the efficacy (fit for purpose) of the BacT/ALERT for sterility screening of variety of high protein coffee, vanilla and chocolate beverages. Secondary mixing of samples could be performed without loss of sensitivity.

Poster

P3-155 Microbial Source Tracking of Fecal Contamination in Maipo and Maule Rivers in Central Chile

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Introduction: Waterborne infectious diseases cause an estimated 2.2 million deaths per year. Contamination with feces is the main source of pathogens in surface water. Therefore, it is very relevant to determine the origin of the fecal sources contaminating surface waters, especially if these have agricultural uses, such as the Maipo and Maule rivers in Central Chile. While fecal coliforms are used as indicators, these do not provide information about the source of contamination. Host specific microorganisms such as *Cryptosporidium*, *Giardia* and *Salmonella enterica* allow tracing of sources of fecal contamination in surface waters.

Purpose: The main objective of this work was to determine the impact of land use on fecal contamination in surface water in the Maipo and Maule rivers in Central Chile

Methods: A total of 21 l of surface water was collected from the Maipo and Maule rivers every three months, starting in August 2017. Twelve sampling sites representing different land uses were selected (natural, agricultural, urban and livestock areas). To determine fecal coliforms we used one liter for the most probable number (MPN) method based on Chilean regulations (1,000 coliform/ml is permitted). The other 20 l were subjected to ultrafiltration to quantify *Cryptosporidium* and *Giardia* by immunomagnetic separation and direct immunofluorescence, and PCR sequencing. *Salmonella* was quantified (CFU) in Agar XLT-4 and bismuth sulfite medium and confirmed by PCR for typhoidal and non-typhoidal *Salmonella*.

Results: Up to 30,000 coliforms/ml were found in the Maipo river in the urban area in January 2018. Zoonotic genotypes of *Giardia* and bovine genotypes of *Cryptosporidium* were detected in urban areas in both rivers. One typhoid *Salmonella* and five non-typhoidal *Salmonella* were also found in urban sites of the Maule river.

Significance: Our results will allow designing better mitigation measures and will lead to preventing infections transmitted by water and the risk for food contamination.

P3-156 Microbial Evaluation of 'Adoyo' Drink Sold in Ogun State, Nigeria

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Introduction: Adoyo is an herbal drink produced by boiling pieces of African yellowood and lemongrass in omidun and adding pieces of grape, pineapple and ice. The drink is believed to cure malaria but it can also serve as a refreshing drink when served cold.

Purpose: Due to the prevalence of foodborne illness, it is important to assess the quality and evaluate the microbial content of adoyo drink that is being sold.

Methods: Samples were purchased from three major car parks in Abeokuta while the control sample was produced in the laboratory. The samples were analyzed for chemical, microbial and heavy metal properties using standard laboratory techniques.

Results: Sample pH ranged from 4.39 to 4.58 as a result of the omidun, which is highly acidic due to fermentation. Vitamin C content of the samples ranged from 33.07 to 38.57 with the control having the highest. There was no detection of lead or mercury but a small amount of cadmium was detected in a safe quantity of 0.06. Zinc, which is likely already existent in fruit was detected below the minimum permissible limits of five mg/l. Staphylococcus, coliforms and fungi ranged from 1.5×10^6 to 4×10^7, 1×10^5 to 3×10^6 and 2.5×10^6 to 5×10^6 respectively. The assessment of the total coliform count in adoyo showed five genera of bacteria in the coliform group that were characterized as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp., and *Proteus* spp. Fungal count ranged from 2.5×10^6 to 5×10^6 CFU/ml with the control having the lowest count of 2.5×10^6 CFU/ml.

Significance: This data suggest that the presence of *Staphylococcus aureus* and *Trichoderma* probably indicates contamination from the raw materials, environment or personnel processing or vending the adoyo.

P3-157 Does the Indigenous Microbial Community of Kombucha Prevent Survival and Growth of Pathogens?

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❖ ***Developing Scientist Entrant***

Introduction: Kombucha is a fermented, acidic beverage that is frequently prepared using home brewing methods. This raises concerns about the food safety risks involved.

Purpose: The objective of this study was to evaluate the microbial ecology of various kombucha blends as well as the potential for growth of foodborne pathogens during brewing.

Methods: Four different commercially available kombucha starter kits were brewed according to suppliers' instructions and then examined over a 14-d period. Aerobic mesophiles, yeasts, lactic acid bacteria, and acetic acid bacteria were enumerated on selective media. Survival of *Salmonella enterica*, *Escherichia coli*, and *Cryptosporidium parvum* were assessed. Culture techniques were used to enumerate and detect (by enrichment) bacteria and an HCT-8 cell line was used to enumerate *C. parvum*. Starter kit sweetened tea blends (BT) were used as controls.

Results: Populations of aerobic mesophiles, yeasts, lactic acid bacteria, and acetic acid bacteria increased more than two log during the first 72 h and then plateaued and gradually decreased after seven d. *Salmonella* and *Escherichia coli* grew and persisted in BT over 14 d but showed a significant difference in the different tea blends (n=72, P<0.0001) (n=60, P<0.0001). *Salmonella* decreased in kombucha 5.61 log CFU/ml (n=207) by seven d, with the most significant reduction at three to five d (n=36, P<0.0001). *Salmonella* was undetected by enrichment in 77.7% of samples (n=18) after seven days of brewing. *Escherichia coli* decreased in kombucha by 4.99 log CFU/ml in seven d, with the most significant reduction in five to seven days (n=20, P<0.001).

Significance: Results of this study provide information concerning fluctuations in the indigenous microbial ecology during kombucha brewing, as well as the capability of *Salmonella*, *E. coli*, and *C. parvum* to survive and grow. This will help to better assess food safety risks during home brewing of kombucha.

P3-158 WITHDRAWN

P3-159 Inactivation of Foodborne Pathogens in Opaque Fluid Using a Thin-film UV Reactor

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Introduction: Although the inactivation of microorganisms in water and high transmittance liquid foods have been studied extensively, the efficiency of the process is rather low for treating opaque fluids using traditional UV treatment apparatus.

Purpose: To evaluate the ability of UV-C light treatment to inactivate food borne pathogens in simulated opaque fluid using thin film reactor.

Methods: The thin film UV apparatus used in this study consists of a three lamp thin-film annular reactor consisted of inlet and outlet tanks, a peristaltic pump, three thin-film reactors each with a monochromatic low-pressure amalgam lamp emitting at a 254 nm wavelength, was positioned at the center of the stainless-steel reactor. UV fluence distribution of reactor was validated by biodosimetry using T1 bacteriophage. Opaque test fluid was prepared by adding concentrated humic acid to water until to get UV transmittance (%/cm) of $7.39\times10^{-12}\pm1.35\times10^{-12}$ and pH of the fluid was adjusted to 7.1±0.2. Test fluid was inoculated separately with test microorganisms (*E. coli*, *Salmonella* Muenchen, *L. monocytogenes* and *B. cereus* endospores) to a concentration of ~7 seven log CFU/mL^{-1} and treated with UV-C light (n=3). After treatment, enumeration of spores was performed by serial dilution plate count method (detection limit two log CFU/mL^{-1}).

Results: Populations of vegetative microorganisms were reduced by more than five log and endospores were reduced by 4.5 log The inactivation kinetics of microorganisms were described by log linear models with low root mean square error (RMSE) and high coefficient of determination (R^2>0.98) confirms a similar dose distribution in each reactor. The D_{10} values were identified as 2.9±0.1, 4.4±0.3, 4.7±0.2 and 9.3±0.2 mJ·cm^{-2} for *E. coli*, *Salmonella* Muenchen, *L. monocytogenes* and *B. cereus* endospores, respectively. This study clearly demonstrated that high levels of inactivation of microorganisms can be achieved in opaque fluids using UV-C light.

Significance: This study advances the feasibility of UV-C light technology to treat opaque liquid beverages.

P3-160 Effects of High Pressure and High Temperature Short Time Processing on Microbiological Shelf Life, Physicochemical Properties, and Non-Enzymatic Browning in Atemoya Juice

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Introduction: Atemoya is one of the important tropical fruits, and its processed products are of high commercial and economic importance. The work reports the effects of high-pressure processing (HPP) as compared to high-temperature short time (HTST) processing on overall quality and shelf life of atemoya juice during cold storage.

Purpose: The purpose of this study was to evaluate HPP treatments that may reduce microbial load and non-enzymatic browning, and maintain a stable quality of atemoya juice.

Methods: Atemoya juice treated by HPP and HTST were evaluated for effects on natural microbial flora, non-enzymatic browning index, hydroxymethylfurfural (HMF), color, invertase, sugars, pH, titratable acidity, cloud, and aroma score, immediately after treatments and during six-week storage at 4°C.

Results: The amount of aerobic, psychrotrophic, *E. coli*/coliform, and yeasts and moulds in the juices were reduced by HPP or HTST to levels below the minimum detection limit, and showed no outgrowth after refrigerated storage for six weeks. However, HTST treatment significantly changed the color of the juice, while no significant difference was observed between the untreated and HPP juices. Both HPP and HTST reduce the invertase activity, which could possibly prevent sucrose conversion to fructose and glucose. The non-enzymatic browning degree and HMF content of HTST treated juice were significantly higher than untreated and HPP juice. There was no significant change in the cloud of atemoya juice after HPP treatment, while a significant decrease after HTST treatment. Aroma score analysis showed that HPP had no effect on aroma, maintaining the highest score during cold storage.

Significance: This work suggests that HPP treatment can achieve the same microbial safety as conventional HTST while maintaining overall quality, and decrease the non-enzymatic browning and HMF content of atemoya juice, which demonstrates its potential for commercial production of high-quality atemoya juice.

P3-161 Prevalence and Characteristics of Selected Foodborne Bacterial Pathogens in Post-Hurricane Florence Floodwaters

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Introduction: In September 2018, Hurricane Florence caused extreme flooding in eastern North Carolina, an area highly dense in industrial poultry and swine production, causing major ecological disruptions potentially including microbial populations.

Purpose: Assess potential impacts of the hurricane on three major foodborne pathogens: *Listeria monocytogenes*, *Salmonella,* and *Campylobacter*.

Methods: We collected 88 water samples from floodplains, streams and ephemeral waterbodies post-hurricane and selectively enriched for the pathogens. *L. monocytogenes* was enriched from water samples and filters using Half and Full Fraser broths, isolated on MOX and confirmed on blood agar and via PCR. *Salmonella* was enriched using buffered peptone water, RV broth, and isolated on XLD agar. *Campylobacter* was enriched using Bolton broth and isolated on mCCDA.

Results: Just one sample (one percent) was positive for *L. monocytogenes,* while other *Listeria* spp. were detected in 18% of samples. Isolates recovered from the *L. monocytogenes*-positive sample were serotype 1/2a (3a), a serotype commonly associated with human illness. *Salmonella* was detected in five percent of samples and *Campylobacter jejuni* was detected in a single sample (one percent). However, *Arcobacter*, a member of the *Campylobacteraceae* family, was detected in 73% of samples via the procedures employed for *Campylobacter*. A subset of 112 *Arcobacter* isolates were identified via multilocus sequence typing (MLST) as *Arcobacter butzleri*, an emerging pathogen associated with gastrointestinal infections, revealing 74 different sequence types (STs), of which 67 were novel and encountered in 91% of those isolates.

Significance: Data provide early evidence for high prevalence of *A. butzleri* in post-hurricane floodwaters. Future sampling efforts, planned for April and August 2019, will provide an opportunity to gather data on these pathogens during non-flooded conditions. As the severity of storms is expected to increase, it is imperative that we attempt to understand their impacts on the prevalence of these human pathogens in affected ecosystems.

Poster

P3-162 The Relationship between *E. coli* Levels and Pathogen Detection in Surface Water Samples is Mediated by Environmental Conditions

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Introduction: While *E. coli* is used as an indicator of microbial water quality, the relationship between *E. coli* levels and pathogen presence in surface water varies widely between studies. We hypothesized that environmental conditions mediate the relationship between *E. coli* levels and pathogen presence in water.

Purpose: To investigate how environmental conditions affect the relationship between *E. coli* levels and pathogen contamination of surface water.

Methods: Five New York streams and two Arizona canals were sampled >30 times in 2017 (total samples=243). At each sampling 30 L of water were collected, and separate 10-L aliquots were tested for *Salmonella*, *Listeria*, and Shiga toxin-producing *E. coli (*STEC). Data on *E. coli* levels and environmental factors (weather and water quality) were also collected. The relationship between *E. coli* levels and pathogen detection was characterized using logistic regression. Random forest and partial dependence plots (PDPs) were used to investigate the impact of environmental factors (e.g., turbidity, rainfall) on the relationship between *E. coli* levels and the likelihood of pathogen detection.

Results: *Salmonella* and *Listeria* were isolated from 41% and 33% of samples, respectively; 63% of samples were STEC-positive based on a PCR-screen. Regression showed that *Salmonella* isolation in AZ (P=0.009), and STEC detection in AZ (P=0.002) and NY (P<0.001) were significantly associated with *E. coli* levels. PDPs indicated that multiple factors affected the relationship between *E. coli* levels and pathogen detection. For example, the likelihood of STEC detection in NY when *E. coli* levels were >200 MPN/100 ml was higher when turbidity was greater than seven NTU than when turbidity was ≤7 NTU.

Significance: Our findings suggest that *E. coli* may not be a reliable indicator of pathogen contamination of surface water. Thus, alternative methods for estimating food safety risks associated with the use of specific water sources for produce production are needed that are based on environmental data and not just on *E. coli* levels.

P3-163 Occurrence and Levels of *Salmonella* Species in Primary Irrigation Water Canals and Return Flows in Arizona and the Risk of Contamination of Lettuce Crops

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Introduction: Studies have detected *Salmonella* in irrigation waters in Arizona; however, few were quantitative and thus the risks of *Salmonella* contaminating crops is unclear.

Purpose: To determine the occurrence/concentrations of *Salmonella* in irrigation water and return flow (drainage) canals in Arizona.

Methods: A total of 355 samples were collected from 53 locations (two regions) during the winter (n=175) and summer (n=180), including samples from primary canals (main canals n=157, lateral/sub-lateral canals n=148) and return flows (n=50). A semi-quantitative most probable number assay determined *Salmonella* concentrations. *Salmonella* occurrence was correlated with other microbial/physical-chemical water quality parameters. The *Salmonella* arithmetic average was used in a quantitative microbial risk assessment (QMRA) (rather than geometric mean) because it is considered a more appropriate QMRA summary descriptor.

Results: *Salmonella* was detected in 41 samples (11.6%; average 4.1 MPN/100 ml). No correlations were observed between *Salmonella* occurrence and microbial/general water quality parameters. Of the 41 positives, 16 were collected during the winter growing season (average 0.9 MPN/100 ml) and 25 during the summer (average 7.1 MPN/100 ml). All but one of 175 growing season samples had ≤10 *Salmonella;* one sample had ~100 MPN/100 ml. Although *Salmonella* was detected more often in return flows than primary canals (14.0% versus 11.2%), no significant difference was observed between concentrations. The QMRA determined that *Salmonella* found in irrigation waters during the growing season were below the acceptable risk level of 1:10,000 infected/year, even if harvesting lettuce within one day following irrigation. Assuming a 0.35-log dieoff per day (from a previous study), water with levels orders of magnitude higher (1,000 to 10,000 *Salmonella*/100 ml) could be used to irrigate lettuce presuming sufficient time has passed post-irrigation (three or six d, respectively).

Significance: The risk of *Salmonella* infection from water used to irrigate Arizona lettuce crops appears to be quite low and could be reduced by ensuring adequate time between final irrigation and harvest.

P3-164 Incidence of Fecal Indicator and Pathogenic Bacteria in Reclaimed and Return Flow Waters in Arizona, United States

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Introduction: The quality of irrigation water used for growing produce that is consumed raw is an important issue with regard to food safety.

Purpose: The objective of this study was to evaluate the microbiological quality and safety of potential irrigation water sources in Arizona by testing for the presence of indicator and pathogenic bacteria.

Methods: Reclaimed water samples were collected from two wastewater treatment plants and return flow samples from two drainage canals and one return flow pond. Standard membrane filtration methods were used for the detection of indicator bacteria. Water samples (n=28) were filtered through cellulose ester membrane filters and bacterial populations were enumerated by placing them on selective agar. For detection of pathogens (*Salmonella enterica, Listeria monocytogenes* and Shiga toxin-producing *E. coli* (STEC), water samples were filtered through Modified Moore swabs and enriched in universal preenrichment broth, followed by selective enrichment broth for each pathogen. The enriched broth was streaked onto agar media selective for each pathogen. Presumptive colonies were confirmed by PCR/real-time PCR.

Results: Among the 14 reclaimed water samples, the ranges of recovered populations of *E. coli*, total coliforms, and enterococci were zero to 1.3, 0.5 to 8.3×10^3, and 0 to 5.5 CFU/100 ml, respectively. No *L. monocytogenes*, *Salmonella* or STEC were found. In the 13 return flow water samples, the ranges of recovered populations of *E. coli*, total coliforms and enterococci were 1.9 to 5.3×10^2, 6.5×10^2 to 9.1×10^4, and 3.7 to 2.9×10^3 CFU/100 ml, respectively. All samples were negative for *L. monocytogenes*. One (7.1%) of the return flow samples was positive for *E. coli* O145. Nine (64.3%) samples were positive for *Salmonella*. Both real-time PCR and culture-based methods were used for the detection of *Salmonella* and *L. monocytogenes*, and results from the two methods were comparable.

Significance: The findings provide evidence that irrigation waters in Arizona, including return flows and reclaimed water, could be potential sources of bacterial contamination of produce.

P3-165 *Listeria monocytogenes* Levels and Population Diversity in Surface Waters in the United States Mid-Atlantic Region

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Introduction: Microbiological quality standards for agricultural water rely on bacterial fecal indicators. The role or human or animal input in *Listeria monocytogenes* concentrations in surface waters is unknown and thus, a better understanding of the major sources of this pathogen in agricultural waters is needed.

Purpose: To characterize the genotypic diversity and levels of *L. monocytogenes* in the Conococheague Creek located in the United States Mid-Atlantic region.

Methods: Water (500 ml) was collected weekly from six sites spread over a 15 mile reach of the creek over the course of 18 months. *L. monocytogenes* populations were quantified by MPN procedure, using a five´100-ml, eight´10-ml, eight one-ml scheme with a lower limit of detection of 0.0021 MPN/ml. *L. monocytogenes* positives were confirmed using VITEK MS and subjected to whole genome sequencing. Core genome multi locus sequence type (cgMLST) and in-silico MLST analysis were performed to analyze the genotypic diversity of all isolates.

Results: Overall, 97.9% (n=504) of samples were positive for *L. monocytogenes*. Levels in water samples ranged from ≤0.21 to 120 MPN/100 ml. cgMLST analysis revealed high genetic diversity among these isolates, representing genetic lineage: I (21.2%), II (48.2%), and III (31.6%), and molecular serogroups: IIa (1/2a, 3a) (48.2%), IIb (1/2b, 3b) (2.1%), IVb (4b, 4d, 4e) (18.1%), and IVb variant (1%). Twenty-five novel clones of *L. monocytogenes* were identified. The strains do not match strains from recent United Staets outbreaks. However, some strains belonged to clones (CC1, CC4 and CC6) that have been strongly associated with clinical cases and are considered hypervirulent.

Significance: This study generated a highly resolved temporal and spatial genetic relationship map among *L. monocytogenes* strains coupled with actual pathogen levels in surface waters that are critical for microbial source tracking, modeling and risk assessment. These novel data are also essential in reassessing the significance of the current bacterial fecal indicators-based microbiological quality standards proposed for irrigation waters.

P3-166 Occurrence and Population Diversity of *Listeria monocytogenes* in Two Irrigation Ponds in Maryland

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Introduction: The ubiquitous nature of *Listeria monocytogenes* results in the presence of this pathogen in a variety of surface waters. *L. monocytogenes* can transfer on produce if contaminated waters used for irrigation. Therefore, in addition to compliance with FSMA water quality standards, it is important to estimate the incidence and potential sources of *L. monocytogenes* in irrigation waters.

Purpose: To evaluate incidence and population diversity of *L. monocytogenes* in irrigation ponds in Maryland during produce growing season.

Methods: Using the pond-specific spatial grids, water samples (500 ml) were collected biweekly (June to July) from 30 and 20 sites of Pond 1 and Pond 2 respectively. *Listeria* detection was conducted by selective enrichment in buffered *Listeria* enrichment broth, followed by streaking onto RAPID'*L.mono* and ALOA agars. Presumptive *Listeria* positives were identified to the levels of species using VITEK MS. All confirmed *L. monocytogenes* isolates were subjected to whole genome sequencing. Core genome multi locus sequence type (cgMLST) and in-silico MLST analysis were performed to analyze the genotypic diversity of all isolates. cgMLST was also conducted to analyze these strains against recent United States outbreak strains.

Results: Overall, 21% of water (n=120) samples were positive for *L. monocytogenes.* Generic *Listeria* spp. detected in water samples were *L. seeligeri*, *L. welshimeri, L. innocua* and *L. ivanovii*. cgMLST analysis revealed high genetic diversity among *L. monocytogenes* isolates, representing genetic lineage: I (22.4%), II (37.5%), and III (36.9%); and molecular serogroups: IIa (12.5%), and IVb variant (25.1%), non-typeable (12.6%), and serotypes of lineage III were not identifiable. Twelve novel clones of *L. monocytogenes* were identified. These water strains do not match strains from recent United States outbreaks.

Significance: The genotypic characterization of *L. monocytogenes* isolates provided novel insights into the sources of this pathogen in irrigation waters. The baseline information on the incidence of *L. monocytogenes* in surface waters is essential to understand how these irrigation sources may influence the microbiological safety of fresh produce.

P3-167 Evaluation of Nontraditional Irrigation Water Sources for Shiga Toxin-producing and Atypical Enteropathogenic *Escherichia coli* in the United States Mid-Atlantic Region

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❖ ***Developing Scientist Entrant***

Introduction: The microbial quality of irrigation water has increasingly become a concern as a source of contamination of fresh produce. Nontraditional irrigation water sources are being used by more growers in smaller, highly diversified farms in the United States Mid-Atlantic. Shiga toxin-producing *Escherichia coli* (STEC) have been responsible for several outbreaks of infections associated with the consumption of leafy greens.

Purpose: This study evaluated the prevalence of the "big seven" STEC serogroups and the associated enterohemorrhagic *E. coli* (EHEC) virulence factors (VF) genes in nontraditional irrigation water sources in the United States Mid-Atlantic.

Methods: Water samples (n=510) from 170 sampling events were collected from eight untreated surface water sites, two wastewater reclamation facilities, and one vegetable processing plant from October 2016 to November 2017. A total of 2,489 presumptive STEC isolates were tested for the presence of the most frequently reported STEC serogroups that cause foodborne illness: O26, O45, O103, O111, O121, O145, and O157, along with VF genes *stx1*, *stx2*, *eae*, and *ehxA*.

Results: STEC isolates were found in 12 (2.35%) of 510 water samples, while 46 (9.0%) of 510 contained an atypical enteropathogenic *E. coli* (aEPEC) isolate. The *eae* gene (n=88 isolates) was the most frequently detected EHEC VF of the isolates screened. The majority of the STEC isolates containing *stx1* or *stx2*

Poster

genes came from either a pond water or reclamation water site on two specific dates, potentially indicating that these isolates were not spatially or temporally distributed among the sampling sites. No samples were positive for O157. None of the isolates containing *eae* were determined to be *Escherichia albertii*.

Significance: This study indicates that STEC prevalence is not spatiotemporally distributed, and is lower than that of aEPEC, in Mid-Atlantic nontraditional irrigation water sources . These data can inform mitigation strategies to improve irrigation water quality in the Mid-Atlantic.

P3-168 Presence of *Salmonella* and *Listeria monocytogenes* in Reclaimed and Surface Irrigation Water Sources on Maryland's Eastern Shore: A Conserve Study

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❖ ***Developing Scientist Entrant***

Introduction: Water scarcity is placing increasing pressure on traditional irrigation waters. This is leading to increased interest in the use of non-traditional water sources for the irrigation fresh produce. Therefore, ensuring the microbial safety of these water sources is paramount.

Purpose: The purpose of this study was to determine the prevalence of *Salmonella* and *Listeria monocytogenes* in non-traditional irrigation water sources.

Methods: Water samples (*n*=489) were collected over a two year period from two reclaimed water (RW) (*n*=105) and four surface water (SW) (*n*=384) sites on Maryland's eastern shore from 2016 to 2018. Samples were collected by filtration using modified Moore swabs. Swabs were enriched in Universal Preerichment Broth followed by secondary enrichment in tetrathionate broth and Rappaport-Vassiliadis broth for *Salmonella* and Buffered *Listeria* Enrichment Broth for *L. monocytogenes*. Bacteria concentrations in the samples were quantified by real-time PCR using a modified MPN procedure using three volumes (10, 1, and 0.1 liters).

Results: Of the 489 water samples collected, 346 (70.8%) of 489 and 160 (32.7%) of 489 were positive for *Salmonella* and *L. monocytogenes*, respectively. For both *Salmonella* and *L. monocytogenes,* MPN values varied among sites over the course of the study, ranging from zero to 11 MPN/l. For *Salmonella*, average MPN/l values for the SW sites were significantly higher ($P<0.05$) than the RW sites (2.47 and 0.84, respectively). For *L. monocytogenes*, average MPN/l values were 0.01 and 0.60 for RW and SW, respectively.

Significance: This study shows that *Salmonella* and *L. monocytogenes* are present in nontraditional irrigation water sources on the eastern shore of Maryland. While these water sources have the potential to be utilized for fresh produce irrigation, development of mitigation strategies ensuring the safety of these water sources is also essential.

P3-169 Evaluation of Survival and Infectivity of Environmental *Listeria monocytogenes* Isolates in Tidal Brackish Irrigation Water

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* has been found in surface water used for irrigation of produce, but critical data gaps remain concerning *L. monocytogenes* survival and infectivity in these environments.

Purpose: To compare the survival and infectivity of *L. monocytogenes* isolated from surface water in environmental waters collected over a growing season.

Methods: Tidal brackish river water samples (TBRW) were collected and analyzed for total plate count (TPC), pH, and conductivity in summer 2018. *L. monocytogenes* isolates (four from Mid-Atlantic surface water, one from vegetable wash water, and one from a 2011 cantaloupe outbreak) were evaluated for survival in TBRW over 10 d. Isolates were individually inoculated in triplicate at 8.55±0.09 log CFU/ml into 10 ml of TBRW or one percent buffered peptone water (BPW), along with uninoculated TBRW controls, for 10 d at 16°C. *L. monocytogenes* was enumerated on Brilliance *Listeria* Agar (BLA) on d zero, three, five, seven, and 10; sub-samples (one ml) were applied to HCT-8 cell-monolayers for infectivity on days zero, seven, and 10. Infection included incubation at 37°C, gentamicin sulfate treatment, and enumeration of infective cells enumerated on BLA. Statistical analysis was performed by one-way ANOVA, t-test, and Tukey-Kramer, with *n*=6.

Results: Initial populations of *L. monocytogenes* (8.55 log CFU/ml) on d zero remained unchanged in BPW or were reduced by up to 3.02 log CFU/ml after 10 d with slight variations by isolate. Significant decreases in viability and infectivity occurred for all isolates ($P<0.0001$) in TBRW compared to BPW. In TRBW samples, *D*-values for survival and infectivity ranged from 3.31 to 8.40 d and 2.31 to 4.40 d, while in BPW ranged from 53.70 to 780.53 d and 13.20 to 62.59 d, respectively. *L. monocytogenes* survival was significantly correlated with lower TPC in TBRW samples. ($P=0.0095$).

Significance: *L. monocytogenes* environmental isolates were shown to survive and remain infective in TBRW, and their survival/infectivity may be affected by the quantity of other bacteria in water samples.

P3-170 Variability of Generic *E. coli* Along the Tualatin River during the 2018 Blueberry Growing Season

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Introduction: Growers remain concerned about the potential economic impact of the agricultural water quality requirements within the Produce Safety Rule (PSR). There is significant interest from growers to be able to share water testing data; however, due to a lack of data , analysis, and guidance it remains unclear when sharing data may be appropriate and allowable.

Purpose: To quantify and estimate variability of generic *E. coli* along a single surface water source used for irrigation in Oregon's Willamette Valley.

Methods: Water samples (100 ml; *n*= four per day) were collected weekly from six agricultural water access points along the Tualatin River during the 2018 blueberry growing season (July through October). Water was analyzed using the IDEXX ColiLert method in combination with the QuantiTray 2000 for enumeration. The temperature at the time of collection along with the pH was also recorded for each water sample. Geometric means and statistical threshold values were calculated for each test site and compared to the standard in the PSR.

Results: Water samples ranged in generic *E. coli* counts from 47.1 to 1046.2 MPN/100 ml. Of the six farm sites tested, only the most downstream site was in compliance with the standards of the PSR. Generic *E. coli* levels were elevated as samples were collected upstream. There was a single day (8/31/18) with

exceptionally high levels (365 to 1046 MPN/100 ml) of generic *E. coli* at five of the test sites. Another sampling day (9/11/18) demonstrated high levels (488 to 613 MPN/100 ml) at two of the test sites.

Significance: These results demonstrate the variability (daily, weekly, monthly) of generic *E. coli* levels along a surface water source that carries risk of not being in compliance with the current PSR standards which can guide sampling strategies and the opportunities to share water testing data.

P3-171 Biofilm Formation by *Pseudomonas aeruginosa* Isolated from Mineral Water Samples Marketed in the State of São Paulo, Brazil

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Introduction: Biofilms are matrix-enclosed microbial communities. Biofilms can also be a major problem for the water industry.

Purpose: This study was undertaken to evaluate the biofilm formation ability of *P. aeruginosa* isolated from mineral water samples marketed in state of São Paulo, Brazil.

Methods: A total of 114 *P. aeruginosa* strains were studied. Biofilm formation was evaluated using different coupons (stainless steel, polypropylene (PP), glass, polycarbonate (PC) and polyethylene terephthalate (PET) - 10 mm by 10 mm by 1 mm). For analysis, 100 µL of culture of *P. aeruginosa* were transferred to 24-well microtiter plates containing three ml of mineral water (sterilized by filtration) and after gentle homogenization, the coupons were added and incubated at 37°C for 24 h. The coupons were removed and transferred to 10 ml 0.85% saline solution for one min. After, they were transferred to five ml of 0.85% saline solution for two min and counts of *P. aeruginosa* were done on Pseudomonas CN agar. The results were expressed as CFU/cm^2.

Results: All *P. aeruginosa* isolates were capable of attachment to all different types of coupons, most of them achieving populations above four log CFU/cm^2. Populations above five log CFU/cm^2 adhered to the coupons were found for 80.7%, 77.19%, 84.21%, 83.33% and 85.96% of the isolates studied on stainless steel, polypropylene (PP), glass, polycarbonate (PC) and polyethylene terephthalate (PET) coupons, respectively. In contrast, few isolates showed adherence above six log CFU/cm^2 (5.26, 3.51, 2.63, 0.88 and 4.39%), respectively.

Significance: These data suggest that *P. aeruginosa* isolates from different mineral water samples can form biofilm on different surfaces and packing materials.

P3-172 Characterization of *Pseudomonas aeruginosa* Isolates in Mineral Water of São Paulo, Brazil, Using Pulsed-Field Gel Electrophoresis

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Introduction: Mineral water is an essential substance for human life and may contain specific microbiota, including opportunistic pathogens such as *Pseudomonas aeruginosa*. *P. aeruginosa* is known to survive and even grow very well in water.

Purpose: The purpose of this study was to determine the pulsotypes of *P. aeruginosa* strains isolated from mineral water marketed in São Paulo state, Brazil.

Methods: A total of 114 *P. aeruginosa* strains were isolated from 93 bottled mineral water samples. Pulsed field gel electrophoresis (PFGE) was done using the enzyme *SpeI*.

Results: Forty-one genetic profiles were defined from all *P. aeruginosa* strains typed with 100% similarity, and the dendrogram showed 12 major clusters (A, B, C, D, E, F, G, H, I, J, L and M) of isolates with 90% similarity. The largest cluster (C) contained 40 isolates, obtained in mineral water from brands A (12 isolates, two lots, one season and two volumes), B (one isolate, one lot, one season and one volume), C (two isolates, one lot, one season and one volume) , D (two isolates, one lot, one season and one volume), E (19 isolates, two lots, two seasons and one volume) and one source (four isolates and three seasons). Cluster B grouped 16 isolates obtained from four brands and five different lots. The clusters A, D, E, F, G, H, I, J, L, and M grouped eight, eight, four, two, two, five, two, nine, four and 11 isolates, respectively.

Significance: These data show that *P. aeruginosa* strains isolated from different mineral water samples bottled in different plants and located in distant cities may present high genetic similarity. This finding provides key data that can be useful for ensuring the quality and safety of mineral water for *P. aeruginosa.*

P3-173 Photodynamic Treatment as an Alternative for *Alicyclobacillus* spp. Inactivation

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❖ ***Developing Scientist Entrant***

Introduction: Heat treatment is commonly employed to ensure the shelf-life stability of fruit juices and beverages, which inactivates the main spoilage bacteria, including *Alicyclobacillus* spp.. Despite this, heat treatment results in significant losses in sensory and nutrients in foods. Photodynamic treatment (PDT) may be an alternative method for *Alicyclobacillus* inactivation without the detriment changes observed when heat treatments are applied.

Purpose: The aim of the present study was to evaluate the effectiveness of PDT to inactivate *Alicyclobacillus* spp. spores.

Methods: Spore suspensions of *Alicyclobacillus acidoterrestris* (DSM 2498) and *Alicyclobacillus acidocaldarius* (ATCC 43034) were prepared. Three photosensitizers were used in this study: porphyrin tetra-Py+-Me, a mixture of five porphyrins, and new methylene blue (NMB). The spores were exposed to a white LED spotlight (400 to 740 nm) and recovered on YSG agar (pH 3.7). The In Vitro experiments were conducted in liquid media consisting of PBS buffer and orange juice. Inactivation curves were plotted and compared using ANOVA.

Results: The In Vitro PDT inactivation led to seven-log reductions (ANOVA, $P<0.05$) of *A. acidoterrestris* (DSM 2498) and *A. acidocaldarius* (ATCC 43034) after six h of treatment at an irradiance of 140 mW/cm^2. In addition, seven-log reductions were also observed for both strains when inoculated in orange juice (ANOVA, $P<0.05$) after 10 h of treatment.

Significance: This work suggests that PDT can be a potential method for *Alicyclobacillus* spp. inactivation under In Vitro conditions and in orange juice. However, further developments and optimization are required to ensure conditions that are feasibly implemented by food industry.

P3-174 Behavior of Silver Nanoparticles under Various Wash Water Conditions for Leafy Green Processing

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❖ ***Developing Scientist Entrant***

Introduction: Engineered nanoparticles (ENPs), such as nanopesticides, are increasing in agroecosystems, particularly from land application of sewage sludge. Exposing fresh produce to ENPs may increase residual levels posing potential food safety concerns. Understanding the fundamental interactions between agriculturally relevant ENPs and commercial washing of fresh produce is important in designing effective mitigation strategies.

Purpose: This study aimed to evaluate the behavior of silver nanoparticles under different wash water conditions for leafy greens.

Methods: Aggregation and dissolution kinetics of silver nanoparticles (AgNPs) were systematically investigated in wash water with or without dissolved lettuce extract (DLE, 0.1% w/v). Wash water was prepared by adding two, 50, or 100 ppm free chlorine followed by five mg/l AgNPs, with five mg/l AgNPs in deionized water serving as the control (~pH 6.5). Average particle size (diameter, nm) and zeta potential (mV) of the AgNP suspensions were measured using a zetasizer, with dissolved Ag concentrations (mg/l) measured using an atomic absorption spectrophotometer initially and again after two, six, 12, and 24 h, and four, seven and 10 days at 4°C. Results from triplicate experiments were statistically analyzed using one-way ANOVA.

Results: For all the chlorine levels tested, the dissolved Ag concentrations were much lower (0.01 to 0.03 mg/l) as compared to the control (0.54 to 0.8 mg/l) (P<0.05). Increased particle size with time (49 to 431 nm) as compared to the control (58 to 60 nm) indicates high AgNPs aggregation in the presence of chlorine, with the zeta-potential values more negative (-39 to -95 mV) compared to the control (-10 to -20 mV) (P<0.05). AgNP treatments with DLE yielded similar findings.

Significance: Since the fate of AgNPs in fresh produce washing systems is affected by both chlorine and organic matter in wash water, these interactions are important in evaluating the sorption of AgNPs to leafy greens.

P3-175 Fabrication of a Metal Oxide Coated Pouch for Alternative Processing of Military Ration Components

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Introduction: Foil-based materials for high-barrier packaging has been the industry standard for decades. The global demand for aluminum and rising energy costs have established the need for non-foil barrier alternatives. The food industry's need to use energy-efficient, high throughput, and cost-effective processing technologies has led to the development of alternative food processing techniques. These alternative technologies can be incompatible with foil-based packaging and therefore require an alternative barrier material solution.

Purpose: The purpose of this study was to analyze the effect of atomic layer deposited (ALD) metal oxide coatings onto substrates similar in composition to packaging used in the military's Meal, Ready-to Eat (MRE) in order to achieve the required barrier performance for extended shelf life.

Methods: Common packaging materials used in MRE packaging were evaluated: polyester terephthalate (PET), nylon, and polypropylene (PP). A multilayer film and pouch structure were also evaluated. Aluminum oxide and titanium oxide coatings were deposited at thicknesses ranging from two to 25 nm/layer using ALD. The coated materials were evaluated for oxygen and water vapor permeation using a MOCON Oxtran 2/21 and Permatran-W 3/33.

Results: The largest reductions in oxygen and water vapor transmission rates were observed on film samples coated with a 25 nm layer of Al_2O_3 and a 25 nm layer of TiO_2. The nylon had a measured oxygen transmission rate (OTR) of 4.73±0.04 cc/m^2-day (97.47% reduction). The PET had a water vapor transmission rate (WVTR) of 0.48±0.16 g/m^2-day (97.91% reduction). The closed multilayer pouch with 12 alternating layers of two nm Al_2O_3/TiO_2 had an OTR of 7.44±2.46 cc/m^2-day (82.28% reduction) and a WVTR of 1.56±0.18 cc/m^2-day (93.62% reduction). End application of the ALD coating will not be applied to a direct food contact layer, so there should not be a concern with food safety.

Significance: These data suggest that ALD of nanoscale metal oxide coatings onto packaging polymers have the potential to provide enough barrier to create alternative processing compatible ration packaging.

P3-176 Antimicrobial Coatings for Improving Safety and Shelf-life of Cherry Tomatoes

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Introduction: Edible antimicrobial coatings have demonstrated their antimicrobial efficacy on several types of food. However, tomatoes have waxy surfaces which require a special surface treatment before coating or a special coating formula, so that the fruit surface could be evenly coated and the antimicrobials in the coating could interact with pathogens on the fruit surface.

Purpose: The objective of this study was to develop new edible antimicrobial coating solutions to effectively reduce Salmonella and spoilage fungi in cherry tomatoes while maintaining their quality.

Methods: Two coating solutions were used: coating 1 was one percent chitosan; coating 2 was one percent chitosan and six percent allyl isothiocyanate (AIT). Both solutions contained two percent tween 80 and four percent lecithin and all ingredients were dissolved in two percent acid solution (acetic acid, lactic acid, levulinic acid). The solutions directly coated on the surface of tomatoes (20 pieces) inoculated with a cocktail of *Salmonella* Stanley, *Salmonella* Panama and *Salmonella* Poona and placed in polyethylene terephthalate boxes, stored at 10°C. The survival of *Salmonella* and the changes in color and texture were determined. Experiments were repeated three times.

Results: Addition of tween 80 and lecithin significantly improved the coating bind ability to tomato surface and both coating solutions formed uniform coatings around the whole surface of the tomatoes. Both treatments reduced *Salmonella* cells from an initial 3.5 log CFU/tomato to 0.7 log CFU/tomato after treatment and through the 14-day storage period. Both coating treatments significantly (P<0.05) increased whiteness and yellowness while not affecting redness. Texture firmness of tomatoes was not significantly (P>0.05) affected by any of the treatments. After 21 days' storage at 10°C, two treated tomatoes still showed fresh-like appearance while non-treated tomatoes had moldy surfaces.

Significance: This study provides some options to develop edible antimicrobial coating solutions for produce with waxy surfaces.

Poster

P3-177 Black Drum (*Pogonias cromis*) Shelf Life Comparing Four Packaging Methods

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✦ ***Undergraduate Student Award Entrant***

Introduction: Seafood is one of the main sources of protein around the world, and its consumption continues to grow annually. Because of its high-unsaturated lipid composition, seafood is highly perishable with a relatively short shelf life. Reduced oxygen packaging technology is recognized to expand shelf life of fresh products by slowing down lipid oxidation and microbial growth.

Purpose: The main goal of this project was to evaluate the shelf life of black drum comparing three different packaging technologies.:

Methods: Fresh Black drum fillets were purchased direct from the dock within 24 h of being caught. Fillets were packed using four methods: polyethylene bags (PB), vacuum packed (VP), Modified Atmosphere Packaging (MAP) (CN – 50% CO_2 and 50% N_2) and MAP (CNO – 40% CO_2, 30% N_2, 30% O_2). The packed fish were stored at 0.5±2°C for 20 d. Shelf life were studied in terms of TVB-N, TBARS, pH, Color, aerobic plate count (APC), *Enterobactereaceae*, yeast, and mold. Microbiological and physical/chemical evaluations were carried out at d zero and every four d thereafter.

Results: A 20-day microbial shelf life based on APC was observed in black drum fillets stored in MAP, which was an increase of eight d compared to PB and VP (P<0.0001). Even though, MAP appeared to extend the shelf life of black drum based on APC, there were no significant differences in TVB-N, TBARS, pH, and color analyses. There were also no significant differences between the two MAP gas combinations used during this study (P<0.6672).

Significance: This study demonstrated that MAP is effective in extending the shelf life of black drum fillets. Being able to expand fish shelf life allows fishermen and processors to reach larger markets.

P3-178 Development of Predictive Models for *Vibrio vulnificus* and *Vibrio cholerae* Growth in Gizzard Shad Sashimi

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❖ ***Developing Scientist Entrant***

Introduction: Although the risks of foodborne illness by *Vibrio vulnificus* and *Vibrio cholerae* have increased recently, regulations for the bacteria in seafood have not been established yet in Korea.

Purpose: The objective of this study was to develop predictive models to predict *V. vulnificus* and *V. cholerae* growth in gizzard shad sashimi.

Methods: Growth patterns of *V. vulnificus* and *V. cholerae* were compared in Luria-Bertani + 2% NaCl (w/v) broth at seven, 10, 15, and 25°C. A mixture of *V. vulnificus* and *V. cholerae* strains were then inoculated on gizzard shad sashimi samples (10 g) at three log CFU/g. The bacterial cell counts were enumerated on CHROMagar Vibrio during storage at 7, 10, 15, and 25°C. The Baranyi model was fitted to kinetic parameters such as μ_{max} (maximum specific growth rate; log CFU/g/h) and *LPD* (lag phase duration; h), which were then analyzed with an inverse second order and a quadratic model, respectively. To validate the model performance, predicted data were compared with observed data, and *RMSE* (root mean square error), A factor (A_f), and B factor (B_f) were calculated.

Results: There was no difference in growth patterns between the two species. μ_{max} of the mixture increased (P<0.05) from 0.08 to 0.50 log CFU/g/h, and *LPD* decreased (P<0.05) from 14.5 to 1.4 h, as storage temperature increased. Moreover, the secondary models were appropriate to evaluate temperature effect on μ_{max} and *LPD* with 0.989 and 0.913 of R^2, respectively. For validation, *RMSE*, A_f, and B_f were 0.409, 1.06, and 1.00, respectively, indicating that the developed models were appropriate for growth prediction.

Significance: The developed models should be useful in predicting cell counts of the bacteria in gizzard shad sashimi, and the models showed the bacteria can survive and grow even at low temperature. Therefore, a technology to control them in the gizzard shad sashimi should be developed.

P3-179 Quantification of Risk for *Vibrio parahaemolyticus* Foodborne Illness by Sea Pineapple (*Halocynthia roretzi*) Consumption

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❖ ***Developing Scientist Entrant***

Introduction: Sea pineapple (*Halocynthia roretzi*) is consumed raw in some countries, and it causes *Vibrio parahaemolyticus* foodborne illness. Because the sea pineapple may accumulate *V. parahaemolyticus* in digestive glands through aquatic respiration, the risk from raw sea pineapple consumption may be high.

Purpose: This study analyzed the risk of *V. parahaemolyticus* foodborne illness by sea pineapple consumption in Korea.

Methods: Thirty sea pineapple samples were analyzed to detect *V. parahaemolyticus*. Predictive models for *V. parahaemolyticus* cell counts in sea pineapple during distribution were developed. The distribution conditions, consumption amount and frequency for the sea pineapple, as well as a dose-response model were also surveyed. With all collected data, a simulation model was prepared, and the probability of foodborne illness for *V. parahaemolyticus* by sea pineapple consumption was calculated by the @Risk program.

Results: The distribution of RiskBeta (7,30) for the prevalence of *V. parahaemolyticus* estimated that the initial contamination level was -1.9 log CFU/g. With this initial contamination level, the developed Baranyi models showed that the *V. parahaemolyticus* cell counts increased under the investigated distribution conditions. For the investigated conditions, appropriate distributions were RiskUniform (0,72) and RiskLogLogistic (-29.283, 33.227, 26.666, RiskTruncate (-5,20)) for time and temperature, respectively. The consumption amount and frequency were 62.14 g and 0.28%, respectively. The combination of these results with the β-Poisson dose-response model [risk=1-(1+dose/1.18×10^5)$^{-0.17}$] showed that the probability of *V. parahaemolyticus* foodborne illness by sea pineapple consumption was 4.03×10^{-7} per person per day.

Significance: This result should be useful in evaluating the risk of *V. parahaemolyticus* foodborne illness by sea pineapple consumption.

Poster

P3-180 Prevalence, Antibiotic Resistance, and Virulence Gene Profiles of *Listeria monocytogenes* Isolated from Smoked Salmon in South Korea

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Introduction: Although listeriosis is rare, *Listeria monocytogenes* infection can cause severe clinical symptoms such as gastroenteritis, septicemia, meningitis, or abortions. Studies have shown that the occurrence of *L. monocytogenes* in ready-to-eat (RTE) smoked salmon is quitely documented, threatens public health, and results in devastating socio-economic losses. Monitoring program to detect *L. monocytogenes* in processing RTE foods and plants is considered important for reducing the burden of *L. monocytogenes* to the food industry.

Purpose: The aim of this study was to determine the prevalence of *L. monocytogenes* in smoked salmon and its processing plants and characterize the isolates by serotyping and the identification of antimicrobial resistance and virulence determinants.

Methods: Three hundred seventy-five and 360 samples of packaged smoked salmon products and swabs were collected through retail markets and the food processing plants, respectively, in South Korea during 2018. Presumptive *Listeria spp.* isolates from the samples were identified using real-time polymerase chain reaction (PCR) and 16s rRNA sequencing. All serotypes were confirmed using multiplex PCR-based serogrouping assay and serotyping kit for O-antigens. Antimicrobial susceptibility and virulence genes of the isolates were determined using disk diffusion assay and PCR, respectively.

Results: Two (0.53%) *L. monocytogenes*, six (1.6%) *L. ivanovii*, and 16 (4.3%) *L. seeligeri* were isolated from 375 RTE smoked salmon products, whereas no *Listeria* spp. was isolated from plant environmental samples. Two *L. monocytogenes* isolates were identified as serotype 1/2a. Both were susceptible to all antibiotics tested in this study. They harbored *inlA*, *inlB*, *plcB*, *hylA*, and *iap.*

Significance: Results show that RTE smoked salmon products sold on retail markets could be potential risk of having *L. monocytogenes* 1/2a with several virulence genes. In conclusion, food safety and quality control programs to eliminate *L. monocytogenes* in RTE food products should be systemically applied for reducing the risk of *L. monocytogenes* contamination.

P3-181 Microbiological Characteristics of Non-eviscerated Smoked Blue Whiting (*Micromesistius poutassou)* Fish during Storage

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Introduction: Smoked fish constitutes an important part of the diet of many consumers in the developing world. However, the safety of the traditionally smoked fish is becoming a major public health concern.

Purpose: This study investigated the microbial qualities of non-eviscerated smoked Blue Whiting fish (*Micromesistius poutassou)* during storage.

Methods: Ten samples each of non-eviscerated smoked fish were randomly and aseptically collected from five different selected processors in Makoko, Lagos, Nigeria. Laboratory prepared eviscerated and non-eviscerated smoked fish served as control samples. The microbial quality (total plate count (TPC), total fungal count (TFC), *Escherichia coli*, *Shigella* and *Listeria* counts) were determined. during zero to five d of storage. Smoked fish samples from processors and the control samples were stored at freezing, refrigeration and ambient temperatures during which they were subjected to microbiological analysis. Data obtained were subjected to analysis of variance.

Results: The control samples (eviscerated and non-eviscerated) had TFC (2.15 and 2.7 log CFU/g) and TPC (2.24 and 3.25 log CFU/g), respectively. During refrigerated and frozen storage, the *E. coli*, *Listeria* and *Shigella* were reduced in the non-eviscerated smoked fish but were not detected in the eviscerated smoked fish during storage. However, *E. coli*, *Listeria* and *Shigella* were lower in eviscerated smoked fish than in the non-eviscerated smoked fish at ambient temperature. The molecular assay identified bacteria including *Klebsiella pneumoniae, Shigella dysentery, Escherichia coli, Samonella enterica, Staphylococcus aureus, Listeria grayi* and *Vibro vulnificus*. The phylogenetic analysis showed that the isolated bacteria were associated with *Klebsiella pneumonia* strain KPCSM-DRIA 1*, Shigella dysentery strain* sd 197, *Acinetobacter oleivorans* strain JC 3 and *Vibro harveyi* strain HW 4 in Genbank.

Significance: The non-eviscerated smoked fish samples were highly susceptible to microbial spoilage irrespective of the storage, thus there is a need for the evisceration of the raw fish prior to processing.

P3-182 Histamine Production by *Photobacterium* spp. in Tuna and Mahi-Mahi Tissue at Various Storage Temperatures

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Introduction: Histamine-producing bacteria (HPB) generate histamine in decomposing scombrotoxin-forming fish tissue. Elevated histamine levels (≥200 ppm) can cause scombrotoxin fish poisoning in humans who consume decomposed seafood products. Determining storage conditions that produce high HPB/histamine is critical to mitigating risks.

Purpose: The objective of this study was to determine the time at four, 10, and 20°C for histamine to reach ≥200 ppm in tuna and mahi-mahi inoculated with psychrotrophic HP *Photobacterium* spp.

Methods: *P. kishitanii* and *P. angustum* were previously found to be the most prolific HPs at 4 (*P. kishitanii)*, 10 (*P. kishitanii* and *P. angustum*), and 20°C (*P. angustum*). A strain mix of three *P. kishitanii* or three *P. angustum* was inoculated at 10^2, 10^4, and 10^6 CFU/ml into 10 g homogenized tuna and mahi-mahi in triplicate and stored at 4 (14 days), 10 (14 days), and 20°C (48 h). Samples were analyzed for histamine with modified AOAC fluorometric method and HPB by MPN real-time PCR.

Results: In tuna inoculated with 10^2, 10^4, and 10^6 CFU/ml *P. kishitanii*, histamine was ≥200 ppm after seven, five, and three days at 4ºC and two, two, and one day at 10ºC, respectively. In tuna inoculated with 10^2, 10^4, and 10^6 CFU/ml *P. angustum* histamine was ≥200 ppm after six, four, and two days at 10ºC and 24, 20, and 12 h at 20ºC, respectively. In mahi-mahi inoculated with 10^2, 10^4, and 10^6 CFU/ml *P. kishitanii* histamine was ≥200 ppm after nine, six, and four days at 4ºC and three, three, and two days at 10ºC, respectively. In mahi-mahi inoculated with 10^2, 10^4, and 10^6 CFU/ml *P. angustum* histamine was ≥200 ppm after 10, nine, and three days at 10ºC and 38, 16, and 12 h at 20ºC, respectively.

Significance: Homogenized inoculated tissues contained ≥ 200ppm histamine after four to nine days under recommended storage (4°C) but 12 h to 10 days depending on conditions. Understanding how storage conditions affect histamine in scombrotoxin-forming fish is important to provide guidance.

P3-183 Metagenomic Evaluation of Methods to Recover of *Vibrio* spp. from Oysters

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Introduction: Outbreaks of *Vibrio parahaemolyticus* have been linked to raw oysters, clams and other shell fish on multiple occasions. Efficient recovery of *Vibrio* spp. from implicated foods is first and foremost in public health endeavors to understand source and route of contamination events via genomic and epidemiological approaches.

Purpose: The purpose of this work is to optimize efficiency and speed of source tracking by improving recovery metrics of *Vibrio* spp. from oysters.

Methods: Five oysters (from the Chesapeake Bay) were purchased locally, shucked and homogenized. Oyster meat homogenate was enriched at 37°C for 24 hours in alkaline peptone water. Every four hours, replicates (*n*=3) of four ml were taken from each enriching oyster (*n*=5). Cells were pelleted and DNA was extracted using the Qiagen DNEasy kit. Libraries were prepared using the Illumina Nextera Flex kit and sequenced (Illumina Nextseq 550). Reads were assigned taxonomy using CosmosID.

Results: Microbiota observed at hour zero included; *Arcobacter*, *Pseudoalteromonas haloplanktis*, *Pseudomonas, Psychrobacter, Flavobacterium* spp*, Limnohabitans, and Propionibacterium*. Over time, the microbiota changed rapidly. At hour eight, there was a significant increase in relative abundance of *Marimonas* spp., and *Shewanella baltica*. At hour 12, *Vibrio* species, including *Vibrio parahaemolyticus, Vibrio ordalii, Vibrio cyclitrophicus* had increased to an average relative abundance of 70% in all but one sample. Interestingly, abundance of *Vibrio* spp. in all samples decreased to a relative abundance of approximately 15% by hour 24.

Significance: The temporal profiles shared here demonstrate that hour 12 of incubation is a good point at which to evaluate *Vibrio* genomics, either with metagenomic sequencing or by additional culture based approaches using selective media. Relationships between endemic microbiota of oysters in traditional *Vibrio* enrichments are complex and likely vary by source microbiome. Using shotgun sequencing to characterize these dynamics contributes to an optimization of recovery methods.

P3-184 Rapid Screening for Finfish Species Substitution Using Chip-based Capillary Electrophoresis and a Web-Based Application

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Introduction: Authenticity is crucial to the seafood industry, as substitution and mislabeling have important economic, environmental, and food safety consequences. Most species substitution is simply economic fraud, but substitution can also increase the risk of health hazards.

Purpose: Water extraction and chip-based microfluidic electrophoresis (Agilent 2100 Bioanalyzer) for the analysis of high abundance fish muscle proteins along with a novel data analysis method for species-specific protein pattern recognition was developed and validated. The resulting protein patterns were used to create a web-based species substitution identification tool.

Methods: A 0.15-g muscle tissue sample from 866 authenticated, raw finfish specimens were added to deionized water for extraction of sarcoplasmic proteins. The extracted proteins were differentiated using chip-based microfluidic electrophoresis. The mean masses of the highest abundance proteins were selected for inclusion in the species-specific protein pattern-matching logic-based algorithms to produce probability matches between unknown samples and authenticated reference species.

Results: The performance validation study for the method's capacity to distinguish often-substituted species from their common illegal replacement species was evaluated using sensitivity (true positive values), specificity (true negative values), and accuracy. Mean results showed 86% sensitivity to recognize the product with a correct species label, 97% specificity to recognize the product with an inaccurate species label and 93% accuracy to correctly link the highest probability match to the labeled product. As an example, for American catfish versus other catfish including Asian catfish, the tool performed at 95% sensitivity, 100% specificity and 97% accuracy.

Significance: The water extraction method combined with the web-based tool provides the seafood industry with an efficient and high throughput alternative to DNA methods.

P3-185 The Effect of Tumbling Processes on the Shelf Life of Whole Octopus (*Amphioctopus kagoshimensis* and *A. marginatus*) Stored in Ice

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Introduction: The traditional way to overcome octopus toughness has been the repeated "slapping" of the freshly caught octopus on the rocks by the sea. This procedure has been adopted by the industry and the mechanical "tumbling" of octopus is performed in a custom-made tumbler.

Purpose: This study evaluated the effects of tumbling on the sensory, microbiological, and physical properties of iced whole octopus (*Amphioctopus kagoshimensis* and *A. marginatus*).

Methods: Fresh octopus (200±20 g) were caught by professional home water fishers with fish traps. After 12 h on ice at sea, octopus samples were landed in the fish port and were slapped by using a piece of tumbling equipment immediately. After tumbling the samples were packaged in a polyethylene bag and kept in ice in a 0°C cooler for a period of 21 d.

Results: The increases in psychrophiles in octopus with slap treatment were higher than those of control treatment during 12 d of storage in ice. The increases in psychrophilic bacteria were in agreement with the increases in total volatile base, trimethylamine and formaldehyde contents. Inosine (HxR) was the main nucleotide present in the *Amphioctopus kagoshimensis* and *A. marginatus* during initial iced storage. During the first days of ice storage of *A. kagoshimensis*, adenine nucleotides were almost completely converted to hypoxanthine. The nucleotide degradation rate of *A. kagoshimensis* was significantly higher than *A. marginatus* compared to both treatments. In addition, the slap treatment showed significant catabolism of HxR with conversion to hypoxanthine. At the same time, we have set up the database of formaldehyde content in *A. kagoshimensis* and *A. marginatus* during iced storage.

Significance: Shelf-life of *A. kagoshimensis* and *A. marginatus* with tumbling treatment were estimated as six d during iced storage, according to sensory, microbiological, and physical properties.

Poster

P3-186 Food-derived Bioactive Peptides on Antioxidative Capacity, Xanthine Oxidase and Tyrosinase Inhibitory Activity

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Introduction: Bioactivities of 11 synthesized peptides derived from fish and egg white were investigated for their antioxidative capacity, xanthine oxidase (XO) and tyrosinase. The XO is an important hepatic enzyme in purine catabolism, catalyzing the oxidation and breakdown of hypoxanthine to xanthine and subsequently to uric acid. Tyrosinase catalyzes and oxidizes the biosynthesis of melanin pigment in melanocytes of human skin from tyrosine or dihydroxyphenylalanine (DOPA) as the substrate.

Purpose: The usage possibilities of peptides as an antioxidant and the mechanism of antioxidant activity of the peptides were studied. In addition to that kinetic activity of the peptides against XO and tyrosinase was also discussed.

Methods: The 11 bioactive peptides were HGAYV, VWWW, IRW, PSYV, NHRYDR, LARL, PHYL, LPHSGY, VKAEFAWTANQQLS, PSKYEPFV, and LPTSEAAKY. Antioxidant assays include 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and reducing power. Inhibition assays include XO and tyrosinase. Kinetic parameters were measured for the Michaelis–Menten equation and Lineweaver–Burk plots.

Results: The antioxidative capacity of peptides showed that VWWW (mackerel) possessed the highest antioxidant activity in DPPH assay, while VWWW, IRW (egg white), and VKAEFAWTAQQLS (tuna) had significantly greater antioxidant capacities among all selected peptides in ABTS test. Furthermore, peptides with higher in-vitro antioxidant activities were selected to be studied their enzyme inhibition. The XO inhibition of three selected peptides exhibited competitive inhibition pattern. The IC_{50} of the peptides for XO inhibition assay was 3.61±0.10, 1.34±0.01, and 2.74±0.02 mg/mL for VWWW, IRW and VKAEFAWTAQQLS, respectively. Selected peptides also inhibited the tyrosinase competitively. Their IC_{50} values of tyrosinase inhibition assay were 0.85±0.03, 0.98±0.01, and 0.90± 0.01 mg/mL for VWWW, IRW, and VKAEFAWTAQQLS respectively. In conclusion, VWWW implied the potential for functional ingredient development.

Significance: This research is expected to shed more light on the antioxidant studies for fish and egg-derived peptides. Furthermore, it may be able to help to bring the understanding of kinetic behavior of the bioactive peptides against xanthine oxidase and tyrosinase.

P3-187 Rapid Concentration and Molecular Detection of *Vibrio harveyii* in Oyster Farm Seawater

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Introduction: Marine aquaculture is an important and expanding economic activity in the United States, and globally; however, instances of shellfish-associated human pathogen outbreaks occur with some regularity. Improved microbial detection of *Vibrio* in oyster nursery and farm water can enable improved event response and reduce seed losses and potential human outbreaks.

Purpose: In this study, the efficacy of sample preparation using the Concentrating Pipette Select, ultrasonic lysis, and DNA purification, for qPCR analysis, was employed to rapidly detect experimentally infected seawater.

Methods: One liter of autoclaved seawater was infected with 100 or 10 CFU/ml of *Vibrio harveyi*. Samples were concentrated and eluted with lysis fluid and lysed by ultrasonication. The lysed samples were subjected to DNA purification and assayed by qPCR using primers designed to amplify the *V. harveyi* 16S rRNA gene.

Results: Experimentally infected seawater (*n*=3, one liter) were concentrated and plated to determine concentration efficiency. On average, 65.5% (±3.16%) of *V. harveyi* were recovered post-concentration in an average time of eight min and 30 s (±0.24 mins) in 221 (±0.018) microliters, and an average concentration factor of 2,983X (±385.3X). Lysis of *Vibrio* using lysis fluid and ultrasonication resulted in a 99.69% (±0.21%) decrease in viability. Experimentally infected seawater spiked at of 10 CFU/ml (*n*=5) and 100 CFU/m (*n*=5), resulted in average cycle thresholds values of 28.5 (±1.3) and 26.2 (±0.7), respectively, in under two h total.

Significance: These data suggest that same-day detection of *Vibrio* in seawater can be performed rapidly and more efficiently than current test methods.

P3-188 Inactivation of *Listeria monocytogenes* in Frozen Cooked Shrimp by High Pressure Processing

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Introduction: *Listeria monocytogenes* is a serious risk in ready-to-eat seafood. High pressure at low temperatures (HPLT) has proved that lower pressures for shorter times are capable of reducing adequately the microbial populations while retaining quality characteristics. This is caused by the ice phase transition that occurs during pressure. Frozen seafood is an ideal substrate for applying this approach.

Purpose: The purpose of this study was to evaluate the effectiveness of HPLT on inactivation of *L. monocytogenes* in frozen cooked pink shrimps (*Pandalus jordani*).

Methods: Shrimps were defrosted and portions of 25 g, inoculated with a cocktail of six *L. monocytogenes* strains, vacuum packed and stored at -35°C for 24 h. Then they pressurized at 250 MPa for 0.5, 1.5, 3 and 10 min. The pressure liquid was added into the pressure vessel at the temperature of -30°C. After the treatment the samples were placed at -20°C for three d before defrosting. Palcam Agar overlaid with Tryptone Soy Agar was used for the enumeration of survivors. The color was also evaluated.

Results: Product temperature after the HPLT was varied from -24 to -28°C, indicating that the water in the product remained frozen and went through ice phase transitions with increasing pressure up to 250 MPa. There were significant differences ($P<0.05$) in the bacterial reduction caused by the different processing times and varied from 1.5 to 3.2 log CFU/g for 0.5 and 10 min respectively. Not substantial color changes occurred even after 10 min of HPLT.

Significance: Data suggest that HPLT can achieve a three-log reduction of *L. monocytogenes* in frozen cooked pink shrimps without affecting substantially the product color.

P3-189 The Inactivation Effect of High Pressure Processing on Histamine-Forming Bacteria

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Introduction: High pressure processing (HPP) is an emerging non-thermal technology that can inactivate pathogenic and spoilage microorganisms in food at room temperature. Histamine fish poisoning is a worldwide foodborne intoxication caused by the ingestion of seafood that contain high levels of histamine. Histamine is formed mainly through the decarboxylation of free histidine by decarboxylase released by histamine-forming bacteria (HFB) such as *Morganella morganii* and *Photobacterium phosphoreum*.

Purpose: The objectives of this study were to evaluate inactivation effects, morphological damage and cellular death in two HPP-treated HFB, *M. morganii* and *P. phosphoreum*.

Methods: Inactivation effects of HPP treatment (200-500 MPa for zero to 10 min) on the enumeration of HFB surviving cells and decimal reduction time were evaluated. Meanwhile, the morphological damage and cellular death of HFB cells after exposure to HPP treatments were observed by using scanning electron microscopy (SEM) analysis.

Results: The result showed that HFB had a higher *D*-value in tuna meat slurry compared with that in phosphate buffer, indicating that the HFB were more resistant to pressure in tuna meat slurry. *M. morganii* had a higher *D*-value than *P. phosphoreum* at the same pressure, indicating it to be more resistant at HPP treatment. In the present study, SEM analysis showed that pressure treatment induced morphological changes and cell wall and membrane damage in HFB cells.

Significance: Our result indicate that HPP can be applied to inactive HFB *M. morganii* and *P. phosphoreum* by inducing morphological changes in internal and external structures in the cell, as well as by causing cell wall and membrane damage.

P3-190 Application of High Pressure Processing for Preservation of Marlin Meat during Storage

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Introduction: High pressure processing (HPP) is an emerging non-thermal technology that can inactivate pathogenic and spoilage microorganisms in food, and extend the shelf life of foods. Histamine is primarily produced in fish through decarboxylation of free histidine by the activity of various species of histamine-forming bacteria (HFB). Fish included marlin, mahi-mahi, tuna, mackerel, and bonito, are the types of fish commonly involved in histamine fish poisoning due to the high levels of free histidine in their muscle tissue.

Purpose: The aim of this study was to determine the effects of HHP treatment on the levels of aerobic plate count (APC), total volatile basic nitrogen (TVBN) and histamine formation in marlin meat, under the controlled storage temperatures of 4, 15 and 25°C.

Methods: Skinless marlin meats treated with different pressures (20, 300, 400, and 500 MPa for five min) and stored at 4, 15 and 25°C were analyzed. During storage, marlin samples were analyzed for color, APC, pH, TVBN, and histamine content.

Results: The APCs of marlin samples after HPP treatment (200 to 500 MPa for five min) decreased with increased pressure from 4.94±0.12 log CFU/g of control (untreated) to 2.36±0.08 log CFU/g of 500 MPa treatment. In color of marlin samples, L* value (lightness) increased significantly with increased pressure, whereas a* value (redness) was significantly reduced with increased pressure. The HPP (>300 MPa, five min) significantly delayed the APC, TVBN and histamine increase in marlin samples, regardless of the storage temperature. In summary, this result suggested the marlin fillets treated with HPP (>300 MPa, five min) and stored at below 15°C could prevent deterioration of product quality and extend shelf life.

Significance: This study demonstrated that 300 MPa for five min for marlin meat is the optimum HPP condition for controlling color, microbial load, TVBN, and histamine changes. Overall, these results prove the usefulness of HPP in seafood processing while enhancing the preservation and safety of marlin fish consumption.

P3-191 Antibacterial and Antibiofilm Mechanism of Eugenol against *Vibrio parahaemolyticus* Clinical and Environmental Isolates

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Introduction: *Vibrio parahaemolyticus* is the leading seafood-transmitted bacterial pathogen causes gastroenteritis. *V. parahaemolyticus* has attracted considerable attention because of its pathogenicity, antibiotic resistance pattern, biofilm formation, and ability to contaminate seafood in the United States and Asia.

Purpose: The objective of this study was to investigate the antibacterial and antibiofilm activity of eugenol in mechanism level against *V. parahaemolyticus* planktonic and biofilm cells.

Methods: The time-kill assay, motility, hydrophobicity, nucleic acids and protein leakage, transmission electron microscope, biofilm formation and eugenol treatment, crystal violet staining, [2, 3-bis (2-methoxy-4-nitro-5- sulfophenyl)-2H-tetrazolium-5-carboxanilide] reduction method, phenol-sulfuric acid method, field emission scanning electron microscopy, confocal laser scanning microscope and high-performance liquid chromatography were used to evaluate the antibacterial and antibiofilm activity of eugenol.

Results: The results indicated that the different concentrations of eugenol (0.1 to 0.6%) significantly reduced biofilm formation, metabolic activities and secretion of extracellular polysaccharide (EPS), with effective antibacterial effects. Eugenol at 0.4%, effectively eradicated the biofilms formed by clinical and environmental isolates by more than 4.5 and four log CFU/cm^2, respectively. At 0.6%, the reduction rate of metabolic activity for ATCC27969 and NIFS29 were 79 and 68% respectively, whereas at 0.6% concentration the reduction rate of EPS for ATCC27969 and NIFS29 were 78.5 and 71% respectively. In the visual evaluation, the meaningful results were observed for biofilm reduction, live/dead cell detection, and quorum sensing through FESEM, CLSM and HPLC-FLD, respectively.

Significance: This study demonstrated that eugenol can be used to control *V. parahaemolyticus* biofilms, biofilm-related infections and can be employed for the protection of seafood.

P3-192 Application of Chlorine Dioxide and Electron Beam Radiation for Reduction of Murine Norovirus-1 in Low Salt Fermented Clam (Jogaejeotgal)

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Introduction: Jogaejeotgal, a traditional Korean salted and fermented clam, can be contaminated by norovirus because there are no additional sterilization steps. Therefore, the sequential treatment of chemical and physical methods such as chlorine dioxide (ClO_2) and electron beams (e-beam) can be applied to inhibit norovirus in Jogaejeotgal.

Purpose: According to the trend which is to reduce high intake of salt, the purpose of this study was to make low-salt fermented clams to examine the effect of ClO_2 during fermentation and sequential treatment of ClO_2 with e-beam irradiation for murine norovirus-1 (MNV-1) inactivation.

Methods: To examine the reduction value of norovirus during the storage period, clams salted with 10 and 20% NaCl were stored for zero, one, three five, seven, 10, 15, 20, and 25 d. To evaluate the effect of ClO_2 on norovirus reduction, inoculated clams were treated with 50, 100, 150, 200, and 300 ppm of ClO_2.

Poster

After ClO_2 treatment, 10% salt was added to the clam. E-beam treatment was carried out after chemical treatment and performed after seven d of storage. The doses were one, three, and 5.5 kGy in e-beam treatment.

Results: Reduction values were 1.03 and 1.11 log PFU/m for 10 and 20% salt, respectively, following 25 d of storage. After the treatment with ClO_2 (50 to 300 ppm) in salted clam at 10%, MNV-1 titers were 4.30 to 3.30 log PFU/ml. The sequential treatment of ClO_2 (300 ppm) and e-beam (5.5 kGy) showed 1.90-log reduction after seven d of storage in the salted clam at 10%.

Significance: This study suggests that norovirus in Jogaejeotgal can be effectively inhibited through hurdle technology using ClO_2 and e-beam irradiation.

P3-193 Nisin Inhibition of *Listeria monocytogenes* in a Smoked Whitefish Salad Blend

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❖ ***Developing Scientist Entrant***

Introduction: Seafood salads are cold stored, ready-to-eat foods with a historically high risk of contamination by *Listeria monocytogenes.* Nisin has known bactericidal effects against *L. monocytogenes* and is commonly utilized within the seafood industry in ready-to-eat formulations. Validation of nisin addition in smoked seafood product formulations is necessary to determine the bactericidal effects of the additive against pathogen contamination.

Purpose: We evaluated the bactericidal effects of nisin against *Listeria monocytogenes* inoculated in a whitefish salad blend during a 42-d shelf-life period.

Methods: Smoked whitefish salad samples (*n*=42), formulated with (0.03%) and without nisin (control), were inoculated with one to two log CFU/g *L. monocytogenes* 10403S and massaged to ensure even distribution of inoculum. Samples were stored aerobically at 4°C for the duration of the study (42 d). Every seven d, including d zero, six samples were randomly selected (*n*=3), temperature and pH recorded, and *L. monocytogenes* was enumerated. Data were analyzed using the mixed procedure in SAS with treatment and sample day as fixed effects.

Results: Samples formulated with nisin had less *L. monocytogenes* CFU/g compared to the control over time ($P<0.05$) with the exception of d zero and d 21. At d 21, there was an increase ($P<0.05$) in *L. monocytogenes* growth in the nisin treated samples, with populations similar ($P=0.1173$) to that of the control. Regardless, after d zero, the control did not significantly inhibit *L. monocytogenes* ($P>0.05$), while 0.03% nisin significantly reduced *L. monocytogenes* from 1.33 log CFU/g to below detection level (0.10 log CFU/g) by d 35 ($P<0.05$).

Significance: These results indicate that addition of 0.03% nisin in a whitefish seafood salad is an effective *L. monocytogenes* control strategy during 42-d aerobic storage under the tested parameters.

P3-194 Cold Chain Applied to Meat Products in Major Mexican Retail Stores

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Introduction: In retail stores, meat products' shelf life depends on the temperature within the showcase used.

Purpose: The aim of this research was to determine temperature within an open refrigerated meat showcase of one deck in five retail stores of major Mexican supermarket chains.

Methods: Temperatures were measured on a weekly basis from April to August 2018, at three sections (front, middle and rear) of the deck. Statistical analysis was with a mixed model under a complete randomized design, with repeated measurements, the mean comparison was with a *t*-test. Overall means for each position within the deck were compared against the temperature set for the Mexican government for meat products conservation (MON 94-SSA1-2004).

Results: In four out of the five stores in front of the deck temperature was above the Mexican standard (4°C) and showed no differences ($P>0.05$) among them. Middle of the deck temperature was different ($P<0.05$) among stores, with one showing temperatures above Mexican standards throughout the study period. The rear of the deck temperature showed no difference ($P>0.05$) among stores and always complied with Mexican standards.

Significance: In conclusion, the temperature in open refrigerated meat showcase of one deck depends on the store, position within the deck and is not always compliant with Mexican standards. Better temperature control mechanisms should be implemented to meet Mexican standards.

P3-195 Strategies for Reducing Foodborne Illness from Consumption of Ethnic Raw Meat Dishes in the United States

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Introduction: Several foodborne outbreaks in the United States have been attributed to dishes served with raw meat. Consumption of these foods is often associated with ethnic and cultural traditions.

Purpose: To understand consumer preferences for eating certain foods served with raw meat and how to educate consumers about the associated risks.

Methods: We conducted focus group research (*n*=34 participants) with i) United States consumers who eat kibbeh nayah, a traditional Middle Eastern appetizer prepared with raw ground beef or lamb, and ii) United States consumers who eat raw ground meat (aka cannibal/tiger meat) sandwiches, a dish popular among German and Polish communities in regions of the upper Midwest.

Results: Participants expressed strong preferences for these foods because they eat them during holidays or other special occasions as part of family traditions. Older adults often prepare and eat these foods and the foods are sometimes served to children. Participants are aware that eating these foods could potentially cause foodborne illness; however, participants are willing to accept this risk and continue eating these foods because of tradition or culture. Participants trust their local butcher to provide them with freshly ground meat that is "safe" and understand the need to keep the dish cold when served and to cook or discard leftovers. Participants were not receptive to the government advising people not to eat these foods and doubted the effectiveness of such recommendations.

Significance: The findings of this research suggests that educators should avoid messaging that is targeted to specific foods or cultures which may be perceived as culturally insensitive. Instead, educators should provide messaging on the risks of eating raw meat in general, especially among at-risk populations. Educators may want to partner with local butchers in areas of the United States where these foods are consumed to disseminate information on the dangers of consuming dishes made with raw meat.

P3-196 Effect of Cranberry Pomace on the Inactivation of *Salmonella enterica* Serovars and Physicochemical Changes during Dry Fermented Sausage Manufacturing

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❖ ***Developing Scientist Entrant***

Introduction: Cranberry pomace (CP) is a cranberry processing byproduct and possesses antimicrobial properties. There is strong consumer interest for products with natural antimicrobials, but there are limited reports examining the practicality of using CP as a natural antimicrobial agent in processed meat products, especially non-thermally processed dry fermented sausages (DFS).

Purpose: Examine the effect of CP incorporation on *S. enterica* inactivation, starter culture growth, and physicochemical properties of DFS.

Methods: DFS containing a five-strain cocktail of *Salmonella enterica* serovars at seven log CFU/g, with the addition of different levels of CP (control, 0%; low, 0.55%; medium, 1.70%; high, 2.25% wt/wt), or liquid lactic acid were manufactured, and subjected to conventional fermentation and drying conditions.

Results: A reduction in initial pH was observed in all CP treatments on day zero as a result of CP natural acidity. Medium and high CP levels showed a lower end-product a_w than the control after drying. DFS incorporated with CP exhibited a faster and greater *S. enterica* inactivation during the first five days; the reduction rate and degree were directly correlated to the CP level. Both CP phenolic compounds and its natural acidity were found to play a key role in *S. enterica* inactivation. In the presence of medium and high levels of CP, *Staphylococcus* spp. growth was suppressed, while *Lactobacillus* spp. and *Pediococcus* spp. exhibited a stimulatory effect. All treatments, except the low CP treatment, showed no significant effect on products' chemical composition, and the moisture to protein ratio. Medium and high CP levels resulted in darker, duller and redder DFS with a softer and more crumbly texture.

Significance: Findings suggest that low CP levels can be utilized by DFS manufacturers as a natural functional ingredient to further minimize the risk associated with *S. enterica* during production without altering final product quality.

P3-197 Comparison of *Listeria* Isolates from Environmental Sampling Using Whole Genome Sequencing, Pulsed Field Gel Electrophoresis, and the Riboprinter® System

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Introduction: Whole genome sequencing (WGS) is transforming food safety as it allows for rapid and reliable detection of foodborne illness culprits. WGS is used by academia as well as regulatory authorities to characterize and link foodborne disease outbreaks, trace pathogen sources, and compare against epidemiological data; however, its use at industry level is still fairly new.

Purpose: The purpose of this study was to compare three subtyping methodologies by analyzing isolates collected from environmental swabs in a manufacturing facility to determine whether there were any differences in results that may impact investigation and influence future method selection.

Methods: Thirty-five *Listeria* isolates were assessed following the PulseNet standard operating procedure for pulsed-field gel electrophoresis (PFGE) of *Listeria monocytogenes* using *Apa*I and *Asc*I restriction enzymes. Next, WGS was completed using the Illumina MiSeq. BioNumerics software from Applied Maths was used for analysis and to apply the whole genome multi-locus sequence typing (wgMLST) technique for allele calling. Lastly, isolates were analyzed on the Hygiena RiboPrinter system using the *Eco*RI restriction enzyme. BioNumerics software was used for comparison and clustering analysis.

Results: Isolates clustered into two groups using PFGE, WGS, and RiboPrinting. Group A included 15 isolates that clustered by PFGE (identical band patterns – deemed indistinguishable), wgMLST (92.4% allele similarity), and RiboPrinting (97.0% average similarity). Group B included 14 isolates that clustered by PFGE (identical band patterns – deemed indistinguishable), wgMLST (84.9% allele similarity), and RiboPrinting (94.0% average similarity). The remaining six isolates did not cluster well by PFGE, wgMLST, or RiboPrinting, with the exception of two isolates, which clustered by RiboPrinting (90.8% average similarity).

Significance: The results demonstrated that WGS offers higher resolution than PFGE and RiboPrinting, and using PFGE or RiboPrinting results alone may potentially link isolates that are not as similar as they appear, which could lead to incorrect root cause analysis.

P3-198 Antimicrobial Effect of Microwave Treatment on Beef Jerky Inoculated with *Salmonella* and *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* and *Listeria monocytogenes* are important pathogenic bacteria that can cause recalls and numerous illness with millions of dollars in related public health costs. These pathogens have the ability to produce life-threatening illness in children, elderly, and other susceptible populations. Furthermore, *Salmonella* can survive for long periods even in dry conditions, making dehydrated beef products, like jerky, an important environment where this pathogen could survive.

Purpose: The objective of this project was to evaluate the effectiveness of microwaves as a post-packaging intervention to reduce *Salmonella* and *Listeria monocytogenes* on flavored beef jerky.

Methods: Three flavors of beef jerky were separately evaluated: teriyaki, korean BBQ, and original. The study had two treatments (treatment 1 and treatment 2, each with different microwave power exposure) and one negative control. For each treatment and control, 15 individual samples of 25 g were analyzed. The samples were first inoculated under an air hood with 50 µl of either *Salmonella* or *Listeria* cocktail (~eight log CFU/ml), allowed to dry for 10 minutes, assigned to microwave treatments, and treated using a conveyorized, custom-made, six magnetron microwave. After treatment, each sample was diluted in 225 ml of buffered peptone water and homogenized at 230 RPM for two min. Serial dilutions were made and spread plated on rifampicin tryptic soy agar for *Salmonella* and Modified Oxford Agar for *Listeria*.

Results: The results showed that treatment 1 can reduce more than 1.9 log CFU/ml of *Salmonella* and *Listeria*, and treatment 2 can reduce more than five log of both pathogens.

Significance: The study demonstrates the effectiveness of microwaves to eliminate *Salmonella* and *Listeria* in beef jerky. Nonetheless, this technology can be implemented on the processing line before or after packaging fortifying the food safety of the product.

Poster

P3-199 Performance Evaluation of Fluorescence Resonance Energy Transfer-based Real Time PCR for *Salmonella* spp. Detection in Nut Matrices (Almonds and Peanuts) at a 375-g Sample Size

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Introduction: Many advancements in PCR have made it possible to use it for food safety testing. Despite robustness, different matrices may present new challenges and should be reviewed for compatibility with the adoption of new technology in the laboratory. Nut matrices present different challenges due to tannins and other leachable chemistry from the nuts.

Purpose: A study was conducted to evaluate the performance of GENE-UP *Salmonella* assay (SLM), a real-time PCR, for the detection of *Salmonella* spp. from two sources of nuts (almonds and peanuts) at 375-g sample size.

Methods: Almonds (375 g) were tested per AOAC validation guidelines using 30 unpaired samples, where five and 20 replicates were inoculated with *Salmonella* Typhimurium at a high (~two CFU/test portion) and low levels (0.2 to two CFU/test portion), respectively. Five samples were tested uninoculated. Unpaired test portions were evaluated by both the GU and the reference (FDA-BAM Ch5). Peanuts (375 g) were also tested for *Salmonella* spp. with <20 CFU inoculation for *n*=5 samples; one sample was tested uninoculated. All samples were enriched in buffered peptone water for 22 to 24h at 42°C. All presumptive results were confirmed with the culture method confirmation.

Results: For almonds, no significant differences (95% CI) were observed from the reference method [$dPOD_r$: 0.0; LCL:-0.28; UCL: 0.43]. For peanuts, GU reported expected results where all the spiked peanuts samples were positive, while the uninoculated samples were reported negative.

Significance: The challenge studies conducted here demonstrate the efficacy (fit for purpose) of the SLM assay for *Salmonella* spp. detection from nut matrix.

P3-200 Performance of a Fluorescence Resonance Energy Transfer-based Real-time PCR Assay for the Detection of *Salmonella* spp. Using a Manual Sampling Device for Beef

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Introduction: The Manual/Continuous sampling device [MSD/CSD] received a no objection letter from the FSIS for the detection of *Salmonella* and/or Shiga Toxin-Producing *E. coli* (STEC) or other indicator organisms on beef trimmings. Matrix extension studies need to demonstrate the compatibility of the MSD/CSD for pathogen detection methods like PCR.

Purpose: To evaluate the performance of fluorescence resonance energy transfer (FRET)-based *Salmonella* RT-PCR assay (GENE-UP, SLM) against *Salmonella* spp. on beef trims using MSD.

Methods: Thirty MSD sheets were used to scrub the beef trim surface for 90 s each to simulate sampling conditions. These MSD were cold stressed at 4°C for 24 h along with a *Salmonella* Typhimurium broth culture. Twenty MSD were spiked with *Salmonella* at less than one CFU/MT. The spiked and the unspiked (*n*=10) MSD were enriched with prewarmed buffered peptone water (200 ml) for eight, 10, and 24 h at 42°C. All enrichments were first tested using SLM. The composited enrichments (*n*=10) were created with equal volumes from five enrichments (one positive and four negatives). These composited enrichments were tested using SLM. All presumptive positive results were confirmed by culture methods.

Results: Among the spiked samples, 13 of 20 samples were positive, while the rest (seven of 20) samples were negative for *Salmonella*. All unspiked samples were found to be negative, and no samples were reported as invalid by SLM. All composited samples gave expected results, where the samples with ≥1 positive containing sample were detected as positive.

Significance: These data demonstrate the compatibility of the SLM assay to detect *Salmonella* from the MSD. This test combination (MSD and SLM) could provide the beef industry with a viable option for highly specific detection of *Salmonella* spp. in beef processing plants.

P3-201 Performance Evaluation of a Fluorescence Resonance Energy Transfer Based Real-Time PCR in a Unit Dose Format for the Detection of *E. coli* O157:H7 in 375 g Ground Beef

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Introduction: A number of commercial PCR systems have been addressing the Shiga toxin-producing *E. coli* O157:H7 (STEC O157) detection needs for the beef industry for a couple of decades now. However, these systems have shown to cross-react with non-pathogenic O157 isolates. In the United States, given that STEC O157 is considered an adulterant, the implications of these PCR-method shortcomings are huge for the beef industry. We have previously shown that fluorescence resonance energy transfer (FRET) based real-time PCR GENE-UP *E. coli* O157:H7 (ECO) does not cross react with non-pathogenic O157 isolates.

Purpose: A study was conducted to evaluate the comparative performance of a traditional ECO against a modified easier-to-use format, a pelleted lyophilized master mix, called unit-dose ECO2. The study was conducted according to the current AOAC validation guidelines.

Methods: Samples of ground beef (375 g) were tested per AOAC validation guidelines using 30 unpaired samples, where five and 20 replicates were inoculated with *E. coli* 0157:H7 at a high (~two CFU/test portion) and low levels (0.2 to two CFU/test portion), respectively. Five samples were tested uninoculated. Unpaired test portions were evaluated using the ECO and ECO2 format and the FSIS/MLG Ch 5 reference method. All ECO/ECO2 samples were enriched in buffered peptone water (one to four) for 10 h at 42°C. All presumptive results were confirmed with the reference method as well as using the alternate confirmation plating to ChromID EHEC, and CT-SMAC.

Results: No significant differences (95% CI) were observed in the performance of the ECO, ECO2 and the reference method [dPODc: -0.25; LCL: -0.49; UCL: 0.04].

Significance: These data indicate i) performance of ECO and ECO2 was equivalent, ii) both ECO and ECO2 performed equivalent to the reference method. ECO2 presents a viable alternative for the beef industry as it combines the sensitivity/specificity of ECO but with an easier-to-use workflow.

P3-202 Estimating the Likelihood of Human Toxoplasmosis from Consuming *Toxoplasma gondii*-contaminated Fresh Cut Meats

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❖ ***Developing Scientist Entrant***

Introduction: *Toxoplasma gondii* is capable of infecting almost all warm-blooded animals including humans and causes a disease condition, known as toxoplasmosis. Although its seroprevalence is high in humans, *T. gondii* is recognized as one of the neglected parasites. Humans become infected by ingesting *T. gondii* tissue cysts in raw or under-cooked meat or oocysts from cat feces mixed in food or water. A major portion of human *T. gondii* infections is acquired through the consumption of poorly cooked meat containing tissue cysts.

Purpose: There is no robust information available on the concentration of viable *T. gondii* in muscle tissues of naturally infected meat animals. The goal of this study was to quantify viable *T. gondii* concentration in meats of naturally infected lambs and goats.

Methods: The shoulder or leg cuts of 44 lambs and 39 goats were serologically tested for *T. gondii* antibodies through MAT (modified agglutination test) and then bio-assayed in mice in different amounts: five, 10, and 50 g, to observe the infection rate. DNA was isolated from positive tissue samples and further genotyped with *T. gondii* specific markers.

Results: Although the seroprevalence was very low in both lambs (4.5%) and goats (2.6%), the likelihood of getting ill from consuming *T. gondii* contaminated meat cuts if consumed under-cooked, is high. The bioassay results showed the probabilities of getting ill from consuming five, 10 and 50 g of *T. gondii* contaminated under-cooked lamb meat as 0.28, 0.45 and 0.81, respectively. There was a 100% chance of getting sick from consuming contaminated goat meat. Detection of 529 base-pair bands in agarose gel confirmed *T. gondii* DNA in one-g muscle samples and one-ml pepsin-digested samples.

Significance: This study indicates that *T. gondii* can be present in naturally infected meat animals and could pose a threat of foodborne illness to consumers.

P3-203 Shiga Toxin-producing *Escherichia coli* Harboring *stx1* or *stx2* genes Isolated from Poultry Meat in Brazil

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Introduction: Shiga Toxin-producing *E. coli* (STEC) is one of the most important foodborne pathogens frequently reported in food products of animal origin worldwide. However, despite its great importance in public health, there is insufficient information regarding the presence of this microorganism in poultry meat in Brazil.

Purpose: The aim of this study was to investigate the presence of STEC in poultry meat samples recovered from poultry slaughterhouses located in different regions of Brazil and characterize its virulence factors: *stx1*, *stx2*, *eae* and *ehx*.

Methods: One hundred and forty-three poultry meat samples collected from slaughterhouses located in southern, southeastern and central-western regions of Brazil, were analyzed to monitor the presence of Shiga toxin-producing *E. coli* by using PCR according to ISO/TS13136 and USDA MLG 5B.05 methods.

Results: Among the one hundred and forty-three chicken meat samples analyzed for STEC strains, one isolate was positive for Shiga toxin-producing gene *stx1,* ten isolates were positive for Shiga toxin-producing gene *stx2* and thirty-three isolates were positive for *eae* gene alone. None of the isolates were positive for *ehx* gene or presented more than two genes.

Significance: The presence of *stx1* and *stx2* genes suggests that chicken meat can be a vehicle for transmission of STEC with an impact on food safety. Moreover, the detection of the *eae* alone in some strains suggests the presence of enteropathogenic *E. coli* (EPEC), also responsible for attaching and effacing lesions. However, more research is required with a larger number of samples, since there is not enough data regarding STEC in poultry meat worldwide.

P3-204 Evaluation of *Listeria monocytogenes* and *Staphylococcus aureus* Survival and Growth on Natural-source Nitrite-cured Ham during Stabilization

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Introduction: USDA recently added guidelines for stabilization for meat products cured using naturally-occurring nitrites. However, small meat processors struggled with insufficient information on whether their meat products using naturally-occurred nitrites cool fast enough to prevent growth of pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*.

Purpose: To investigate the temperature profiles of alternatively cured hams during refrigerator cooling and determine the survival of *S. aureus* and *L. momocytogenes* on ham during these cooling periods.

Methods: Whole, half and quarter hams were cured with celery powder and cherry powder containing 100 ppm nitrite and 250 ppm ascorbate, cooked in a smokehouse until internal temperature reached 140°F, and immediately transferred into a walk-in cooler (38±3°F). Surface and internal temperatures were recorded. Small cooked ham samples (25 g) prepared using identical formula were inoculated with *S. aureus* or *L. monocytogenes* (~four log CFU/g) to simulate post-lethality contamination, then gradually cooled from 130 to 45°F over 10 h to simulate internal ham temperature during cooler-stabilization. Bacteria were enumerated from 48 samples at 12 different time-temperature intervals during cooling by plating onto appropriate selective media.

Results: Overall, post-cooking whole (14 lbs), half (six lbs), and quarter hams (three lbs) were all cooled to 45°F within requirements of USDA Appendix B with cooling rate at 0.142, 0.195, and 0.232 °F/min. Mean internal temperature decreases over time can be best approximated using a polynomial curve (R^2>0.99). Time spans that ham temperatures permitting pathogen growth (130-45°F) were 598±16, 369±18 and 303±23 min, respectively. During cooling, neither bacterium showed significant growth, with *L. monocytogenes* from initially 4.34 to finally 3.95 log CFU/g, and *S. aureus* from 3.93 to 3.37 log CFU/g.

Significance: This study reveals effect of size on cooling time of meat products and growths of pathogens on alternatively cured ham, helping small meat processors arrange production and quality control.

P3-205 Comparison Effect of NaCl and KCl on *Clostridium sporogenes* PA3679 as Surrogate for *C. botulinum* in Shelf-stable Mortadella

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Introduction: Botulism is a disease of high severity associated with meat products. A factor that inhibits the germination of *Clostridium botulinum* spores is the water activity (a_w). In meat products the reduction of a_w can be carried out by addition of NaCl or KCl.

Poster

Purpose: The aim of this study was to evaluate the effect of a_w (0.95, 0.96 and 0.97) adjusted with different salts (NaCl and KCl) on *C. sporogenes*, used as a surrogate of *C. botulinum*, in shelf-stable mortadella.

Methods: The mortadella emulsion was inoculated with two log CFU/g of a *C. sporogenes* PA3679 spore suspension. The a_w was adjusted in the emulsions to 0.95, 0.96 and 0.97 with NaCl or KCl. The samples were heated at 85°C until the internal center of the product reached 72°C. The concentration of the *C. sporogenes* was determined in the product after one, 15 and 30 d storage at 35°C. The results were analyzed by ANOVA and Tukey's test for comparison between means ($P<0.05$). The experiment was repeated twice with $n=6$.

Results: None of the treatments showed spore germination after one d of storage (two log CFU/g of spores). After 15 d, in products with 0.97 a_w a high *C. sporogenes* count (ca. eight log CFU/g) was observed using both NaCl and KCl. In the same period of time, for 0.96 a_w, a significant difference ($P<0.05$) was verified between KCl (7.1 log CFU/g) and NaCl (5.1 log CFU/g). After 30 days, only the treatment with NaCl adjusted to 0.95 a_w did not show spore germination.

Significance: In shelf-stable mortadella the germination of *Clostridium sporogenes* spores was influenced by the type of salt used to adjust the a_w.

P3-206 Effect of Different Dry Aging Temperatures on *Listeria innocua* as Surrogate for *Listeria monocytogenes*

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Introduction: Dry aging is one of the main types of meat aging. However, few studies assess the impact of this process on food safety. *Listeria monocytogenes* is a microorganism that causes foodborne diseases and is able to develop at low temperatures. Thus, studies that evaluate the behavior of this microorganism during dry aging are extremely important.

Purpose: The objective of this study was to evaluate the effect of different dry aging temperatures on the behavior of *Listeria innocua* used as a surrogate of *L. monocytogenes*.

Methods: Striploin pieces (1.5 kg) were inoculated with six log CFU/g of *L. innocua* ATCC 33090 and subjected to the dry aging process for up to 42 days at 2 and 8°C. *Listeria* counts were determined in Modified *Listeria* Oxford agar. The data obtained were evaluated using ANOVA and the Weibull model.

Results: The process conducted at 8°C resulted in a greater reduction of *Listeria innocua*. After 42 d, reductions of 2.4 and 3.4 log CFU/g were obtained on the surface of the samples aged at 2 and 8°C, respectively. The final values of water activity were 0.926 and 0.879. According to data predicted by the Weibull model, the samples aged at 2°C would achieve a four-log reduction with twice the time required for the process conducted at 8°C. After trimming, 66.7% of the samples aged at 2°C were positive for *L. innocua*, whereas at 8°C the rate was 33.3%.

Significance: The behavior of *L. innocua* is influenced by the aging temperature and time, as well as by the water activity on the meat surface.

P3-207 Fat Contributes to the Effect of Heat against *Salmonella* in Red Meat Juice

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Introduction: *Salmonella enterica* is a foodborne pathogen of public health concern worldwide. The role of fat in the survival of *Salmonella* during red meat processing when subjected to heat treatment is unclear.

Purpose: This study investigated the effect of fat on heat treatment of *Salmonella* Bovismorbificans, Heidelberg, Newport, Typhimurium, and Virchow in beef, lamb and goat meat juice.

Methods: Meat juices were made from the exudate of the fatty layer and meat mixed (FLM), fatty layer (FL) and meat (M). *Salmonella* was inoculated at ~10^8 CFU/ml into meat juices and gradually heated at 70°C for five min. Untreated controls were included. Bacterial numbers in meat juices were determined on thin layer xylose lysine deoxycholate agar.

Results: In beef juice (FLM) heat treatment significantly ($P<0.05$) reduced *Salmonella* numbers by ~4.20 log CFU/ml or in some cases to less than the limit of detection (<LOD=1.49 log CFU/ml). Heat treatment significantly ($P<0.05$) reduced *Salmonella* numbers by ~2.10 to ~3.90 log CFU/ml in beef juice (FL) and to <LOD in beef juice (M). In lamb juice (FLM) heat treatment significantly ($P<0.05$) reduced *Salmonella* numbers by~4.80 to 6.80 log CFU/ml. Heat treatment significantly ($P<0.05$) reduced *Salmonella* numbers by~2.30 to ~3.40 log CFU/ml in lamb juice (FL) and by ~4.30 to ~5.30 log CFU/ml in lamb juice (M). In all goat meat juices heat treatment significantly ($P<0.05$) reduced numbers of *Salmonella* by ~3.50 to <LOD. In beef and lamb juice (FL) high survival (~5.07 to 6.50 log CFU/ml) was apparent while low (<LOD) and moderate survival (LOD to 5.00 log CFU/ml) were apparent in all other meat juices (FLM and M).

Significance: Fat content in red meat juices may protect *Salmonella* against the effect of heat. This study has implications for the persistence of *Salmonella* during red meat processing.

P3-208 Using Model Miniature Ham and Response Surface Methodology as a High-throughput Tool to Screen Antimicrobials Targeting *L. monocytogenes*

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Introduction: *Listeria monocytogenes* causes the foodborne disease listeriosis and is transmitted through eating contaminated ready-to-eat foods, such as deli meats. Antimicrobials in high-risk food products are used in combination with environmental sampling to limit the presence of this zero-tolerance organism.

Purpose: This study evaluates use of a miniature ham model, combined with a statistical experimental design, response surface methodology (RSM), as a tool for the food industry to identify effective and cost-efficient antimicrobial combinations.

Methods: Miniature hams were made by cutting 4.8 mm circular disks from ham slices made in the University of Illinois Meat Science Laboratory. Ham slices and miniature hams were surface-treated with commercially available and validated antimicrobials then inoculated with *Listeria* strains at 10^5 CFU/ml. The indicator of interest was growth after 12 d at 4°C, measured by serially diluting the hams in PBS and plating on PALCAM. The RSM was designed using JMP with an on-face design and three center points in a central composite design (27 total runs, done in triplicates). Three levels of four antimicrobials were used in combination: nisin at zero, 110, and 220 ppm, potassium lactate sodium diacetate (PLSDA) at zero, 1.75, and 3.5%, lauric arginate (LAG) at zero, 100, and 200 ppm, and Listex P100 at zero, 2×10^6, and 2×10^7 PFU/cm^2.

Results: An ANOVA indicates that the growth of *L. monocytogenes* after twelve days does not differ between untreated and nisin-treated traditional sliced ham and miniature hams ($P>0.05$). Multiple formulas of ham, differing by salt and water content, indicate combinations of potential synergistic antimicrobials ($P<0.05$ for interaction affects), such as nisin/PLSDA and nisin/LAG. The versatility of RSM was tested using *L. innocua* ATCC 33090, *L. monocytogenes* 10403S, and a cocktail of *L. monocytogenes* strains isolated from foodborne outbreaks. These indicated the same significant combinations of antimicrobials with synergistic effects ($P<0.05$), including nisin/PLSDA and nisin/LAG.

Significance: This project developed a high-throughput way of analyzing many antimicrobials in a food system, which can be applied to novel antimicrobials targeting *L. monocytogenes*.

P3-209 Effectiveness of Organic Acid Interventions for Reduction of *Escherichia coli* on Pork Carcasses in a Small-scale Pork Harvest Facility

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❖ ***Developing Scientist Entrant***

Introduction: Food safety interventions are widely used in the beef and poultry industries within the United States, but less work has been done to validate effectiveness in the pork industry.

Purpose: The objective of this study was to determine the most effective intervention for reduction of *E. coli,* for application prior to chilling of pork carcasses.

Methods: Split carcasses ($n=8$) were railed off prior to the hot box in a small-scale pork harvest facility. A five-strain cocktail of non-pathogenic ATCC surrogate strains of *E. coli* that mimic *Salmonella* behavior were sprayed on carcasses in the shoulder and ham area. Interventions, as assigned, were sprayed onto the carcass surface. Intervention treatments included: control (no intervention), water, Titon (sulfuric acid and sodium sulfate), PAA+Acetic acid (peracetic acid acidified with a two percent acetic acid solution), PAA+Titon^T (peracetic acid acidified with sulfuric acid and sodium sulfate solution), LA (three percent lactic acid solution), HBR (hypobromous acid 300 ppm), and LAE (lauramide arginine ethyl ester). Swabs on pork carcasses were used to determine microbial concentration after inoculation (attachment), and one and 24 h after intervention application. Microbial enumeration was performed by plating onto MacConkey agar with a tryptic soy agar overlay. Bacterial enumeration data were converted into log values for statistical analysis relative to CFU/100cm^2 and data were analyzed using SAS.

Results: All treatments began with 6.5 to 6.9 log CFU/100 cm^2 of *E. coli* attachment. Intervention application showed significant reduction of 1.7 to 4.9 log CFU/100 cm^2 of *E. coli* on pork carcasses 24 h after intervention application and chilling ($P< 0.05$).The most effective intervention, PAA+acetic, reduced *E. coli* by 4.9 log 24 h after application.

Significance: This work provides valuable information for the effectiveness of selected interventions if implemented in the pork harvest process prior to chilling, for reduction of *E. coli* on pork carcasses.

P3-210 Effectiveness of Organic Acid Interventions for Reduction of *Escherichia coli* on Pork Carcasses in a Large-scale Pork Harvest Facility with Blast Chilling

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Introduction: Intervention applications are employed in the beef and poultry industries, less work has investigated their effectiveness in the pork industry, especially in a commercial harvest facility.

Purpose: The objective of this study was to identify the most effective intervention for incorporation in a commercial pork facility to reduce *E. coli* and total bacteria prior to fabrication.

Methods: Carcasses ($n=32$) were railed off for inclusion and inoculation of non-pathogenic ATCC surrogate strains of *E. coli.* Prior to inoculation a 100cm^2 area on the right shoulder of each pork carcasses was swabbed to determine the aerobic plate count. Carcasses were then inoculated on the left shoulder with the *E. coli* surrogate cocktail. For intervention application carcasses were put back on the rail and traveled through a commercial spray cabinet. Interventions included were: PAA +Acetic acid (peracetic acid acidified with a 2% acetic acid solution), PAA + Titonä (peracetic acid acidified with sulfuric acid and sodium sulfate solution) and LA (3% lactic acid solution), Swabs on pork carcasses were used to determine microbial concentration after inoculation (attachment), 1 hour after, and 24hrs after intervention application. Microbial enumeration was performed by plating onto MacConkey agar with a tryptic soy agar overlay. Bacterial enumeration data were converted into $\log_{10}$ for statistical analysis relative to CFU/cm^2 and data were analyzed using SAS.

Results: Aerobic plate counts were reduced by 3.6, 3.0 and 2.1 log CFU/100cm^2 by lactic acid, PAA + Acetic, and PAA + Titonä respectively. While lactic acid, PAA + Acetic, and PAA + Titonä significantly reduced *E. coli* by 3.0, 4.8 and 3.1 CFU/100cm^2 respectively on pork carcasses 24hrs after intervention application and chilling ($P < 0.001$).

Significance: This work shows promising results for the pork industry in utilizing an effective intervention prior to chilling, for the reduction of *E. coli* and aerobic bacteria.

P3-211 Impact of Storage Temperature on the Survival of *Salmonella* in Finished Salami

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❖ ***Developing Scientist Entrant***

Introduction: While there are no performance standards for dry, fermented sausages, FSIS recommends the use of a process that achieves a five-log reduction of *Salmonella*. Achieving this targeted reduction during fermentation and drying has been difficult. Recent research has suggested that elevated storage temperature of the finished sausage could contribute to significant reductions in *Salmonella* that would support the microbial safety of these products.

Purpose: To determine the impact of storage temperature on the survival of *Salmonella* in finished salamis.

Methods: A cocktail of 10 *Salmonella* strains was inoculated between layers of four commercial sliced salami products (Italian dry, hard, Genoa, and uncured Genoa) and stored for seven d at 4, 15, and 22°C. *Salmonella* was enumerated on tryptic soy agar with Hektoen enteric agar overlay on days zero, one, four, and seven ($n=3$). Moisture content, pH, a_w, and lactic acid bacteria cell density were measured on uninoculated samples on d zero.

Results: *Salmonella* was stable in all salami products with storage at 4°C; however, storage at 15 and 22°C led to significant reductions after four and seven d in all four commercial products. Storage at 15°C for seven d led to an average reduction of 0.77 log CFU. Increasing the storage temperature to 22°C led to an average reduction of 1.85 log CFU by d seven. The inactivation of *Salmonella* at elevated storage temperatures was consistent across all four product types.

Significance: Short-term storage of salami after fermentation and drying could be an important step to achieve the targeted five-log reduction of *Salmonella* for these products. This study demonstrates that elevated storage temperatures (15 and 22°C) lead to significant reductions in *Salmonella* which could assist salami processors in achieving food safety goals.

Poster

P3-212 Microbiological Safety of *Staphylococcus aureus* and *Escherichia coli* in Dry-aged Beef Requiring Long Aging Time

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❖ ***Developing Scientist Entrant***

Introduction: Dry-aging is one of the aging processes that takes a longer time than others, especially for beef. This aging process is popular, because it produces flavor and texture that consumers prefer. However, food safety concerns for the process have been raised.

Purpose: This study investigated the growth and the survival of *Staphylococcus aureus* and *Escherichia coli* in dry-aged beef.

Methods: A mixture of *S. aureus* isolates or a mixture of *E. coli* isolates from dry-aged beef were inoculated on dry-aged beef (25 g) at 2.5 log CFU/g. The samples were stored at four to 15°C for three d in triplicate. *S. aureus* and *E. coli* cell counts were then enumerated on Baird Parker agar and 3M Petrifilm$^{T.E.\ coli}$/Coliform Count Plates, respectively. The Baranyi models were fitted to the *S. aureus* and *E. coli* cell count data to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD*; h).

Results: *S. aureus* and *E. coli* growth were not observed at 4°C, but the cell counts were maintained at initial concentration during storage periods. The *S. aureus* and *E. coli* cell counts were increased ($P < 0.05$) at 10°C and 15°C. μ_{max} increased ($P < 0.05$) from 0.06 to 0.08 log CFU/g/h, and *LPDs* decreased ($P<0.05$) from 21.72 h to 3.51 h, as storage temperature increased. During storage, *S. aureus* grew up to 5.3 log CFU/g, and *E. coli* grew up to 6.2 log CFU/g after 48 h at 15°C. *S. aureus* showed longer ($P < 0.05$) *LPD* and slower ($P < 0.05$) growth than *E. coli* during storage at low temperature.

Significance: These results indicate that a food safety regulation about temperature and period needs to be established to improve food safety of dry-aged beef, because the dry has a longer aging period than wet-aging beef, and its surface is trimmed with a knife, which may allow cross-contamination and foodborne pathogen growth.

P3-213 Comparative Evaluation of Sanitizers for the Control of *E. coli* O157:H7 in Ground Beef

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7 is a common contaminant of ground beef, resulting in numerous outbreaks and recalls. Ground beef processing, which involves grinding and packaging of beef-trims, relies primarily on carcass decontamination for pathogen control. Furthermore, temperature abuse (>5°C) of ground beef during retail could influence pathogen growth. Although conventional sanitizers are used as beef-trim interventions, their efficacy at abuse temperatures, especially during retail storage, is questionable. It is therefore important to devise intervention strategies that would maintain effectiveness throughout retail storage.

Purpose: Evaluate the efficacy of lactic acid, peracetic acid and pelargonic acid in reducing *E. coli* O157:H7 during ground beef processing and storage.

Methods: A total of 24 beef trims (four by four inch) were inoculated with a cocktail (~three log CFU/g) of five *E. coli* O157:H7 strains, with an attachment time of 15 minutes. The trims were spray-washed (20 ml) with either water, lactic acid (five percent), peracetic acid (400 ppm) or pelargonic acid (five percent) and allowed a dwell time of one min. Positive and negative controls were also used. The treated samples were then ground in a meat grinder and stored in retail packages at 4 and 8°C. Surviving pathogen populations were determined on days zero, one, two, and three. Experiments were repeated three times and data analyzed using one-way ANOVA ($P<0.05$).

Results: Of all the treatments tested, pelargonic acid was the most effective and resulted in significant ($P<0.05$) reductions in *E. coli* O157:H7, at both 4 and 8°C. Pelargonic acid reduced pathogen populations by 0.8 log immediately (d zero) and maintained its antimicrobial activity over four d storage. It demonstrated significantly ($P<0.05$) higher antimicrobial activity than lactic acid (0.4-log reduction) on d zero and peracetic acid (zero to 0.1-log reduction) on d two and three of storage at both 4 and 8°C.

Significance: Use of plant-based fatty acids, such as pelargonic-acid, might result in better control of foodborne pathogens in ground beef.

P3-214 Comparison of Culture Preparation and Inoculum Levels of *Listeria monocytogenes* in Challenge Studies Applied to Cooked Ready-to-Eat Meat Products

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Introduction: Challenge testing of RTE cooked meat with *Listeria monocytogenes* is required to assess growth during refrigerated storage and validate effectiveness of antimicrobial agents in accordance with USDA regulations. According to the USDA, "a ready-to-eat (RTE) product is defined as a meat or poultry product that is in a form that is edible without additional preparation to achieve food safety and that may receive additional preparation for palatability or aesthetic, epicurean, gastronomic, or culinary purposes."

Purpose: The purpose of this study was to compare the effect of culture preparation and inoculum levels when challenging nine RTE meat products with *L. monocytogenes* to assess growth at 4°C for 18 weeks.

Methods: Three culture preparations were evaluated. In the first preparation, *L. monocytogenes* strains were grown in Brain Heart Infusion (BHI) broth, subsequently transferred once into BHI, and incubated at 35°C for 24 h. Final average inoculum level was 2.3 log CFU/g.

In a second culture preparation, strains were started in tryptic soy broth with 0.6% yeast extract (TSBYE), subsequently transferred two consecutive times into TSBYE and incubated for 24 h at 35°C. The average inoculum level was 3.1 log CFU/g.

For a third culture preparation, strains were started in TSBYE, subsequently transferred into TSBYE at 35°C for 24 h and followed by a single transfer into TSBYE at 7°C for seven d. Average inoculum levels were 3.7 log CFU/g and 2.7 log CFU/g.

The acceptance criteria was greater than one log CFU/g increase of *L. monocytogenes* over inoculum levels during storage.

Results: Greater than a one log CFU/g increase of *L. monocytogenes* was observed in nine meat samples using the second culture preparation compared to two meat samples using the first culture preparation. With the third inoculum preparation, greater than one log CFU/g of *L. monocytogenes* counts was observed in six products with the higher inoculum level compared to five products with the lower inoculum level.

Significance: Culture preparation or inoculum levels can impact the growth of *Listeria monocytogenes* in RTE meats during refrigerated storage

Poster

P3-215 Comparison of Clean Label Antimicrobials with Nitrite on the Inhibition of *Clostridium perfringens* during Extended Cooling of a Model Deli-Style Ham Product

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Introduction: In response to consumer demands, processors are developing no-nitrite ready-to-eat meats alternatives. The efficacy of these clean label antimicrobials require validation against *Clostridium perfringens* during extended cooling.

Purpose: To evaluate the efficacy of antimicrobial blends containing dried vinegar (DV) and fruit and spice extracts [FSE, FSE-S (containing salt)] on *C. perfringens* in uncured ham compared to traditionally cured ham.

Methods: Batter for nine ham formulations (target 75% moisture, pH 6.3; 1.5 or 1.12% salt) were inoculated with a 3-strain mixture of *C. perfringens* spores, cooked to 75°C for 5 minutes, and cooled according to USDA Appendix B-Option 3. Formulations included 1.5% salt uncured control, 1.5% and 1.12% salt cured controls (100 mg/kg NO_2 and 547 mg/kg erythorbate), three uncured 1.5% salt formulations with 1% DV-FSE-A, 1% DV-FSE-A + 0.5% DV or 1.0% FSE-S-A + 0.5% FSE-S-C and three uncured 1.12% salt formulations with 0.6% DV-FSE-B + 0.5% DV, 0.6% DV-FSE-B + 0.75% DV or 0.6% FSE-S-B. Triplicate samples were assayed for populations by enumerating on tryptose-sulfite-cycloserine at 0, 2.5, 5, 7.5, 10, and 15 hours; experiments were replicated three times.

Results: After 15 hours, *C. perfringens* populations in the uncured control increased by >5-log CFU/g whereas populations in the cured controls remained unchanged regardless of salt concentration. Formulations targeting 1.5% salt and containing 1.0% DV-FSE-A or 1.0% FSE-S-A + 0.5% FSE-S-C supported an average 1.74 and 1.51 log increase, respectively, but no growth was observed using 1% DV-FSE-A + 0.5% DV. *C. perfringens* increased 2.80 log after 15 hours in 1.12% salt formulation containing 0.6% FSE-S-B; no growth was observed using 0.6% DV-FSE-B + 0.5% DV or 0.6% DV-FSE-B + 0.75% DV.

Significance: This study confirms that combinations of clean label antimicrobials in uncured ham show similar inhibition of *C. perfringens* compared to traditionally cured ham during extended cooling.

P3-216 Humidity Affects *Salmonella* Lethality and USDA FSIS Appendix A Compliance for Impingement-cooked Meat and Poultry Products

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❖ ***Developing Scientist Entrant***

Introduction: A recent revision of USDA FSIS Appendix A articulates humidity requirements for meat/poultry cooking processes. However, information/data documenting the impact of humidity on the microbial safety of impingement cooked meat and poultry is currently limited.

Purpose: This study aimed to quantify the impact of humidity on *Salmonella* lethality in impingement cooked meat and poultry products, particularly as related to Appendix A compliance.

Methods: Beef strips, ground beef patties, chicken breast filets, and breaded chicken patties were inoculated with an eight-serovar cocktail of *Salmonella*. Each product was cooked (in triplicate) in a pilot-scale, moist-air impingement oven at a specific temperature (218 or 232°C), absolute humidity (0.7, 30, or 70% v/v), and fan speed (20 or 80% power), to an endpoint center temperature of 70 or 73°C for beef and poultry products, respectively, then immediately cooled in liquid nitrogen, dissected to remove surface and core samples, and plated on differential media to enumerate *Salmonella* survivors. Sample surface/core temperatures and final moisture content also were measured.

Results: The addition of process humidity significantly ($P<0.05$) affected *Salmonella* lethality. For example, when processed with dry heat, the required 6.5-log reduction was not achieved for beef products at any condition. However, increasing the humidity to 30% resulted in greater than seven-log reduction for all beef strips. Additionally, greater *Salmonella* survival in samples from the surface was observed ($P< 0.05$) for multiple products cooked with dry air at the high fan speed.

Significance: Compliance with USDA FSIS Appendix A is critical for the meat and poultry industry to provide a safe product; however, the relationship between humidity and *Salmonella* survival includes complicated interactions between time, temperature, and air velocity. More research is necessary to confirm specific levels of humidity needed to achieve the required *Salmonella* lethality.

P3-217 The Effect of Recurring Cooling and Reheating on *Clostridium perfringens* Growth in Uncured Turkey and Cured Beef

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Introduction: Heat-resistant spores of *Clostridium perfringens* may germinate and multiply in cooked meat and poultry products when the rate and extent of cooling do not occur in a timely manner. There is a lack of information in the literature on the subsequent reheating/smoking and cooling of meat products that have already achieved lethality.

Purpose: The purpose of this study was to determine the survival and outgrowth of *C. perfringens* spores during the subsequent cooling and reheating of cooked cured beef and uncured turkey.

Methods: Raw cured beef (nitrite along with lactate and diacetate) and uncured turkey (1.5% salt with no preservatives) samples (n=4) were inoculated with *C. perfringens* spores (ATCC 112915, 12916, and 13124) to yield about two log CFU/g. Individual 25-g samples were vacuum packaged, and cooked in a water bath at 71°C for 15 min followed by a complete cooling to 7°C in 15 h. After one, two, and five days of storage at 4°C, individual sets of samples were re-heated to 49°C for 30 min and slowly cooled to 7°C in eight h. The specific temperature and time parameters were selected to resemble the worst-case scenarios that could happen in the processed meat industry. *C. perfringens* outgrowth was determined at the end of cooling cycles by plating on tryptose-sulfite-cycloserine agar with no supplementation.

Results: The recurring heating and cooling cycles followed in this study in cured beef demonstrated no significant *C. perfringens* growth (≤1 log) in compliance with the FSIS' Stabilization Compliance Guidance, Appendix B. In uncured turkey, the highest relative *C. perfringens* growth was ≤2 log after a second reheat and chill cycle, which is not a prevalent practice in the industry.

Significance: These data suggest that there is no potential *C. perfringens* growth concern for both cooked cured beef and uncured turkey going through a series of reheating/smoking and cooling cycles.

Poster

P3-218 The Effect of Pulsed Light Energy Delivery Mode on Inactivating *Salmonella* spp. in Vitro

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Introduction: Despite improved practices on the farm and in food processing facilities, *Salmonella* contamination is a big concern for the food industry and consumers. To mitigate this problem, effective methods for surface decontamination of raw chicken, meat or fruits and vegetables are continuously sought out. Pulsed light (PL) technology has shown great potential in inactivating bacteria on the surface of many foods, including raw chicken, without compromising their freshness, but the effectiveness of the treatment needs to be improved.

Purpose: The effect of energy delivery mode of PL inactivation of *Salmonella* on an agar model system for chicken breast was systematically investigated.

Methods: Four *Salmonella* serovars (*Salmonella* Typhimurium, Heidelberg, Enteretidis, and Minnesota) were used in this study due to their prevalence on chicken. Stationary phase inoculum of each strain was serially diluted, and each dilution spread onto tryptic soy agar for PL treatment. The inoculated agar plates were subjected to a combination of three voltage levels (2000 V, 2500 V, and 3000 V) and seven pulse durations (50 µs to 420 µs), with fluence ranging from 0.05 to 0.82 J/cm^2. The treated plates were incubated, and colonies enumerated. Log reduction was calculated for each condition compared to untreated controls. All experiments were conducted in triplicate, and results were analyzed statistically using ANOVA.

Results: Maximum reductions of *Salmonella Typhimurium* achieved after PL exposure to 2000 V, 2500 V, and 3000 V were 5.8, 6.5, and 7.1 log, respectively (average values). Similar results were obtained for the other three serovars. At a fixed PL fluence, pulses delivered in high-voltage-short-duration mode resulted in greater *Salmonella* inactivation than in low-voltage-long-duration mode (P<0.05).

Significance: The fact that PL delivery mode has a significant effect on *Salmonella* inactivation can be used for the development of PL treatments with higher effectiveness for the decontamination of a variety of foods, including raw chicken breast.

P3-219 Independent Performance Evaluation of VIDAS-Spt for the Detection of *Salmonella* spp. in Poultry Primary Production Samples

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Introduction: The disease burden due to *Salmonella* remains a persistent issue, and despite several interventions, poultry products remain as a major food source implicated in salmonellosis. It has been hypothesized that an increased understanding of the *Salmonella* prevalence at the primary production sites could help mitigate the overall *Salmonella* prevalence on the processed poultry products. VIDAS-based *Salmonella* assay (SPT) has been used for *Salmonella* detection in raw/processed poultry for many years. However, the candidate method has not been extensively evaluated as a method for *Salmonella* detection in poultry primary production samples (PPS).

Purpose: The purpose of this study was to independently evaluate the performance of the candidate method for the detection of *Salmonella* spp. in PPS from diverse sources.

Methods: Three independent labs evaluated n=270 PPS obtained from the turkey and broiler facilities against the buffered peptone water method (USDA-NPIP PS; §147.54 Jan 2017). For the candidate method, the PPS were enriched in buffered peptone water+supplement (1:10) at 42°C for 18 to 22 h, then 0.1 ml of this primary enrichment was transferred into SX2 and incubated at 4°C for 18 to 22 h . The enriched SX2 was processed on the candidate method . All presumptive results were confirmed with the buffered peptone water method and plating on ASAP media (biomerieux). The two by two contingency table analysis was performed to compute the sensitivity, specificity with 95% CI, and Cohen Kappa statistic.

Results: The candidate method sensitivity and specificity were 99% (true positive=93; true negative=175; false positive=1; false negative = 1). The positive predictive value, negative predictive value, and the overall accuracy of the candidate method were ~99% and the Cohen Kappa statistic was 0.98.

Significance: The candidate method provides highly sensitive and comparable results to the culture methods and a significant time advantage (~48 h) over the culture methods, and thereby presents a viable alternative for *Salmonella* detection in PPS.

P3-220 Detection of Multiple Serotypes of Salmonella on Pre-chilled Chicken Carcasses by Whole Carcass Rinse or Whole Carcass Incubation

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Introduction: Chicken carcasses and meat products may be contaminated with *Salmonella* and have been implicated in disease outbreaks. Generally, only one or two *Salmonella* colonies are selected and characterized for any one positive carcass; it is not clear how many serotypes of *Salmonella* may be present.

Purpose: The objective of this study was to compare the number of *Salmonella* serotypes detected from prechilled chicken carcasses sampled by standard carcass rinse to those sampled by whole carcass incubation.

Methods: Eight broiler carcasses, four from each of two flocks, were collected pre-chill at a commercial slaughter plant. Each carcass was subjected to a whole carcass rinse in 400 ml buffered peptone water, an aliquot was removed for incubation and the carcass was incubated in the remaining fluid. Plating was conducted on BG-sulfa, XLT4 and Hectoen Enteric agars before and after buffered peptone water preenrichment and after selective enrichment in RV, TT or RV to TT broths. Up to three typical *Salmonella* colonies were picked from each plate from each broth (potential 45 picks per sample) and characterized by serogrouping and smart PCR serotyping.

Results: Serotypes detected from 268 total picks included: *Salmonella* Kentucky, Typhimurium, Schwarzengrund, Enteritidis and Hadar. On average, significantly more (P<0.05) serotypes were detected by whole carcass enrichment than from rinse of the same carcass (three vs 1.9, respectively). *Salmonella* Schwarzengrund was the only serotype detected significantly more often from whole carcass enrichment than from rinse samples (seven vs two, respectively).

Significance: Sampling chicken by analysis of a carcass rinse aliquot may limit the diversity of serotypes detected. Tightly bound salmonellae may be more likely detected by incubation of the whole carcass.

Poster

P3-221 Survival of *Salmonella* Typhimurium and *Salmonella* Enteritidis after Treatment with Stress Conditions: Heating, Chilling, Salt and Freezing Temperatures

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Introduction: *Salmonella* spp. are a serious pathogen and are able to grow over a wide range of temperature, water activity (a_w) and pH. Poultry and poultry products are at high risk of *Salmonella* contamination. *Salmonella* spp. can be eliminated during food production. However if they survive the applied stress condition and if the laboratories have limited ability to detect injured cells, they may cause foodborne outbreaks and disease.

Purpose: To examine the fate of *Salmonella* spp. under conditions that occur during poultry processing and transportation.

Methods: A suspension of two *Salmonella strains* were treated with simulated poultry production conditions; heating, chilling, reduced a_w and freezing in duplicate. Samples were taken from freezer during three months of an experiment from October to December 2018. Viable *Salmonella* were counted on TSA and XLD. Data were plotted and lethality rates were analyzed by regression statistics.

Results: After heat and chill, the number of *Salmonella* Typhimurium and Enteritidis reduced rapidly by 3.17 log CFU/ml and 3.31 log CFU/ml, respectively. After adjusting a_w to 0.98 until 140 min of frozen, the number of *Salmonella* Typhimurium and Enteritidis increased to an estimated 0.5 log CFU/ml and started to decrease after 160 min of frozen storage. After 21 days of frozen storage, the two strains of *Salmonella* increased by 0.5 log CFU/ml again and then reduced slowly during 72 days of frozen storage to a level of 0.3 log CFU/ml of *Salmonella* Typhimurium and zero log CFU/ml of *Salmonella* Enteritidis. The level of injury differed during storage time, however, the trends of injury were slightly stable between 0.5 to 3 log CFU and 0.5 to two log CFU for *Salmonella* Typhimurium and *Salmonella* Enteritidis, respectively.

Significance: Laboratory and quality control department in poultry industries can use data from this study to improve and develop challenge tests for determination injured cell of *Salmonella* and reduce risk of *Salmonella* in finished products.

P3-222 The Effects of Feeding Original XPC on Reducing *Salmonella* Prevalence and Numbers in Ceca Samples and Carcass Rinses Taken from Commercial Broilers

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Introduction: *Salmonella* prevalence and load entering abbatoirs with broiler flocks challenge production system interventions and may be associated with increased food safety risk to end consumers. Preharvest technologies reducing *Salmonella* colonization and shedding load may contribute to multi-hurdle food safety risk mitigation.

Purpose: Evaluate qualitative and quantitative *Salmonella* reduction in ceca and carcass rinse samples of commercial broilers fed with or without Diamond V Original XPC (XPC).

Methods: Five commercial broiler farms from a single United States company were selected. Birds were fed either a control (standard commercial diet) or treatment diet (control diet with 1.25 kg/tonne XPC) from hatch to processing at 58 d. Control samples were taken prior to application of the treatment diet on the subsequent chick placement in the same houses. Sixty ceca and 30 re-hang carcass rinses per house were collected and shipped to an ISO 17025 accredited commercial laboratory. Cecal contents (one g) were prepared at 1:10 dilutions in buffered peptone water and rinsates according to USDA FSIS MLG 4.09. Samples were serially diluted, spread-plated to XLT4 agar and primary suspensions enriched following MLG 4.09. Presumptive *Salmonella* isolates were confirmed with Poly-O agglutination and enumeration plates counted. Data were analyzed in SAS using GLM with treatment as the main effect and farm as a random effect with significance at P<0.05.

Results: Ceca *Salmonella* prevalence in control birds (38.1%) were significantly (P<0.0001) higher than *Salmonella* from the ceca of birds fed XPC (7.3%). Of positive control fed birds' ceca samples, 29.0% were enumerable (2.9 log CFU/g) as compared to 9.1% of XPC fed birds' ceca samples (1.43 log CFU/g). Carcass rinse prevalence from control farms (69.3%) was significantly (P<0.0001) higher than XPC (27.3%). Similarly, 29.8% of control positives carcass rinses were enumerable (2.30 log CFU/g) compared to 2.44% of XPC samples (0.7 log CFU/g).

Significance: These data indicate including XPC in commercial broiler diets may support risk reduction strategies associated with *Salmonella* entering the abattoir.

P3-223 Control of *Salmonella* in Chicken Meat Using the Combination of a Commercial Bacteriophage and Plant-based Essential Oil Antimicrobial Compounds

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Introduction: Poultry and poultry products are among the most important vehicles for human *Salmonella* infections. The contamination of broiler carcasses with *Salmonella* during slaughter and subsequent processing poses significant concerns for consumers and the poultry industry.

Purpose: The objectives of this study were to evaluate the antimicrobial activity of 10 essential oil compounds and to determine the combined effects with a commercial bacteriophage (SALMONELEX) against *Salmonella* on chicken products.

Methods: The antimicrobial effect of essential oil compounds against *Salmonella* was evaluated using the standard micro-broth dilution method. The effect of bacteriophage, thymol and carvacrol or their combinations against *Salmonella* on the chicken breast was determined using the dipping method, followed by enumeration on XLD agar plates. The antimicrobial efficacy experiments in chicken included four replicates. The mean values were compared using the one-way ANOVA followed by Tukey's honest significant difference (HSD) tests (P<0.05). Statistical analysis was performed using SPSS.

Results: Thymol and carvacrol were the most active essential oil compounds tested, with the minimum inhibitory concentration (MIC) at 0.25 to one mg/ml and the minimum bactericidal concentration (MBC) in the range of 0.5 to one mg/ml. A multiplicity of infection (MOI), which refers to the bacteriophage/pathogen ratio (PFU/CFU), significantly affected the bactericidal effect of bacteriophage treatment in tryptic soy broth and in chicken products. The bacteriophage preparation decreased *Salmonella* Typhimurium 3001 by five log CFU/ml at low MOI (≥1.7) in tryptic soy broth, but a higher bacteriophage concentration was needed to exhibit a bactericidal effect in chicken products. The bacteriophage was more active against a single strain, *Salmonella* Typhimurium 3001 than a cocktail of three *Salmonella* strains in chicken products. Dipping of inoculated chicken in thymol and carvacrol emulsions at 1.6% (w/v) for three min inactivated 1.3 and 1.6 log CFU/g, respectively, of a cocktail of three *Salmonella* strains. The sequential treatments by dipping the inoculated chicken in bacteriophage (1.1×10^8 PFU/ml) for three min and then in thymol or carvacrol at 1.6% (w/v) for three min resulted in 1.9 to 2.0-log CFU/g reduction of the cocktail strains in chicken meat.

Significance: The combined treatments were significantly (P<0.05)better than the corresponding single bacteriophage or essential oil compound treatments.

Poster

P3-224 Food Safety and Inspection Service Nationwide Raw Pork Products Sampling Study

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Introduction: Consumption of undercooked pork products containing *Salmonella* has been implicated as the cause of outbreaks and recent reports suggest foodborne illnesses linked to consumption of pork containing Shiga toxin-producing *Escherichia coli* (STEC).

Purpose: The purpose of the Raw Pork Product Exploratory Sampling Study was to determine the National Prevalence of *Salmonella* and ascertain the presence of STEC in raw pork products.

Methods: In Phase I, 1200 samples (comminuted, non-intact, and intact cuts) were tested for *Salmonella* from slaughter and processing-only establishments in 2015; 200 of these samples were analyzed for the top seven STEC. Phase II occurred from June 2017 through May 2018; 4145 raw pork samples (comminuted, non-intact, and intact cuts) were collected from slaughter and processing-only establishments and tested for *Salmonella*. Only slaughter establishments were tested for STEC (1,476 samples). Samples were analyzed using FSIS Microbiology Laboratory Guidebook methods.

Results: In Phase I, 200 samples (16.7%) were positive for *Salmonella*. Of the 200 samples tested for STEC 10 positive samples resulted in 27 independent isolates. Seventeen of these isolates were recovered from processing establishments and 10 from slaughter establishments. From Phase II, the National Prevalence of *Salmonella* was 26.74% for comminuted, 10.03% for intact cuts, and 5.99% for non-intact cuts. Three STEC isolates were recovered. Comminuted pork samples contained the most *Salmonella* and STEC, suggesting that fabrication and processing augmented the levels of pathogens.

Significance: *Salmonella* associated with raw pork products is a public health concern. Data from this study will be used to develop standards or policies to reduce the levels of *Salmonella* in raw pork products. Further investigation is warranted to determine if STEC is an emerging public health concern in raw pork products.

P3-225 *Salmonella* and *Campylobacter* in Religious-exempt and Low-volume Poultry Products

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Introduction: In 2017, FSIS began two new exploratory sampling projects for religious exempt chicken carcasses (head, feet, and/or viscera intact; 0.4% of the poultry industry) and low-volume poultry products (average daily production less than 1,000 pounds; one percent of the poultry industry), which are currently exempt from routine sampling and performance standard categorization.

Purpose: This sampling program assesses the *Salmonella* and Campylobacter percent positive in religious exempt chicken carcasses and in low-volume poultry products, to evaluate if these products represent a significant public health risk.

Methods: From June 2017 through September 2018, FSIS collected and analyzed 89 religious exempt chicken carcass rinses from 25 establishments and 965 low-volume chicken parts rinses, 607 low-volume comminuted chicken products, and 226 low-volume comminuted turkey products from 522 establishments. *Salmonella* (MLG Chapter 4) and Campylobacter (MLG Chapter 41) percent positives and confidence intervals in religious exempt chicken carcasses and in low-volume poultry products were compared to percent positives for conventional chicken carcasses or poultry products in establishments that produce over 1,000 pounds per day (high-volume), respectively.

Results: Religious exempt chicken carcasses (*n*=89) had an average *Salmonella* percent positive of 30% (confidence interval 23 to 39%) and an average *Campylobacter* percent positive of 21% (confidence interval 15 to 30%) compared to five percent and two percent, respectively, for conventional chicken carcasses. Low-volume chicken parts and chicken and turkey comminuted products had *Salmonella* and *Campylobacter* percent positives comparable to or lower than their high-volume counterparts, with statistical differences noted in *Salmonella* percent positives for comminuted chicken ($P<0.001$) and comminuted turkey ($P<0.02$).

Significance: Like any poultry, poultry slaughtered under religious exemptions or in low-volume establishments may be contaminated with *Salmonella* or *Campylobacter*. This exploratory sampling program indicates a higher prevalence in some products, though the overall volume of such products in the food supply is low.

P3-226 Viability of *Listeria monocytogenes* and Shiga Toxin-producing *Escherichia coli* Cells on Slices of Commercially-produced Bresaola, a Dry-Cured Beef Product, during Extended Storage at 4° and 10°C

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Introduction: Despite an increasing demand across the U.S. for dry-cured, RTE meats, there is a lack of information about the safety of these products. In addition, surface contamination of these RTE products during packaging and/or slicing raises a serious public health concern, especially for products with an extended shelf life and/or that are consumed without further cooking/processing.

Purpose: Monitor viability of multi-strain cocktails of genetically-marked strains of *Listeria monocytogenes* and Shiga toxin-producing *Escherichia* (STEC) on bresaola, an Italian dry-cured beef product, during extended storage at 4° and 10°C.

Methods: Two slices (ca. eight g each) of commercially-sliced bresaola were layered horizontally into a nylon-polyethylene bag. The outer surface of each slice was inoculated (50 µl total; ca. three log CFU/package) with a five-strain cocktail of rifampicin-resistant (100 mg/ml) cells of *L. monocytogenes* or an eight-strain cocktail of rifampicin-resistant cells of STEC. Bags were vacuum-sealed and stored at 4 or 10°C for 150 or 90 days, respectively. In each of three trials, three bags were analyzed at each sampling interval. Pathogens were recovered from the surface of the meat via the USDA-ARS package rinse method.

Results: The average pH and water activity (a_w) of the product was pH 6.5 and a_w 0.9130, respectively. Bresaola did not support growth of *L. monocytogenes* or STEC during extended storage. More specifically, *L. monocytogenes* or STEC numbers decreased by 1.2 to 1.8 log CFU/package after 150 or 90 days when bresaola was stored at 4 or 10°C, respectively.

Significance: Bresaola does not provide a favorable environment for outgrowth of *L. monocytogenes* or STEC if present on the surface of bresaola as a result of inadequate processing and/or post-process contamination.

Poster

P3-227 Recovery of *Enterobacteriaceae* Indicator Organisms in Raw Poultry Rinse Testing Using Buffered Peptone Water and Neutralizing Buffered Peptone Water

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Introduction: For pathogen recovery from raw poultry carcasses in the presence of sanitizers, FSIS uses neutralizing buffered peptone water (nBPW) rather than buffered peptone water (BPW). The industry is slowly adopting nBPW in its New Poultry Inspection Study (NPIS) for pathogen and indicator tests.

Purpose: This study evaluated the influences of nBPW vs. BPW on recovery of *Enterobacteriaceae* indicator organisms from poultry carcasses using different test methods and sanitizer residuals.

Methods: Whole organic chickens (*n*=9) were extracted in 400 ml Butterfield's Phosphate buffer, then diluted in BPW and nBPW to a countable range. Each sample was exposed to FSIS approved sanitizers; 500 ppm peracetic acid (PAA), 1.5% acidified sodium chlorate solution (Ach), and control conditions. The samples were plated on Charm Peel Plate *Enterobacteriaceae* test (AOAC OMA 2018.05), 3M Petrifilm *Enterobacteriaceae* (AOAC OMA 2003.01), and VRBGA (ISO 21528-2). *Enterobacteriaceae* colonies were confirmed by isolation and oxidase negative, pH agar positive results. Paired statistical analysis was employed in data comparison.

Results: *Enterobacteriaceae* recovery using BPW and nBPW had no significant difference between OMA 2018.05 and ISO method with P=36% and P=24%, respectively, in control and PAA sanitizer. OMA 2003.01 had a -0.66 log difference (P=1%) in *Enterobacteriaceae* recovery under the same conditions. Bacteria recovered by all methods using nBPW were one-log greater than with BPW in Ach P<0.1%. Results between Peel Plate and ISO method with PAA sanitizer were not significantly different in either buffer or between methods, P=10%. Results showed 56% confirmation of *Enterobacteriaceae* in BPW compared to 70% confirmation in nBPW.

Significance: These limited data suggest nBPW is more effective than BPW in recovery and selection of *Enterobacteriaceae* indicators when sanitizers are present, depending on the method chosen. Data indicate that industry should consider using nBPW with an appropriately validated *Enterobacteriaceae* method, as improved recovery and selection may be more predictive of gram-negative pathogens.

P3-228 Fate of Spore-forming Pathogens in High and Reduced-moisture, Shelf-stable Processed Meat and Poultry Products Subjected to Post-packaging Pasteurization

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❖ ***Developing Scientist Entrant***

Introduction: Sporeforming bacteria can survive lethality schedules directed to destroy vegetative pathogens and rapid cooling is necessary to inhibit germination and growth in storage of low moisture processed meat and poultry products.

Purpose: This study was designed to assess the fate of sporeforming pathogens when subjected to post packaging pasteurization after lethality and stabilization schedules on high and reduced moisture, shelf-stable meat and poultry products, and to evaluate the effect of storage conditions, antimicrobial formulation and water activity during shelf-life.

Methods: Three strains of *C. perfringens* spores were used to inoculate high and reduced-moisture meat and poultry products (*n*=30 per treatment) before exposure to validated lethality processes. Different incremental stabilization schedules (time zero, 60, 120, 180 min) were evaluated to determine spore survival and germination rates with and without subsequent exposure to post-packaging pasteurization (six min, 77°C).

Results: Viability of germinated sporeformers was significantly decreased ($P<0.05$) when extended stabilization rates schedules were evaluated in high moisture products. On the other hand, post packaging pasteurization applied after short-term stabilization schedules had no effect on inactivation of spore survivors.

Significance: Extended stabilization schedules, that support spore germination after lethality exposure, may allow for significant inactivation of sporeforming pathogens by post-packaging pasteurization in reduced-moisture meat and poultry products.

P3-229 Detection of Chicken Vaccine Strain *Salmonella* Enteritidis 441/014 (ade-/his-) and Differentiation between *Salmonella* Field Strains and the Vaccine Strain

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Introduction: *Salmonella* can be present either in poultry meat or on eggs or the feathers of the animals. The detection of *Salmonella* in primary poultry production is relevant for control of poultry farms.

Purpose: For poultry production sites using live vaccines differentiation between the live vaccine strain and *Salmonella* field strains is of importance e.g., for newly vaccinated poultry.

Methods: Enrichment of chicken samples have been performed 18±2 h in buffered peptone water. DNA preparation has been performed with the foodproof StarPrep Three Kit. Real-time PCR was performed with the new four-channel real-time PCR assay.

Results: We detected the live vaccine strain *Salmonella* Enteritidis 441/014 (ade-/his-) in 66 different samples (channel 1), and we could differentiate between this strain and *Salmonella* field strains (channel 2). Samples containing the vaccine strain were positive in the vaccine channel and in the SE/STM channel (channel 3). In 59 samples with *Salmonella* Typhimurium (STM) we could differentiate between *Salmonella* Enteritidis and Typhimurium serotypes in a one-tube-reaction by melting curve analysis in the *Salmonella* Enteritidis and Typhimurium channel (channel 3). In addition, we use an internal positive amplification control in one channel (channel 4). We positively tested different cyclers.

Significance: BIOTECON Diagnostics developed a new multiplex Real-time PCR assay - vetproof SE Vaccine Detection 1 Kit - which enables specific detection of the live vaccine strain *Salmonella* Enteritidis 441/014 (ade-/his-) (e.g., present in Salmovac SE, Salmovac 440 und Gallivac SE), and can differentiate between this strain and *Salmonella* field strains plus additionally allows differentiation between *Salmonella* Enteritidis and Typhimurium serotypes in a one-tube-reaction. The vetproof SE Vaccine Detection 1 Kit is prefilled and lyophilized within individual strip tubes for direct loading into the real-time PCR instruments.

Poster

P3-230 Thermal Inactivation of *Salmonella, Campylobacter jejuni* and *Listeria monocytogenes* in Moisture Enhanced Non-intact Chicken Patties by Double Pan-broiling Under Dynamic Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Pathogens may translocate from the surface to internal tissue during non-intact chicken moisture enhancement processes. Limited information is available regarding the thermal inactivation of *Campylobacter* spp. in chicken products.

Purpose: This study aims to determine the thermal inactivation kinetic parameters of *Salmonella*, *Campylobacter jejuni* and *Listeria monocytogenes* in moisture enhanced reconstructed non-intact chicken patties double pan-broiled for various time periods.

Methods: Fresh 1.5-kg coarse-ground chicken breast, inoculated with *Salmonella* Typhimurium and Tennessee, *C. jejuni* (three-strain mixture), or *L. monocytogenes* (three-strain mixture), were mixed with NaCl (2.0%)+Na-tripolyphosphate (0.5%) solution to reach a 10% pump rate. Inoculated samples were manufactured (hamburger patty maker) into patties (2.1-cm thick and 10.4-cm diameter) followed by aerobic storing (air permeable plastic film, 4°C, 42 h) before double pan-broiling (Farber-waregriller, set at "Grill" 400°F) for up to 210 s. Temperatures were monitored with type-k thermocouples. Survivals of *Salmonella* and *L. monocytogenes* were analyzed on XLT-4 and MOX agar seeded with tryptic soy agar layers. *C. jejuni* were analyzed on *Brucella* agars under microaerophilic conditions. The pH values and cooking losses of chicken samples were also tested. Data (three replicates/three samples each) were analyzed using the Mixed Model of SAS and the USDA-Integrated-Predictive-Modeling-Program software.

Results: Double pan-broiling reduced counts of *Salmonella* (6.5 log CFU/g), *C. jejuni* (5.1 log CFU/g), and *L. monocytogenes* (6.3 log-CFU/g) ($P<0.05$) from the original to <0.3 log-CFU/g after 180, 210, and 210 s. Linear-model only fits survival curves of *C. jejuni* with calculated *D*-value of 45.63 s. According to the Weibull-model, the *D*-value of *C. jejuni* (98.67 s) was lower ($P<0.05$) than those of *L. monocytogenes* (111.6 s) and *Salmonella* (136.1 s). Buchanan-Two-Phase-model calculated "shoulder" time increased from 85.67 s (*C. jejuni*) to 113.18 s (*L. monocytogenes*) and 115.5 s (*Salmonella*). Cooking losses increased from 0.37 to 4.37% and pH values increased from 5.56 to 5.90 after 210 s.

Significance: These findings will be useful by USDA-FSIS to develop risk assessments of foodborne pathogens in chicken products.

P3-231 Systematic Review and Meta-Analysis on the Effects of Processing Stages and Interventions to Control *Campylobacter* Contamination in Broiler Chickens

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Introduction: A considerable number of primary research studies are documented in the literature on the effect of different processing stages and interventions to control pathogen contamination. However, individual studies often fail to address the overall effect and variation among different set of conditions. Systematic review and meta-analysis approach is a useful tool to combine and evaluate data from multiple sources resulting in a summarized outcome while minimizing selection bias and evaluating quality of the evidence.

Purpose: The purpose of this study is to quantify and compare the efficacy of multiple processing stages and mitigation strategies that can be implemented throughout the processing chain to control *Campylobacter* spp. in broiler chickens.

Methods: Six electronic databases were searched with specific syntax in order to capture relevant publications. Retrieved records were deduplicated, screened for relevancy by titles, abstracts and full texts. Data were then extracted from relevant studies, including prevalence, concentration, and sample size and study characteristics. Meta-analyses were conducted to summarize data from individual studies into the overall change in prevalence (quantified as odds ratio) or concentration (quantified as log reduction) for each stage or intervention. Quality of evidence was assessed by following GRADE guidelines.

Results: Information on 10 different processing stages (from scalding to storage) and 13 interventions (chemical and physical decontamination methods) were collected from 86 articles. Among the processing stages, defeathering and evisceration were identified to increase prevalence and concentration while other stages estimated to decrease prevalence, concentration or both. Almost all of the interventions are estimated to be effective in reducing contamination.

Significance: This study identifies data gaps and reporting flaws in current literature on interventions controlling *Campylobacter* in broilers. In addition, results of this study provide data for a quantitative microbial risk assessment model in a farm-to-fork continuum addressing campylobacteriosis in human due to broiler chicken consumption.

P3-232 Effect of Ozonated Water on the Microbiological Profile of Chicken Parts

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Introduction: Poultry meat represents an important part of the United States economy and diet. However, it remains one of the food categories responsible for the most outbreak-associated foodborne illness cases. Ozone treatment has become an attractive decontamination option for food products, including poultry, due to its potential antimicrobial properties and minimal effects on quality.

Purpose: To evaluate the effectiveness of ozonated water for the reduction of microbial indicators in chicken parts.

Methods: Skinless chicken thighs (average 206.5±33.7 g) were immersed in ozonated water (1.33±0.12 ppm) for different exposure times (five, 10, 15, and 20 min). Aerobic plate counts (APC) and coliform counts (EC) were determined on Petrifilm plates for rinsates (100 ml/sample) of treated and untreated samples. Treatments were repeated for skin-on chicken thighs (average 225.3±33.7 g) in ozonated water (1.25±0.23 ppm). Experiments were performed in triplicate (one thigh per treatment per replicate).

Results: APC of treated skinless chicken thighs ranged from 2.30±0.13 to 2.76±0.49 log CFU/ml rinsate. EC ranged from 1.82±0.06 to 2.40±0.49 log CFU/ml rinsate. APC and EC of untreated samples were 2.66±0.72 and 1.88±0.40 log CFU/ml rinsate respectively. For treated skin-on chicken thighs, APC ranged from 3.33±0.92 to 4.08±1.22 log CFU/ml rinsate, with counts of 3.57±0.86 log CFU/ml rinsate in untreated samples. No significant reductions in counts ($P>0.05$) were observed for any of the treatments.

Significance: The data suggest that an ozonated water treatment at 1.30 ppm for zero to 20 min is not effective in controlling native microflora in chicken parts. Studies are underway to determine the effect of higher ozone concentration on the microbial profile of chicken parts and the potential application to control *Salmonella* and *Campylobacter* in poultry.

Poster

P3-233 Inactivation of *Listeria monocytogenes* in Model Chilling Brines for Hard Cooked Eggs

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Introduction: Hard-cooked, deshelled, ready-to-eat eggs are commonly chilled using brine chill systems. This system can become contaminated with *Listeria monocytogenes* and serve as potential harborage source. Moreover, the egg residues collected in the recirculating brine may create a microenvironment supporting the survival and growth of *L. monocytogenes*.

Purpose: Evaluate the survival of *L. monocytogenes* in model test brines and a 30-day-old recirculated/reused plant spent brine.

Methods: Sixteen test brines were formulated at four levels each (four by four) at target pH values of 3.4, 3.2, 3.0, 2.8 (adjusted with citric acid) and hydrogen peroxide (800, 1000, 1200, 1400 ppm). A 30-day old plant spent brine (pH 2.3, 1500 ppm) was also evaluated. All contained 2000 ppm potassium sorbate. Finely crushed eggs (approx. two g per 100 ml) were also added to the test brines to mimic the reused recirculating brines. Brines were inoculated with a five-strain cocktail of acid and cold stress-adapted *L. monocytogenes* to deliver approximately six log CFU/ml, held at 35°F, and enumerated for surviving *L. monocytogenes* populations at one min, 60 min, and five, 24, 48, and 120 h. The experiment was replicated twice.

Results: *Listeria monocytogenes* populations remained unchanged at one and 60 min of exposure in test brine and spent brine. At five h, test brines and spent brine exhibited less than one log and 2.7-log reductions, respectively. By 24 hours, ≥4-log reduction (reached a minimum detection limit of one log CFU/ml by plating) were observed in all brines. At 120 h, viable *L. monocytogenes* was not detected in all samples (one ml enriched and tested) exhibiting ≥5-log reductions.

Significance: Inactivation of *Listeria monocytogenes* was not observed in brines until ≥5 hours suggesting requirements for strict GMPs, environmental *Listeria* monitoring, and/or a post-lethality treatment. A ≥4-log reduction by 24 h supports practices for the end-of-run brine storage and next day reuse as long as the brine is maintained within formulation parameters.

P3-234 Optimizing Ozone Use in a Heat-Ozone Combined Treatment Designed to Inactivate *Salmonella* Enteritidis Grown in the Yolk of Shell Eggs

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❖ ***Developing Scientist Entrant***

Introduction: Salmonellosis outbreaks, attributed to shell eggs, have been a great risk to public health in the United States. Recent studies introduced ozone use (at 10 to 11% level) in combination with mild heat, to inactivate *Salmonella* Enteritidis in shell eggs while attempting to maintain the quality and functionality of fresh eggs. Currently, research is needed to optimize this method for maximizing product quality.

Purpose: This study was conducted to decrease ozone concentration during shell egg processing by heat-ozone treatment, while maximizing the inactivation of *Salmonella* Enteritidis in the yolk.

Methods:: *Salmonella* Enteritidis, pre-adapted to egg yolk, was inoculated into the yolk of shell eggs by using a calibrated syringe. After 48 h incubation at 30°C, the eggs were cooled and subjected to heat-ozone processing. The selected process involved submerging shell-eggs in a 57°C water bath for 28 min, cooling, and treating heated eggs with ozone at five, six, seven, and eight percent for 25 min at 10 psig. The changes in *Salmonella* populations were measured by spread-plating onto a non-selective medium. Experiment was repeated independently three times and significance of ozone treatment on *Salmonella* inactivation (log-reduction) was determined by ANOVA and Tukey HSD.

Results: Ozone treatments significantly affected the log reduction of *Salmonella* in the yolk of shell eggs ($P=0.03$). Treatments producing five, six, seven, and eight percent ozone reduced *Salmonella* populations in shell egg yolk by 6.5±0.47, 7.1±0.05, 6.5±0.51, and 5.6±0.41 log CFU/ml of egg contents, respectively. Reduction in *Salmonella* population was significantly higher at six than at eight percent ozone. However, these results should be cautiously interpreted considering that the adjustment of ozone concentration may change other processing parameters such as treatment come-up time.

Significance: Results showed the possibility of decreasing the severity of ozone usage during the heat-ozone combined treatment, yet attaining high level of inactivation of *Salmonella* in the shell egg yolk.

P3-235 Effect of Percent NaCl and Incubation Temperature on the Growth of *Salmonella* and Background Flora in Raw Chicken Samples from Thailand

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Introduction: Poultry is a significant source of salmonellosis. Buffered peptone water (BPW) is the most commonly used primary enrichment medium for *Salmonella* testing. As BPW is non-selective allowing growth of both *Salmonella* and competitive background flora (BF), better approaches are needed. This study examined the effect of increasing NaCl levels and temperature on *Salmonella* growth and suppression of BF in raw chicken.

Purpose: To study the effect of percent NaCl and incubation temperature on *Salmonella* growth and BF suppression in raw chicken.

Methods: Stage 1: To identify conditions for further study, 60 blank BPW and 12 raw chicken samples were supplemented with zero, one, five, seven, 10 or 12% NaCl, spiked with *Salmonella* Enteritidis and incubated at 37 or 41.5°C for 20 h. BF was enumerated using 3M Petrifilm Aerobic Count (AC) plates and *Salmonella* was detected using a modified ISO method.

Stage 2: 80 raw chicken samples spiked with *Salmonella* Enteritidis or *Salmonella* Typhimurium were enriched in BPW with zero or five pecent NaCl at 37 or 41.5°C for 20 h. BF was enumerated using 3M Petrifilm AC plates and *Salmonella* was detected using a modified ISO method.

Results: Stage 1 showed that one percent NaCl had no effect on BF or *Salmonella* regardless of temperature. Five percent NaCl at 41.5°C showed better BF suppression than at 37°C. seven percent or greater NaCl completely suppressed all growth**.**

Stage 2 showed that 100% of samples with no NaCl were positive at both temperatures for *Salmonella*. Regardless of temperature, at five percent NaCl 100% of samples spiked with *Salmonella* Enteritidis were positive while only 10% of samples spiked with *Salmonella* Typhimurium were positive.

Significance: This work shows that a combination of increased NaCl and incubation temperature is promising for suppression of BF in raw chicken. However, due to different sensitivities of *Salmonella* species, additional studies are needed.

Poster

P3-236 Inactivation of Several Fruit Spoilage Molds Using Visible Light Emitting Diodes

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Introduction: Spoilage molds are an important causative agent for fruit spoilage, especially because of their ability to grow in a variety of conditions. Existing preservation technologies such as refrigeration cannot either kill molds or suppress their growth.

Purpose: The purpose of this study was to develop a visible light emitting diode (LED) technology that could kill fruit spoilage molds, study the factors influencing its efficacy and determine its mechanism of action.

Methods: Seven mold strains that have been implicated in fruit spoilage – *Alterneria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium moniliforme*, *Penicillium expansum*, *Rhizopus stolonifer* and *Stemphyllium botryosum* were illuminated with a range of light emitting diodes with emission spectra in the blue region (400 to 470 nm) of the electromagnetic spectrum. Using a combination of plating and imaging, the most potent wavelength was selected for time-kill assays. The effect of temperature (seven, 16 and 25°C) and relative humidity (RH; 40, 60 and 80%) was studied on *B. cinerea* and *R. stolonifer* using a factorial design. Determination of the endogenous photosensitizers inside the mold cells was performed using high performance liquid chromatography (HPLC).

Results: The most potent wavelength was observed to be 405 nm. Illumination with 405 nm LEDs brought about inactivation of 90 to 99% (n=3) in *A. niger*, *B. cinerea*, *F. moniliforme*, *R. stolonifer* and *S. botryosum*. No significant reductions (P>0.05) were observed in *A. alternata*. Temperature and RH both influenced the antifungal effect of the LEDs, with a higher temperature (25°C) and a higher RH (60 and 80%) producing a faster antifungal effect. HPLC analysis revealed the presence of porphyrins in the mold strains.

Significance: These results demonstrate that the use of 405 nm LEDs might be a promising preservation technology against fruit spoilage molds and could help to reduce their wastage during storage.

P3-237 Efficacy of Different Disinfectants against Isolated and Biofilm Associated Yeasts from a Fruit Juice Production Facility

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Introduction: In the fruit juice production industry, disinfectants are routinely used to disinfect product contact surfaces. Disinfectants provide a necessary step to ensure that the juices produced and consumed are as free as possible from microorganisms that can cause foodborne illness and spoilage. Selection of a suitable type of disinfectant and correct dosage are therefore very important to ensure proper disinfection.

Purpose: Investigate the efficacy of different disinfectants to inhibit yeast growth on laboratory scale as well as the influence of selected disinfectants on biofilm associated yeast diversity in an industrial scale trial.

Methods: Minimum inhibitory concentration (MIC) broth dilution was performed in triplicate to determine the efficacy of eight disinfectants with different active ingredients (paracetic acid, quaternary ammonium compounds, iodine, chlorine, dimethyl ammonium chloride and essential oils) to inhibit growth of 20 different yeast species isolated from the production environment. Two disinfectants, quaternary ammonium compounds (QAC's), were then subjected to facility trial for six months. Four sampling events took place in which swab samples of the nozzles in each filler line were used for yeast enumeration and cultureable diversity assessed with denaturing gradient gel electrophoresis (DGGE).

Results: The MIC results yielded only two disinfectants which displayed growth inhibition of all 20 yeast species at a maximum concentration of 0.1%, both with didecyl dimethyl ammonium chloride ($DDAC_2$) as active ingredient. During the facility trial one disinfectant was able to reduce the yeast population in the filler nozzles by 94.5% within three months. Culturable yeast diversity data indicated that $DDAC_2$ effectively inhibited *Candida sojae*, *Pichia occidentalis, Rhodotorula dairenensis, Sporidiobolus* spp. and *Rhynchogastrema noutii* in a biofilm associated environment. *Lodderomyces elongisporus* and *Kazachstania exigua* were, however, not inhibited and remained prominent.

Significance: The industrial trial demonstrated a QAC disinfectant rather than an acid based product yielded better disinfection results in this fruit juice producing facility. Furthermore, the persistent presence and potential tolerance or resistance of *L. elongisporus* and *K. exigua* requires further investigation.

P3-238 Bioaerosols in a Fruit Juice Manufacturing Facility – Harmful, Harmless or Perhaps Helpful?

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Introduction: Bioaerosols are defined as aerosols comprising of particles of biological origin or activity. Bioaerosols and their components could pose an environmental hazard when presented in high concentrations in indoor environments, resulting in spoilage/contamination of food products or occupational health risks. It is difficult to determine a set standard of acceptable limits in a specific industry, especially since very little information is available regarding different types of bioaerosols and their effects.

Purpose: Determine the culturable bioaerosol diversity in a food juice manufacturing facility and possible effects associated with these bioaerosols on the product and food handlers.

Methods: Air samples were collected by active sampling on selective and non-selective agar during two sampling seasons in five different sites in the processing section. The effect of physical parameters on the bioaerosol count was also determined. Purified isolates from were identified using 16S and 18S rRNA gene sequencing.

Results: The culturable bacterial aerosol concentration were generally high (>300 CFU/m^3) and exceeded that of yeast and mould. A total of 239 bacteria, 41 yeasts and 43 moulds were isolated from the air in the production environment. Eighty-six different species were identified and classified as i) harmful (45%), represented by the genera *Staphylococcus*, *Pseudomonas* and to a lesser extent *Aerococcus*, *Acinetobacter*, *Penicillium*, *Candida*, *Cryptococcus* and *Rhizopus;* ii) harmless (32%), with species from two dominant genera, *Bacillus* and *Staphylococcus;* and iii) helpful microbes (23%), with various potential applications (medical and environmental).

Significance: The presence of a diverse group of harmful microbes able to cause disease or spoilage highlights the need to improve bioaerosol control in this facility. Although harmless and helpful bioaerosols do not negatively influence human health, it is critical that their presence still serve as indicator that an ideal environment is present for possible harmful bioaerosols to emerge.

Poster

P3-239 Inactivation of *Alicyclobacillus acidoterrestris* Spores in Different Types of Juices by 222-Nanometer Krypton-Chlorine Excilamp Irradiation and Identification Sporicidal Mechanism

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Introduction: ***Alicyclobacillus acidoterrestris*** is a thermoacidophilic, spore-forming bacterium that causes spoilage of juice products. It is difficult to inactivate *A. acidoterrestris* spores in juice products using thermal pasteurization without quality loss. Therefore, alternative non-thermal techniques are needed to control the remaining spores.

Purpose: The purpose of this study was to examine the efficiency of a 222-nm krypton-chlorine (KrCl) excilamp for inactivation of *A. acidoterrestris* spores in different types of juices and to identify the sporicidal mechanisms.

Methods: *A. acidoterrestris* ATCC 49025 spores were inoculated into different types of juices (apple, grape, tomato) and treated with a 222-nm KrCl excilamp ranging from 100 to 1500 mJ/cm^2. To analyze the factors influencing inactivation of spores, experiments were performed at different value of pH, sugar concentration, UV absorption coefficient, and turbidity. To identify the sporicidal mechanisms, the fluorescent dye propidium iodine (PI) or diphenyl-1-pyrenylphosphine (DPPP) was used to quantitatively assess the change of cell membrane permeability or the incidence of peroxidation, respectively. Also, CM-H_2DCFD was used to measure intracellular total reactive oxygen species (ROS).

Results: As irradiation dose increased, UVC irradiation caused significantly (P<0.05) more inactivation of *A. acidoterrestris* spores in juice samples. There were no significant (P>0.05) differences of inactivation levels between the phosphate-buffered saline adjusting different value of pH, sugar concentration. However, it was concluded that UV absorption coefficient and turbidity were inversely proportional to the inactivation levels effect of 222-nm KrCl excilamp. Both the loss of membrane integrity measured by PI and lipid peroxidation in the cell membrane measured by DPPP increased as irradiation dose increased. Additionally, ROS was generated, but there were no significant (P >0.05) differences.

Significance: These results provide valuable baseline data for inactivation of *A. acidoterrestris* spores by 222-nm KrCl excilamp in the juice industry.

P3-240 WITHDRAWN

P3-241 Food Safety Knowledge, Attitudes and Practices of Street Food Vendors in Thailand

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Introduction: Street foods are largely appreciated for their flavors, convenience, low cost and their cultural and social heritage link. Due to a lack of basic infrastructure such as water connections and refrigeration, the sanitary quality at these venues may be compromised. As a result, street-vended food has the potential to become contaminated, thus exposing people who consume this food to the potential risk of food borne diseases.

Purpose: To establish a model for improvement of the food safety vended in Thailand.

Methods: Several selected street food vendors in many provinces in Thailand were interviewed, and the vendors were educated and trained in food safety knowledge. For one selected province, the team collected raw materials and foods at the point of sales for testing total plate count, coliforms, *Escherichia coli*, *Staphylococcus aureus,* lactic acid bacteria, yeast and mold with 3M Petrifilm products and *Salmonella* and *L. monocytogenes* with 3M molecular detection system. Conventional methods were used for testing *Vibrio cholerae and V. parahaemolyticus*. The results were shared with the vendors to initiate the awareness. On the job training (OJT) was conducted at cooking and selling sites. The effectiveness of the OJT was monitored by sampling and detecting microorganisms as above.

Results: Twenty-seven raw materials and 35 food samples were tested initially. Twenty-one raw materials and 15 food samples exceeded Thai governmental standard. After the education and OJT including several friendly site visits, the vendors in many provinces became familiar with food safety practices and maintained the safe food handling. The final evaluation showed that most selected vendors improved their food safety behavior. Microbiological results of the selected province also showed that most of the foods complied to the standard.

Significance: Street food vendors need appropriate education and OJT including friendly advice to adopt food safety behaviors to provide safe food to customers.

P3-242 Validation of the Use of Acetic Acid Incorporated with Chitosan to Prolong Shelf Life of Grass-fed Ground Beef

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✦ ***Undergraduate Student Award Entrant***

Introduction: The food industry loses billions daily due to the loss of food products from spoilage organisms and pathogenic bacteria. The industry is looking for natural products that will inhibit microbes and extend the shelf life of meat products.

Purpose: The objective of the study was to determine the effect of acetic acid combined with chitosan on shelf life of grass-fed ground beef.

Methods: Various concentrations of acetic acid/chitosan mixture (zero, 1.7, 3.3, 5.0, 6.7, and 10%) were added to grass-fed beef patties (30 g) to test the response of the background microflora of beef that had reached its sell by date. The beef patties were stored at 4°C for 12 d and tested in triplicate intermittently (d zero, four, eight, and 12) for microbial analysis.

Results: In a dose-dependent manner, the growth of background microflora was inhibited by the acetic acid/chitosan mixture. The control had at least one log of growth throughout the 12 days reaching over six log CFU/g while none of the treated samples reached six log CFU/g. By the end of the experiment, both the 3.3 and 6.7% samples had a lower microbial load than the pretreatment value (4.70 log CFU/g) with 4.30 CFU/g, which was significantly lower than the control's value of 5.30 log CFU/g (P<0.05).

Significance: The results show that the use of acetic acid combined with chitosan has the ability to extend shelf life of grass-fed ground beef without adversely impacting product quality.

Poster

P3-243 Evaluation of the Microbiological Quality of Minced Pork Using Visible and Fluorescence Spectroscopy Methods in Tandem with Multivariate Analysis

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Introduction: Spectroscopic methods coupled with multivariate data analysis have attracted considerable research interest in the last decade as alternative (to microbiological analyses) means of assessing the microbiological status and estimating the spoilage of various perishable foods.

Purpose: The objective of this study was the evaluation of the potential of visible (VIS) and fluorescence (FLUO) spectroscopy-based methods, in tandem with appropriate algorithms, for the quantitative estimation of the microbiological quality of minced pork.

Methods: Minced pork patties were stored under modified atmosphere packaging at isothermal (four, eight and 12°C) conditions. At regular time intervals during storage, duplicate samples were subjected to i) microbiological analyses for the determination of the total viable counts (TVC), and ii) acquisition of VIS and FLUO spectra using a UV-VIS spectrometer. Two independent experimental replicates (different meat batches) were conducted (n=4). The VIS and FLUO data were treated as two distinct datasets, and partial least squares regression (PLSR) models were calibrated with the collected spectral and microbiological data constituting the independent and dependent variables, respectively. Model validation was performed using independent data corresponding to minced meat samples stored at dynamic temperature conditions (in the range of 4 to 12°C).

Results: Both of the developed PLSR models exhibited a good performance when externally validated. The slope parameter of the linear regression between predicted and observed TVC was 0.77 and 0.89 for VIS and FLUO, respectively. The estimated values of the coefficient of determination (r^2) for the VIS and FLUO model were 0.64 and 0.78, respectively, whereas the root mean square error was in both cases low with its corresponding values being 0.66 and 0.51 log CFU/g.

Significance: The investigated spectroscopic methods seem promising for the rapid and non-invasive evaluation of the microbiological quality of minced pork.

P3-244 Application of Fluorescence Spectroscopy as a Tool for Microbial Spoilage Assessment in Fresh-cut Pineapple

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Introduction: Pineapple is one of the most popular tropical fruits, commonly found in the market in the form of ready-to-eat (RTE) products. Fresh-cut commodities frequently represent a paradox, with the applied processing procedures tending to decrease shelf-life and compromise their microbiological quality and safety.

Purpose: The objective of this study was to monitor the microbiological quality of fresh-cut RTE pineapple during storage at different temperatures using fluorescence (FLUO) spectroscopy.

Methods: Trays of fresh-cut pineapple were stored aerobically under isothermal (four, eight, and 12°C) and dynamic temperature conditions for a maximum time period of 10 d. At regular time intervals, duplicate samples (originating from different packages) were analyzed for the determination of the populations of yeasts (specific spoilage organisms of this food commodity) using conventional microbiological approaches, in parallel with FLUO spectroscopy measurements. Three independent experimental replicates were conducted. Partial least squares regression (PLSR) was applied for establishing the correlation between spectral data and microbial counts. Due to high variability among replicate samples, the average values (spectral data and microbial counts) of the duplicate samples were used for model development. The data collected at isothermal (117 samples) and dynamic temperature (42 samples) conditions were used for model calibration and validation (prediction), respectively.

Results: The developed PLSR model exhibited an overall satisfactory performance when externally validated. The predicted and actually observed populations of yeasts were well correlated, with the slope parameter of the linear regression and the correlation coefficient being 0.77 and 0.83, respectively. The coefficient of determination (r^2) was 0.67, whereas the root mean square error of prediction was estimated to be low, namely 0.45 log CFU/g.

Significance: FLUO spectroscopy may constitute a suitable analytical technique for the rapid and non-invasive assessment of the microbiological quality of fresh-cut pineapple.

P3-245 Comparison of Six Methods for Quantification of Lactic Acid Bacteria in Spoiled Sliced Turkey

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Introduction: Lactic acid bacteria are known to cause spoilage in many food products. Different quantification methods of lactic acid bacteria are used in food industry, and the recovery can vary in different food matrices. A study was conducted to compare methods for quantifying lactic acid bacteria in ready-to-eat, spoiled sliced turkey.

Purpose: To compare six methods for quantifying lactic acid bacteria in spoiled sliced turkey to optimize recovery and enumeration.

Methods: Six quantification methods for lactic acid bacteria were utilized to analyze twelve sliced turkey samples. The methods used for analysis were MRS with APT overlay (CMMEF 5TH Edition, Chapter 19.521), Acidified MRS (CMMEF 5TH Edition, Chapter 19.522), APT with sucrose and 0.2% bromocresol purple (CMMEF 5TH Edition, Chapter 19.528), 3M LAB Petrifilm (AOAC Performance Tested Method, Certificate #041701), 3M APC Petrifilm (AOAC 990.12) and 3M APC Petrifilm with MRS broth as diluents (CMMEF 5TH Edition, Chapter 19.571; 3M Insert). The total weight of each sliced turkey sample was homogenized with an equal amount of 0.1% peptone buffer (1:1), and a subsequent dilution (1:5) was prepared to make a 1:10 dilution. Ten fold serial dilutions were prepared with 0.1% peptone, except for one method utilizing MRS broth as dilutions (3M APC Petrifilm with MRS broth, CMMEF 19.571). Each dilution was plated according to the six methods (five methods with peptone diluent and one method with MRS broth as the diluent). The plates and Petrifilm were incubated and read according to each method reference. Confirmation (Gram stain and catalase) and identification (ID by VITEK MS) was performed on the colonies recovered from each sample for each method. Additionally, five sliced turkey samples were analyzed by APT with sucrose and 1.6% bromocresol purple and 3M LAB Petrifilm following the same procedure above.

Results: Lactic acid bacteria was recovered at high levels when using 3M LAB Petrifilm for 11 out of 12 samples analyzed. The remaining five methods showed variable counts. Additional analysis showed that lactic acid bacteria was recovered at high levels when using APT with sucrose and 1.6% bromocresol purple and 3M LAB Petrifilm for all five samples.

Significance: Based on the results of this study, 3M LAB Petrifilm and APT with sucrose and 1.6% bromocresol purple are preferred methods when quantifying lactic acid bacteria in RTE meats.

P3-246 Microbial Profiling of Subprimals Before and After Water Spray and Dry Chilling of Beef Carcasses Subjected to Hot Water Rinses during Long-term Storage

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Introduction: As the world meat market moves to never-frozen alternatives, meat processors seek opportunities for increasing the shelf life of fresh meats by combinations of barrier technologies and non-chemical interventions.

Purpose: To determine the impact of spray- and dry-chilling combined with hot water carcass treatments on indicator organisms in the long-term shelf life of beef cuts.

Methods: Beef carcass treatments were arranged in a completely randomized design with factorial arrangement on three different factors (feed, hot water wash and chilling method) at two levels (grass vs. grain, washed vs. not washed, dry vs. spray chilled). Samples were taken using EZ-Reach^T sponge samplers with 25 ml buffered peptone water over a 100 cm^2 area on the striploin. Sample collection was conducted before hot carcass wash, after hot carcass wash, and after 24 h carcass chilling. Chilled striploins were cut in four sections and individually vacuum packaged and sampled at zero, 45, 70 and 135 days (n=200). Aerobic plate counts, *Enterobacteriaceae*, *Escherichia coli*, coliforms and psychrotroph counts were determined.

Results: Not enough evidence (P>0.05) was found indicating the hot water wash intervention reduced bacterial concentration on the carcass surface. Generic *Escherichia coli* was below detection limits (<10 CFU/ml) in all the samples taken. No significant difference (P>0.05) was found between coliform counts throughout the sampling dates. Feed type did not seem to influence (P>0.05) microbial load of the treatments. Even though no immediate effect is seen from spray or dry chilling at d zero, as product aged, we could observe significantly lower (P<0.05) concentration of aerobic and psychrotrophic organisms in dry chilled samples.

Significance: Data collected can be used to select chilling systems to maximize shelf life. Hot water wash prior to carcass chilling may not significantly reduce indicator organisms. Understanding the best parameters for beef carcass processing will allow the beef industry to select optimized chilling processes for long-term storage.

P3-247 Impact of Carcass Spray-Chilling, Dry Chilling and Hot Water Washes on the Shelf Life and Microbial Profiles of Beef Ribeye Rolls

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Introduction: Microbial interventions such as a hot water washes and various chilling techniques have been utilized for bacterial reduction on beef carcass surfaces. Extended aging of spray-chilled beef sub-primal cuts has been shown to reduce shelf-life when compared to dry-chilled counterparts.

Purpose: This in-plant study was performed to evaluate bacterial indicator loads during extended aging of Australian beef ribeye rolls collected from carcasses subjected to dry and spray-chilling conditions.

Methods: Ribeye rolls of Australia beef carcasses (n=120) were sampled with a sterile, prehydrated sponge on a 100 cm^2 area. Four treatments were evaluated in this study: hot water wash (85°C), no wash, spray chilling, and dry chilling. Each treatment was evaluated on individually packaged cuts obtained from ribeye rolls sampled on d zero, 35, 45, 55, and 65 of refrigerated storage. The study was conducted in triplicate. Swabs were evaluated for total plate counts, psychotrophic plate counts, *Escherichia coli* and coliform counts, *Enterobacteriaceae*, and lactic acid bacteria levels. Colony counts were converted to log CFU/100 cm^2 and statistically analyzed within sampling day to determine significant differences at P≤0.05.

Results: On d zero, no significant differences were observed between treatments and all indicator organisms were below detectable limits. Though no immediate effect of the chilling type was observed on day 0; dry chilled ribeye rolls showed significantly lower total plate counts and psychotropic levels *(P≤0.05)* over 65 d of refrigerated storage.

Significance: Dry-chilling of beef carcasses increases the shelf life of ribeye rolls over extended refrigerated storage under vacuum as compared to spray-chilling counterparts

P3-248 WITHDRAWN

P3-249 Predictive Microbiology Analysis of Dairy Products Stored in Home Refrigerators

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Introduction: At present, government performance tests for home refrigerators focus on assessing energy consumption. Procedures testing the impact on food freshness and safety of refrigerator temperature setting, refrigerator temperature recovery, ambient temperature, door openings, and other factors affecting product temperature need to be developed, validated and harmonized with existing regulations for energy consumption.

Purpose: Microbial growth and other product degradations must be considered when minimizing energy consumption of residential refrigerators.

Methods: While mathematical models are available, much of past research has focused on temperature abuse and thus published model parameters are not in the temperature range of properly-operated refrigerators limiting this work to products for which model parameters were available. This presentation will highlight a predictive microbiology analysis of milk and cheese temperature as affected by room temperature ($T_{Chamber}$, simulating 21 and 32°C homes), refrigerator load (low/high), door (closed/opening cycle), compressor operating mode, temporary room exposure (simulating consumer product removal) for a refrigerator set to operate at 5°C.

Results: Based on ~four million time-temperature data points collected, estimates of the microbial counts increase after 48 h (Δlog_{N48h}) were obtained for *Listeria monocytogenes* and *Pseudomonas putida* in pasteurized milk and only *L. monocytogenes* in fresh cheese. Higher values were observed for the high $T_{Chamber}$-high load and product removal test conditions. In the case of milk never removed from the refrigerator, Δlog_{N48h} ranged from 1.3 to 1.6 for *L. monocytogenes* and 4.3 to 5.0 for *P. putida.* Similarly, *L. monocytogenes* Δlog_{N48h} estimates in fresh cheese ranged from 1.0 to 1.3 using Uhlich's model and 2.1 to 3.2 with Østergaard's model.

Significance: These results highlight the need to improve temperature control for refrigerated products. These and ongoing studies will support developing new standardized testing procedures to guide the design of new refrigerators balancing energy consumption, food waste, and the frequency of foodborne diseases.

P3-250 Evaluation of the Consumption and Contamination Level of Vegetables and Fruits in Ethiopia

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Introduction: Vegetables and fruits have major acceptance in the modern scientific world nowadays, and it is advisable to include more in daily food consumption. Their different antioxidants and vitamins, which are capable of preventing health risks associated with consuming more protenatious and fatty foods (animal products), result in them being selected more in recent times. Although these food products have gained more acceptance, their preparation and sanitation before consumption at the household level needs more attention.

Purpose: The main objective of this study was to assess the microbial load of vegetables and fruits which were submitted to the public health microbiology laboratory of the Ethiopian Public Health Institute, from 2008 to 2013.

Methods: Vegetable and fruit samples were collected and submitted for the purpose of monitoring and regulating their microbial quality. All samples were analyzed and checked for mold/yeast count, aerobic plate count, total coliform count, fecal coliform count and *E. coli* count to determine their microbial contamination level. The NMKL and APHA protocols were used).

Results: Sixty three processed and final products were received in five to six years, which is around four percent (63 of 1554). Referring to acceptable reference protocols (ICMSF) for each test parameter under this food category, out of all tested samples eight (12.7%) of 63 showed unacceptable microbial quality by mesophilic aerobic plate count, followed by total coliforms with four (6.3%) of 63, fecal coliforms with two (3.2%) of 63, *E. coli* with one (one percent) of 63, and mold with one (one percent) of 63.

Significance: Although vegetables and fruits are proven to be the best healthy foods worldwide and are available at low cost, in developing countries like Ethiopia, their consumption rate is overwhelmed by cereals and animal products based on the socioeconomic status of the community. In addition, even though the submitted food products are processed and ready to use for human consumption, the presence of both fecal coliform indicators and mesophilic aerobic plate count further proves that around one-fifth of the samples were microbiologically contaminated.

Therefore, joint efforts have to be exerted by different branches of the Ministry of Health to encourage the community to use vegetables and fruits as a main food source for better health. However, while doing so, series health education has to be provided on sanitation procedures and environmentally associated risks.

P3-251 Microbial Quality during Storage, Prevalence of Foodborne Pathogens and *Salmonella* Colonization Based on Variances in Netting Densities of Melons Grown in Different Regions of the United States

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Introduction: Melons are a widely consumed commodity and industry needs to ensure that consumers get safe and good quality melons.

Purpose: Assess background microflora and prevalence of pathogens on honeydews and cantaloupes grown in different regions in the United States.

Methods: For the microbiological quality study, six experimental honeydew and cantaloupe varieties were sampled from NC, TX, GA, AZ and CA on d zero and three at room temperature. Samples were sonicated, serially diluted and plated on TSA (aerobic plate count-APC), MacConkey (coliforms) and DRBC agar (fungi). For the prevalence of pathogens study, 228 cantaloupes were sampled for prevalence of *Salmonella enterica* and *Listeria monocytogenes*. Fifteen soil, 15 root, three water and three air samples per field were analyzed. Selective enrichment, plating and confirmation of presumptive positives using API were done. For the study of pathogen colonization on melons of varying netting patterns, rinds (dense, medium, light netting) were inoculated with bioluminescent *Salmonella* and biophotonic imaging was done to understand colonization variability based on netting density.

Results: Honeydew rinds had lower microbial population than cantaloupes. DaVinci-SAKATA (NC) had highest APC (6.49 log) and OC164 (TX-Weslaco), the lowest (3.26 log) on d zero. Infinite-GOLD (AZ) had the highest (4.65 log) and HD252 (NC) the lowest APC (3.62 log) on d three. F39 (CA) had the highest (6.89 log) coliforms on d zero and DaVinci-SAKATA (CA) (5.10 log) on d three. F39 (CA) had the most fungi (3.62 log) on d zero and DaVinci-SAKATA (GA) (3.46 log) on d three. No pathogens were found on melons and environmental samples. Populations of enterococci ranged from 2.79 to 3.23 log and coliforms ranged from 3.04 to 4.57 log. Imaging showed that rinds with dense netting had most colonization followed by medium and light netting.

Significance: Understanding natural prevalence of pathogens, and selecting varieties that harbor lower microbial burden will help growers produce high-quality, safe melons.

P3-252 Identification of Tomato Paste Spoilage Bacteria Using Vibrational Spectroscopy Technologies

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial spoilage of shelf-stable tomato products results in product waste and consumer dissatisfaction. Detection methods that rapidly discriminate among the specific spoilage bacteria of concern enable enhanced control over this issue.

Purpose: Development of reproducible and distinct spectral signature profiles for bacterial spoilage genera relevant to tomato paste spoilage using an accurate high-throughput vibrational spectroscopic technique combined with multivariate data analysis.

Methods: Individual colonies of bacterial isolates associated with spoilage of tomato paste were transferred from streak plates to vibrational spectroscopy units including the Agilent 4500 series FT-IR with triple-reflection ATR and a palm-size NIR (Neospectra) unit. The spectral data were analyzed by Soft Independent Modeling of Class Analogy to generate predictive classification models using Pirouette software. The culture collection contained seven *Bacillus* (*B. subtilis*, and *B. coagulans*), and 10 *Lactobacillus* (*L. acidophilus*, *L. brevis*, *L. plantarum*, *L. casei, L. helveticus*, *L. cellobiosus*, *L. rhamnosus*, and *L. bulgricus*) isolates.

Results: A second derivative transformation of the spectra resolved unique differences among the species of the selected spoilage bacteria. The major discriminating factor among the *Bacillus* spp. was associated with carbonyl groups in the 1765/1737 cm^{-1} range. The major discriminating factor among the *Lactobacillus* species was associated with phospholipids at approximately 1171 cm^{-1}. A palm-size NIR also generated classification models that allowed discrimination at the species level, however for sample preparation MIR required less bacterial biomass (~five µg) to produce consistent spectra when compared to NIR.

Significance: Combination of infrared spectra and pattern recognition analysis offers a rapid and reliable tool for discriminating bacteria associated with tomato paste spoilage. Application of this technique would allow for prediction of the vulnerability of the food product to spoilage during storage.

P3-253 A Machine Learning Approach to Analyze Micro-Isothermal Calorimetry as a Function of Microbial Growth in Fresh and Processed Foods

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Introduction: Calorimetry is a useful tool to assess the shelf life stability of foods rapidly. The technique involves the measurement of heat generated in a food system as a result of chemical and biological changes captured in the form of heat flow (µW) and heat energy (µJ). This approach offers a reliable and less labor-intensive estimation of viable counts as an alternative to traditional plate count. The heat data is modeled and interpreted using exponential models ($Q = Q_o e^{\mu t}$). However, the models are only applicable to the initial vertical portion of the curve over a short period. The remainder relatively constant part of the heat flow curve does not add any useful information to the model, leading to an inaccurate estimation.

Purpose: This work reports the application machine learning theory as an alternative method to interpret micro-isothermal calorimetry data in a supervised learning environment.

Methods: A sufficient number of support vectors (heat data, biomass and CFU/ml) were generated to build a training database (*n=60*). Useful features (specific growth rate, peak time, maximum heat) were extracted from the data, followed by statistical learning procedure of relationship present. The microorganisms used were *Lactobacillus rhamnosus, E. coli and Streptomyces cerevisiae* in growth media.

Results: The Support Vector Regression (SVR) yielded optimized kernel (RBF*: ε = 0.001, C = 0.13, γ = 0.123*) which was found to be the most suitable in terms of best fit and lower cost of calibration. A cross-validation procedure of the developed model yielded high R^2 values (>0.87) with low RMSE (<0.03) for all the organisms confirming the validity of the SVR technique. The model was applied to RTE foods including chicken soup, sauces, cake, seafood, cassava syrup, and beans yielding significant results *(P*<0.05*)*.

Significance: The ML approach offers a reliable new way to determine product shelf life with less need for routine microbial enumeration.

P3-254 Extraction and Characterization of Extracellular Polymeric Substances (EPS) of *E. coli* O157:H7 ATCC 43888 and *Listeria monocytogenes* ATCC 7644 Molecular Biofilms Grown under Different Growth Conditions

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Introduction: *Listeria monocytogenes* and *Escherichia coli* can produce biofilms as survival method in food processing environments. The biofilm complex contains aggregates of polymers which provide numerous benefits to cells inside the biofilm, including adhesion, protection, morphology, and physiology.

Purpose: To extract and characterize the extracellular polymeric substance (EPS) produced by *E. coli* O157:H7 ATCC 43888 and *L. monocytogenes* ATCC 7644 at different food related environmental conditions.

Methods: Various spectrophotometric, chromatographic and microscopic techniques were used to decide the composition of the EPS harvest from different growth conditions of pH, temperature, and nutrient availability.

Results: Colorimetric methods were used to determine the biochemical composition of the biofilm EPS matrix: protein (Bradford Protein Assay) and polysaccharides (Phenol-Sulphuric Acid method). Composition varied with different growth conditions and culture type. Fourier Transformed Infrared (FT-IR) spectra confirmed coupling attributed to polysaccharides, proteins, and lipids. Nuclear magnetic resonance (NMR) reveals the presence of protons of anomeric carbons and protons of primary amine and aromatic compounds. High-Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC) revealed the presence of glucose, xylose, arabinose, mannose, and galactose. Scanning electron microscopy (SEM) was performed to observe the morphology of the EPS.

Significance: Biofilm formation is a serious potential hazard in food hygiene and a source of cross-contamination, contributing to the prevalence and occurrence of pathogenic and spoilage microbes. Biofilms are therefore a leading source of food spoilage and transmission of diseases. Understanding the composition of the biofilm is important in initiating prevention, removal, and inactivation measures of controlling biofilms.

P3-255 Antibacterial Properties of High Voltage Cold Atmospheric Plasma and Its Effect on Quality of Asian Sea Bass Slices

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❖ ***Developing Scientist Entrant***

Introduction: Plasma generated when sufficient energy is applied for the ionization of a neutral gas has promising antibacterial properties. However, working gas composition (WGC), treatment time (TT), and post-treatment time (PTT) are factors determining the antibacterial efficacy of high voltage cold atmospheric pressure (HVCAP). Thus, these factors should be considered and optimized.

Purpose: The effect of WGC, TT and PTT on the bacterial inhibition efficiency of in-package dielectric barrier discharge HVCAP against both spoilage and pathogenic microorganisms was investigated. The impact of HVCAP on quality of Asian sea bass slices was also monitored.

Methods: *Pseudomonas aeruginosa, Escherichia coli, Vibrio parahaemolyticus, Listeria monocytogenes* and *Staphylococcus aureus* were treated with HVCAP under different WGC, TT, and PTT conditions. The cells recovery on tryptic soy agar supplemented with one percent NaCl was determined. Quality changes in ASBS treated with HVCAP generated using argon and oxygen (90:10) as WGC for 2.5, 5, 7.5, and 10 min were also monitored. Data were subjected to AVOVA.

Results: WGC had an influence on the concentration of ozone formed, however it had no significant effect on bacterial inhibition of HVCAP against tested microorganisms (*P*>0.05). Nevertheless, TT and PTT influenced the bacteria inhibition of HVCAP (*P*<0.05). HVCAP treated ASBS had a lower microbial load as compared to the control. The efficacy was generally increased with increasing TT. Lipid oxidation was more pronounced with coincidental decrease in polyunsaturated fatty acids in the slices treated with HVCAP. Myosin heavy chain and actin band intensity were also decreased in Asian sea bass slices treated with HVCAP for more than five min

Significance: HVCAP had an excellent bacterial inhibition efficacy. HVCAP under appropriate conditions could reduce the microbial load of Asian sea bass slices, thus extending its shelf life and assuring safety.

Author and Presenter Index

Chintapenta, Karuna, *Delaware State University* (T9-10)
Chirnside, Anastasia E. M., *University of Delaware* (P2-228)
Chiu, Elaine, *Eurofins Food Analytics NZ Ltd.* (P3-26*)
Chiu, George, *Purdue University* (P3-93)
Chiu, Pei, *University of Delaware* (T1-04)
Chiu, Yen-Chuan, *National Kaohsiung University of Science and Technology (NKUST)* (P2-62)
Cho, Gun Hee, *BK21 Plus, Chung-Ang University* (P3-09)
Cho, Yurim, *Korea University* (P2-117, P2-118, P2-11*)
Choi, In Young, *Kyungpook National University* (T6-09*, P2-68)
Choi, Joseph, *University of Tennessee* (P2-122*)
Choi, Jungmin, *Oregon State University* (P2-259)
Choi, Kyoung-Hee, *Wonkwang University* (P3-108, P1-13)
Choi, Seung-Ho, *3M Korea, Food Safety Division* (P3-134, P3-133, P3-61)
Choi, Tae Ho, *Dyne soze Co., Ltd* (P2-03)
Choi, Yukyung, *Sookmyung Women's University* (P1-134*)
Choi, Yun Hui, *Korea Food Research Institute* (P1-130)
Choi, Yuna, *Sookmyung Women's University* (P3-61)
Choiniere, Conrad, *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition* (S28*)
Chollet, Renaud, *Merck* (P3-21, P3-22)
Choo, Kai Wen, *University of Missouri* (P2-29*)
Chou, Quin, *SafeTraces* (T7-01)
Choudhary, Ruplal, *Southern Illinois University* (P2-206, P2-163)
Chowdhury, Shahid, *Public Health Microbiology Laboratory, Tennessee State University* (P1-104, P1-96, P2-60, P2-59, P1-99)
Christian, Candice, *North Carolina State University* (P1-74*)
Christophe, Natalie, *Louisiana Office of Public Health–Infectious Disease Epidemiology* (P1-181)
Chu, Weiping, *University of California Irvine* (P3-39)
Chuang, Emily, *Purdue University* (P1-73, P1-71)
Chucheep, Kamonwan, *King Mongkut's Institute of Technology Ladkrabang* (P3-241)
Chun, Hyang Sook, *BK21 Plus, Chung-Ang University* (P3-08, P3-09, P3-07)
Chung, Aeri, *Ryerson University* (T8-01)
Chung, Taejung, *The Pennsylvania State University* (P1-187*, P1-186*)
Cinar, Hediye Nese, *U.S. Food and Drug Administration* (P3-124)
Clark, Carrie, *U.S. Department of Agriculture – FSIS* (P1-197*)
Clark, Dorn, *ALS-Marshfield* (P3-114)
Clark, Greyden, *Brigham Young University* (P2-245)
Clark, Katharine, *North Carolina State University* (P1-67*)
Clark, Mike, *Bio-Rad Laboratories* (P3-109)
Clarke, Andrew, *Loblaw* (S25*)
Clarke, Jennifer, *University of Nebraska-Lincoln* (P3-231)
Clemens, Nathan, *SGS Vanguard Sciences* (P3-54, P3-106)
Clifford, David, *Nestlé USA, Inc.* (S16*)
Clotilde, Laurie, *ScanX Technologies* (T3-06, T7-01)
Cloyd, Tami Craig, *U.S. Food and Drug Administration – CFSAN, Coordinated Outbreak Response and Evaluation Network* (T12-02)
Cluster, Jane, *U.S. Food and Drug Administration* (P3-147)
Cobar, Joshua, *Louisiana State University* (P3-177*)
Coelho, Inês, *National Health Institute Dr. Ricardo Jorge-Department of Health and Nutrition* (P3-04)
Colavecchio, Anna, *McGill University* (T2-01, P2-241)
Coleman, Sara, *Health Canada – Communications and Public Affairs Branch* (P1-56)
Coleman, Shannon, *Iowa State University* (P1-102, P1-112, P1-83*, T2-01)
Colorado-Suarez, Stephanie, *University of Puerto Rico* (P2-196)
Colson, Bertrand, *QuoData GmbH* (P3-103, P1-177)
Combrisson, Jerome, *Biofortis Mérieux NutriSciences* (T7-05)
Comeau, Genevieve, *Canadian Food Inspection Agency* (T10-04)
Commins, Scott, *University of North Carolina* (S22*)
Concepcion, Anoushka, *Connecticut Sea Grant and Department of Extension, University of Connecticut* (RT3*)
Conrad, Amanda, *Centers for Disease Control and Prevention (CDC)* (P1-181, T10-07)
Constantino, Cristina De Abreu, *3M Company* (P3-68)
Constanza Díaz, Constanza, *Universidad Andres Bello* (P2-75)
Cook, Kimberly, *USDA ARS, U.S. Department of Agriculture-ARS-US PRC* (S17*, P3-220)
Cook, Roger, *New Zealand Ministry for Primary Industries* (RT13*, RT21*)
Cooksey, Kay, *Clemson University* (P2-243)
Cooley, Michael, *U.S. Department of Agriculture – ARS, WRRC* (P2-227)
Cooper, Charlotte, *Thermo Fisher Scientific* (P3-37, P3-50, P3-36, P1-237)
Cooper, Karen, *Neogen Corporation* (P3-20)
Cooper, Kerry, *The University of Arizona* (P2-213*)
Cooper, Margarethe, *The University of Arizona* (P2-213)
Coorey, Ranil, *Curtin University* (P3-207, T9-02)
Corby, Joseph, *Association of Food and Drug Officials* (RT18*)
Coroller, Louis, *University of Brest- UMT 14.01 SPORE RISK* (P3-44)
Coronel, Claudia, *Purdue University* (P1-227)
Corradini, Maria, *University of Guelph* (T3-05, P2-202)
Corridini, Maria, *University of Guelph* (T3-02)
Corrigan, Nisha, *Qualicon Diagnostics LLC, A Hygiena Company* (P2-134*)
Cosansu, Serap, *Sakarya University* (P2-63)
Councell, Terry, *U.S. Food and Drug Administration* (P1-216)
Covernton, Garth, *University of Victoria, Dept. of Biology* (S39*)
Cox, Nelson, *U.S. Department of Agriculture-ARS-USNPRC* (P3-220)
Cozien, Emeline, *ADRIA Food Technology Institute - UMT14.01 SPORE RISK* (P3-41, P3-42)
Crabtree, David, *Thermo Fisher Scientific* (P3-37, P1-237, P3-36)
Craddock, Hillary, *Maryland Institute for Applied Environmental Health, University of Maryland* (P3-167, T1-02)
Craig, Jackson, *University of Tennessee* (P2-126*)
Craighead, Shani, *University of Delaware* (P2-89*, T6-03*, P2-95, P3-169, P1-258, P3-167, T1-02)
Crews, Mary Katherine, *U.S. Department of Agriculture – FSIS* (P1-197)
Crincoli, Christine, *Cargill, Inc.* (S49*)
Crist, Courtney, *Mississippi State University* (RT11*, P1-88*)
Critzer, Faith, *Washington State University* (P2-215, T12-05, P2-186)
Crossey, K., *Randox Food Diagnostics* (P2-251)
Crowe, Jason, *Florida Department of Agriculture and Consumer Services* (P3-69)
Crowley, Erin, *Q Laboratories, Inc.* (P3-91, P3-23, P3-21*, P3-22*, P3-20*, P3-16)
Crowley, Sally, *Cargill, Inc.* (S11*)
Cucic, Stevan, *Agriculture and AgriFood Canada* (P2-107)
Cuellar, Darvin, *Texas Tech University* (P3-95, P3-198*)
Cui, Li, *Jiangsu Academy of Agricultural Sciences* (P2-242)
Cui, Yan, *Shanghai Jiao Tong University* (P2-85, P2-80, T6-06)
Cunningham, Ashley, *Conagra Brands* (P1-248)
Curry, Phillip, *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition* (P1-180)
Cushen, Maeve, *CremeGlobal* (S53*)

Cusimano, Maria Grazia, *University of Palermo* (P2-02)
Cutter, Catherine, *Penn State University* (RT11*)
Czuprynski, Charles, *University of Wisconsin-Madison* (P1-235, P2-254)
D'Amico, Dennis, *University of Connecticut, Department of Animal Science* (S31*, T7-07, P2-256)
D'Souza, Doris, *University of Tennessee* (P2-122, P2-126, P1-224)
da Cruz Almeida, Erika Tayse, *Federal University of Paraíba* (T2-10, P2-17, P2-18)
da Silva de Souza, Rafael, *Termomecanica Technology College* (P1-268)
Da-Silva, Yejide, *Federal University of Agriculture* (P3-156)
Dagher, Fadi, *Agri-Neo Inc.* (T6-04, P1-213, P1-212)
Dagres, Evangelos, *Agricultural University of Athens* (P3-244)
Dalgaard, Paw, *Technical University of Denmark* (P2-15)
Daliri, Eric Banan-Mwine, *Kangwon National University* (P2-123*)
Danaher, Martin, *Teagasc* (P2-255)
Dankwa, Adwoa, *University of Maine* (P2-165*)
Danyluk, Michelle, *University of Florida CREC* (P1-89, T8-10, P1-85, T1-12, P2-181, P2-218)
Daryaei, Hossein, *Illinois Institute of Technology / IFSH* (P3-86*)
daSilva, Alexandre, *U.S. Food and Drug Administration – CFSAN, Office of Applied Research and Safety Assessment* (P3-124, P3-100)
Datta, Atin, *U.S. Food and Drug Administration - CFSAN* (P2-198)
Daube, Georges, *University of Liège* (P2-115)
Davedow, Taylor, *University of Manitoba* (T4-04*)
David, John, *3M Food Safety* (P3-53)
Davidson, Gordon, *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition* (P1-175)
Dávila-Aviña, Jorge, *Universidad Autonoma de Nuevo Leon* (P2-01, P1-05)
Davis, De Ann, *Church Brothers Farms* (S38*)
Davis, Megan, *SC-BOL* (P2-109)
Davis, Shurrita, *North Carolina A&T State University-Center of Postharvest Technologies (CEPHT)* (P2-170, P2-179*)
Dawson, Kelly, *Conagra Brands* (P1-143)
Dawson, Simon, *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University* (P1-61*)
De, Jaysankar, *University of Florida* (P1-122*, P2-235, P1-119, P2-185, P2-234)
De Aquiar Veloso, Vanessa, *Kansas State University* (P1-54)
De Bock, Thomas, *Ghent University* (P2-177*)
De Coninck, Dieter, *bioMérieux Data Analytics* (P1-49)
De Donato, Marcos, *Tecnologico de Monterrey* (P2-99)
De Jesus, Antonio J, *U.S. Food and Drug Administration* (P2-212*)
De la Cruz Quiroz, Reynaldo, *Tecnologico de Monterrey* (P3-249)
De la Rosa, Elva, *FoodChek Laboratories Inc.* (P3-72)
De la Torre-Anaya, Angélica Alejandra, *3M Food Safety México* (P3-18)
De Meulenaer, Bruno, *Ghent University* (P1-169)
De Smet, Kris, *European Commission* (S5*)
De Smet, Stefaan, *Ghent University* (T9-03)
de Smidt, Olga, *Center for Applied Food Security and -Biotechnology (CAFSaB), Central University of Technology, Free State* (P3-238*, P3-237*)
De Souza, Evandro L., *Federal University of Paraíba* (P2-18, T6-07, P1-243, T2-10, P2-17)
de Souza Pedrosa, Geany Targino, *Federal University of Paraíba* (P2-18, P2-17)
de Vegt, Bert, *Micreos Food Safety B.V.* (T2-07*, P2-71, T4-06)
DebRoy, Chirita, *The Pennsylvania State University* (P3-87)
Dechamma, MM, *University College Dublin* (P1-256)
Deck, Joanna, *U.S. Food and Drug Administration* (P1-185)
Deeds, Jonathan, *U.S. Food and Drug Administration – CFSAN* (P3-85)
Deering, Amanda, *Purdue University* (P3-93)
DeFranco, Agnes, *University of Houston* (P2-135)
Degen, Olaf, *Biotecon Diagnostics* (P3-229*)
DeGuzman, Veronica, *SnapDNA, Inc.* (P3-88)
Dehalle, Laurent, *REALCO* (S57*)
Deibel, Charles, *Deibel Laboratories, Inc.* (P3-14)
Del'Angel, Jorge, *Florida Agricultural and Mechanical University* (P1-160)
Delaquis, Pascal, *Agriculture and Agri-Food Canada* (P2-176)
Delaunay, Louis, *LUBEM UBO University - UMT14.01SPORE RISK* (P3-41)
Delhalle, Laurent, *University of Liege* (P2-115*)
Deliephan, Aiswariya, *Kansas State University* (P2-13*)
Dell'Aringa, Joy, *bioMérieux Inc.* (P3-23*)
Dellaringa, Joy, *bioMérieux Inc.* (P3-63)
DeMarco, Daniel, *Eurofins* (P2-253*)
DeMent, Jamie, *Florida Department of Health* (S24*, P1-46)
Demmings, Elizabeth, *Cornell University* (P1-91*)
Demokritou, Philip, *Center for Nanotechnology and Nanotoxicology, Harvard T. H. Chan School of Public Health* (P1-106)
den Bakker, Henk, *Center for Food Safety, University of Georgia* (S13*, T4-03, P3-92)
den Bakker, Meghan, *Research Specialist* (P1-04*)
Den Besten, Heidy, *Wageningen University* (S42*, P3-44, T8-03*)
Denes, Thomas G., *The University of Tennessee* (P1-226, P2-65)
Deng, Ta, *MilliporeSigma* (P3-49)
Deng, Wenjun, *University of Arkansas* (P2-102*)
Deng, Xiangyu, *University of Georgia, Center for Food Safety* (P1-229, P3-92, P1-06, P1-278, P1-184)
Derra, Firehiwot, *EPHI* (P3-250*)
Desai, Dhananjay, *ICAR-Central Coastal Agricultural Research Institute* (P2-239)
Desiree, Karina, *Kansas State University* (P2-149, P2-150*)
Desta, Hiwot, *International Livestock Research Institute* (P2-249)
Destro, Maria Teresa, *bioMérieux Inc.* (RT8*)
Detwiler, Darin, *Northeastern University* (RT16*)
Dev Kumar, Govindaraj, *University of Georgia Center for Food Safety* (P3-213*, T2-12*, P2-168*)
Devlieghere, Frank, *Ghent University* (T6-08)
DeWitt, Christina, *Seafood Research & Education Center, Oregon State University* (P3-188)
DeZutter, Lieven, *Ghent University* (T9-03)
Dhakal, Janak, *Kansas State University* (P2-13, T4-07*)
Dharmasena, Muthu, *Clemson University* (P1-266, P2-125*)
Di Marco Lo Presti, Vincenzo, *Istituto Zooprofilattico Sperimentale of Sicily* (P1-55, P2-02)
Diaz, Claudia, *National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University* (T11-03*)
Díaz, Constanza, *Universidad Andres Bello* (P3-155)
Diaz-Amaya, Susana, *Purdue University* (P3-93*)
Diaz-Perez, Juan Carlos, *The University of Georgia* (P2-155)
DiCaprio, Erin, *University of California-Davis* (P2-175, T6-02)
Diei Ouadi, Yvette, *UN Food and Agriculture Organisation* (P1-169)
Diez-Gonzalez, Francisco, *University of Georgia, University of Georgia, Center for Food Safety* (P1-04, P3-92)
DiMenna, Lauren, *Kraft Heinz Company* (P3-197*)
Ding, Qiao, *University of Maryland* (P1-250, P1-206)

Developing Scientist Competitors

Abe, Hiroki, *Hokkaido University* (T10-09)
Aboubakr, Hamada, *University of Minnesota, College of Veterinary Medicine* (T6-01)
Acuff, Jennifer, *Virginia Tech* (P1-17)
Adeniyi, Ayodeji, *Texas Tech University* (P3-95)
Adhikari, Jayashan, *Public Health Microbiology Laboratory, Tennessee State University* (P1-99)
Aditya, Arpita, *University of Maryland* (P2-06)
Aguayo-Acosta, Alberto, *Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León* (P1-05)
Ahmad, Nurul Hawa, *Michigan State University* (P1-07)
Alarape, Selim, *University of Ibadan* (P2-97)
Allison, Abimbola, *Public Health Microbiology Laboratory, Tennessee State University* (P1-96)
Alvarado-Martinez, Zabdiel, *University of Maryland* (P2-52)
Anast, Justin, *Iowa State University* (T7-11)
Anderson-Coughlin, Brienna, *University of Delaware* (P2-95)
Aras, Sadiye, *Public Health Microbiology Laboratory, Tennessee State University* (P2-60)
Atis, Lordwige, *University of Georgia* (P1-46, P2-94)
Badmos, Amina, *Federal University of Agriculture Abeokuta Ogun State, Nigeria* (P3-10)
Baker, Christopher, *University of Florida* (P2-235)
Bardsley, Cameron, *Virginia Tech - Eastern Shore AREC* (P2-182)
Barone, Nicholas, *The Ohio State University* (P1-117)
Barrett, Tressie, *Purdue University* (P1-70, P1-68, P1-69)
Belias, Alexandra, *Cornell University* (P2-159)
Bertoldi, Bruna, *University of Florida* (P2-185)
Bhandare, Sudhakar, *McGill University* (P2-241)
Bhandari, Devendra, *Tennessee State University* (P3-60)
Bhullar, Manreet, *Iowa State University* (P2-142)
Bhusal, Arjun, *Oklahoma State University* (P2-14)
Boucher, Cara, *Oregon State University* (P2-103)
Brewer, Sheridan, *University of Georgia Center for Food Safety* (P3-157)
Britton, Brianna, *Purdue University* (P3-193)
Broten, Codi Jo, *University of Wyoming* (P3-94)
Bulut, Ece, *University of Nebraska-Lincoln* (T12-06)
Cai, Shiyu, *The Ohio State University* (P2-147)
Campbell, Emily, *The Ohio State University* (P2-08)
Cao, Wanying, *University of Nebraska-Lincoln* (P3-02)
Chantapakul, Bowornnan, *University of Guelph, CRIFS, Department of Food Science* (P2-51)
Chase, Jennifer A., *University of California-Davis* (T1-05)
Chavez, Ruben, *University of Illinois at Urbana-Champaign* (P3-05)
Chen, Jinru, *Department of Food Science and Technology, The University of Georgia* (P2-112)
Chen, Long, *University of Nebraska-Lincoln* (P1-18)
Chen, Yuan Yao, *Agriculture and Agri-Food Canada* (P2-74)
Cheng, Xianbin, *University of Illinois at Urbana-Champaign* (P1-124)
Chhetri, Vijay Singh, *Louisiana State University AgCenter* (P2-173)
Cho, Yurim, *Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University* (P2-11)
Choi, In Young, *Kyungpook National University* (T6-09)
Choi, Joseph, *University of Tennessee* (P2-122)
Choi, Yukyung, *Sookmyung Women's University* (P1-134)
Chung, Taejung, *The Pennsylvania State University* (P1-187)
Clark, Katharine, *North Carolina State University* (P1-67)
Craighead, Shani, *University of Delaware* (T6-03, P2-89)
Cuellar, Darvin, *Texas Tech University* (P3-198)
Davedow, Taylor, *University of Manitoba* (T4-04)
Dawson, Simon, *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University* (P1-61)
Deliephan, Aiswariya, *Kansas State University* (P2-13)
Desiree, Karina, *Kansas State University* (P2-150)
Dev Kumar, Govindaraj, *University of Georgia Center for Food Safety* (P3-213)
Diaz, Claudia, *National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University* (T11-03)
Diaz-Amaya, Susana, *Purdue University* (P3-93)
Dogan, Onay Burak, *University of Nebraska-Lincoln* (P3-231)
Dong, Lianger, *University of Hawaii at Manoa* (P2-219)
Dong, Mengyi, *University of Illinois at Urbana-Champaign* (P2-161, P2-162)
du Plessis, Erika, *University of Pretoria* (P2-77, P2-76)
Ekli, Rejoice, *University for Development Studies* (P2-78)
Engstrom, Sarah, *Food Research Institute, University of Wisconsin-Madison* (T7-08)
Estrada, Erika, *Virginia Tech - Eastern Shore AREC* (P2-186)
Farquharson, Emma, *Cornell University* (P1-81)
Ferelli, Angela Marie C., *University of Maryland* (T6-10)
Fong, Karen, *Food, Nutrition and Health, University of British Columbia* (P2-70)
Gartley, Samantha, *University of Delaware* (P3-169)
Gelda, Krishna S., *University of Guelph, CRIFS, Department of Food Science* (P2-42)
Gensler, Catherine, *University of Connecticut, Department of Animal Science* (T7-07)
Glaize, Ayanna, *North Carolina State University* (T1-08)
Golden, Chase, *University of Georgia* (P1-129)
Gunathilaka, Gayathri, *Michigan State University* (P3-174)
Gutierrez, Alan, *University of Florida* (P2-234)
Ha, Jimyeong, *Sookmyung Women's University* (P3-61)
Hamilton, Alexis M., *Washington State University, School of Food Science* (P2-215)
Hanlon, Keelyn, *Texas Tech University* (P3-209)
Harrand, Anna Sophia, *Cornell University* (P2-223)
Haymaker, Joseph, *University of Maryland Eastern Shore* (P3-167)
Henry, Monica, *Public Health Microbiology Laboratory, Tennessee State University* (P1-255)
Hildebrandt, Ian, *Michigan State University* (P3-216)
Ho, Jordan, *University of Guelph* (P2-216)
Horr, Taryn, *University of Maryland* (P1-128)
Huang, Runze, *Center for Nanotechnology and Nanotoxicology, Harvard T. H. Chan School of Public Health* (P1-106)
Hussein, Walaa, *The Ohio State University* (P2-57, P2-56)
Hwang, Daizy, *University of Georgia* (P1-165)
Igo, Matthew, *Rutgers University* (P1-159)
Jacxsens, Liesbeth, *Ghent University* (P1-169, P1-170)
Jayeola, Victor, *North Carolina State University* (P1-201)
Jiang, Wentao, *West Virginia University* (P3-230)
Jin, Yuqiao, *Washington State University* (P1-190)
Jones, Amy, *University of Florida* (P3-120)
Jorgensen, John, *Oregon State University* (T3-04)

Jurusik, Anna, *University of Delaware* (T10-11)
Kang, Joohyun, *Sookmyung Women's University* (P3-179, P1-135)
Karolenko, Caitlin, *Oklahoma State University* (P1-101)
Keet, Rochelle, *Stellenbosch University* (P2-25)
Kelly, Emily, *California Department of Public Health* (P1-111)
Kenney, Annette, *University of Maryland Eastern Shore* (P2-230)
Kgoale, Degracious, *University of Pretoria* (P2-154)
Kharel, Karuna, *Louisiana State University AgCenter* (P1-215)
Kim, Yeon Soo, *School of Food Science and Biotechnology, Kyungpook National University* (P2-68)
Kim, Yujin, *Sookmyung Women's University* (P3-178)
Kirchner, Margaret, *North Carolina State University* (P1-92)
Lamba, Sakshi, *UCD Centre for Food Safety, UCD Institute of Food and Health, UCD School of Agriculture and Food Science, University College Dublin* (P1-256)
Lane, Kristin, *University of Massachusetts* (P2-131)
Lau, Soon Kiat, *University of Nebraska-Lincoln* (P1-09)
Lau, Tsun Yin Alex, *University of Guelph* (P3-196, P2-27)
Lee, Heeyoung, *Korean Food Research Institute* (P3-212)
Lee, Jeeyeon, *Sookmyung Women's University* (P1-13)
Lee, Jeongmin, *Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University* (P2-117, P2-118)
Lee, Soojin, *Hospitality Management* (P1-118)
Lee, Soomin, *Sookmyung Women's University* (P3-108)
Lee, Yewon, *Sookmyung Women's University* (P1-138, T7-06)
Li, Ka Wang, *West Virginia University* (P2-151)
Li, Shaoting, *University of Georgia, Center for Food Safety* (P1-184)
Li, Yanbin, *Department of Biological & Agricultural Engineering, University of Arkansas* (P1-166)
Liao, Chao, *Auburn University* (P1-265)
Liu, Xiaohan, *University of Hawaii at Manoa* (P2-49, P2-48)
Liu, Xiyang, *IFSH* (P1-210)
Loku Umagiliyage, Arosha, *Southern Illinois University* (P2-206)
Lv, Ruiling, *Zhejiang University* (P1-261)
Ma, Luyao, *Food, Nutrition and Health Program, Faculty of Land and Food Systems, The University of British Columbia* (P3-83, T5-12)
Magdovitz, Brittany, *University of Georgia* (P2-114)
Maggio, Stephanie, *North Carolina State University* (T8-04)
Magossi, Gabriela, *Kansas State University, Food Science Institute* (P1-40, P1-41)
Maher, Joshua, *Kansas State University* (P2-174, P1-54)
Maillet, Aurelien, *UMR 1014 Secalim, UBL, INRA, Oniris* (T7-05)
Marik, Claire M., *Virginia Tech* (P2-148)
Mayton, Holly, *University of Virginia* (T2-11)
McDaniel, Austin, *Kansas State University, Food Science Institute* (P2-207)
McDaniel, Conner, *Oklahoma State University* (P2-138)
Mendez, Ellen, *KSU Food Science Institute* (P2-106, P1-82)
Mercado, Victor, *Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León* (P2-83)
Mohammad, Zahra, *University of Houston* (P1-269)
Monge, Ana, *Iowa State University* (T8-09)
Moorman, Eric, *Department of Food, Bioprocessing, and Nutritional Sciences, North Carolina State University* (T7-04)
Moreira, Juan, *Louisiana State University* (P2-157)
Munoz, Sara, *Texas Tech University* (P3-228)
Murphy, Sarah, *Cornell University* (T7-12)
Nawawi, Azrina, *Michigan State University* (P3-45)
Neale, Rosalind, *University of Vermont* (P2-257)
Oats, Michael, *Purdue University* (P1-227)
Oguadinma, Ikechukwu, *University of Georgia* (P2-155)
Oh, Hyemin, *Sookmyung Women's University* (P1-150, T4-11)
Okorie-Kanu, Onyinye, *University of Nigeria* (P2-73)
Olatunde, Oladipupo, *Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai* (P3-255)
Oloso, Nurudeen Olalekan, *University of Pretoria* (P1-51, P1-52)
Omar, Alexis, *University of Delaware* (P2-228)
Oni, Eniola, *Federal University of Agriculture Abeokuta* (P1-114)
Ou, Chujun, *Shanghai Jiao Tong University* (T9-09)
Overbey, Katie, *Johns Hopkins Bloomberg School of Public Health* (P2-98)
Oyedeji, Ajibola, *Durban University of Technology* (P2-252)
Pabst, Christopher, *University of Florida* (P1-119)
Pahariya, Prachi, *Southern Illinois University* (P2-163)
Park, Eunyoung, *Sookmyung Women's University* (P1-151, P1-152)
Parlindungan, Elvina, *RMIT University* (T2-08)
Parraga, Katheryn, *Louisiana State University* (P1-113)
Patwardhan, Mayuri, *University of Tennessee* (P1-224)
Perez Garza, Janeth, *University of Connecticut* (P2-183)
Pérez-Garza, Janeth, *Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León* (P2-81)
Perry, Bridget, *Iowa State University* (P1-112)
Pilch, Hannah, *University of Wisconsin-Madison, Department of Pathobiological Sciences* (P1-235)
Pletcher, Dennis, *Oklahoma State University* (P2-146)
Qi, Hang, *University of Georgia* (P2-111)
Quintela, Irwin, *Western Regional Research Center, Agricultural Research Service, USDA* (P3-76, P3-77)
Rana, Yadwinder Singh, *The Ohio State University* (P3-252)
Rane, Bhargavi, *Western Regional Research Center, Agricultural Research Service, USDA* (P2-35)
Rani, Surabhi, *University of Maryland* (T10-12)
Rani, Surabhi, *University of Maryland* (P3-202)
Reed, Alyxandra, *Center for Food Safety, University of Georgia* (T4-03)
Reuben, Rine, *Department of Science Laboratory Technology, Nasarawa State Polytechnic, Lafia* (T9-08)
Riggio, Gina, *University of Arkansas* (P2-82)
Rolfe, Catherine, *Institute for Food Safety and Health, Illinois Institute of Technology* (T8-07)
Romero, Ana, *Clemson University* (P2-243)
Saha, Joyjit, *Oklahoma State University* (T9-04)
Sant'ana, Anderson de Souza, *Department of Food Science, College of Food Engineering - University of Campinas* (P3-173, P1-100)
Schwan, Carla, *Kansas State University* (P2-149)
Selover, Brandon, *Oregon State University* (P3-211)
Seo, Yeongeun, *Sookmyung Women's University* (P1-139, P3-110)
Shah, Manoj, *North Dakota State University* (P2-233)
Shin, Hyejung, *Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University* (P2-121)
Singh Hamal, Shreya, *Tennessee State University* (P3-62)
Sisney, Amanda, *Conagra Brands* (P1-157, P1-158)
Sloniker, Natasha, *Michigan State University* (P2-158)
Steinbrunner, Philip, *Michigan State University* (P1-204, P2-209)
Sudagar, Varalakshmi, *Ghent University* (T9-03)
Sullivan, Genevieve, *Cornell University* (T3-03)
Sun, Lang, *University of Connecticut* (P2-256)
Suther, Cassandra R., *University of Massachusetts, Amherst* (P3-98)

Tabashsum, Zajeba, *University of Maryland* (T4-02)
Takeoka, Kohei, *Hokkaido University* (P1-154)
Thomas, Merlyn, *University of Georgia* (P1-153)
Thomas-Popo, Emalie, *Iowa State University* (P1-102)
Tikekar, Rohan, *University of Maryland* (P1-206)
Trudelle, Danielle, *The University of Tennessee* (P2-65)
Vega, Daniel, *Kansas State University* (P1-203)
Vengarai Jagannathan, Badrinath, *University of Kentucky* (P2-64)
Verma, Tushar, *University of Nebraska-Lincoln* (P1-15)
Wadhawan, Kirty, *University of Wisconsin- Madison, Department of Pathobiological Sciences* (P2-254)
Wang, Hongye, *Clemson University* (P1-266)
Wang, Kaidi, *Food, Nutrition and Health Program, Faculty of Land and Food Systems, The University of British Columbia* (P1-236)
Wang, Peien, *Department of Food Science and Technology, The University of Georgia* (P2-214)
Wang, Qingyang, *University of Maryland* (P1-108)
Wang, Wenqian, *University of Arkansas,Â Department of Poultry Science* (T5-09)
Weerarathne, Pabasara, *Oklahoma State University* (P2-67)
Wei, Xinyao, *University of Nebraska-Lincoln* (P1-22)
West, Molly, *The University of Tennessee* (T12-05)
White, Chanelle, *University of Maryland Eastern Shore* (P3-168)
Wong, Catherine, *Food, Nutrition and Health, University of British Columbia* (P2-176)
Wu, Biyu, *University of Hawaii at Manoa* (P3-97, P3-96)
Wu, Sophie Tongyu, *Purdue University* (P1-126)
Wu, Xueyang, *University of Guelph* (P2-191)
Xu, Jie, *Washington State University* (P1-202)
Xu, Yumin, *The Ohio State University* (P3-234)
Yan, Jia, *University of California, Davis,Â Food Science and Technology Dept.*, (P3-153)
Yan, Runan, *The Pennsylvania State University* (P1-199)
Yang, Ren, *Washington State University* (T11-02, T11-01)
Yao, Shiyun, *University of Delaware* (T3-08)
Yeom, Woorim, *Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University* (P2-116)
Yi, Jiyoon, *University of California-Davis* (T2-03)
Zhao, Luyao, *Department of Food Science and Technology, The University of Georgia* (P2-240)
Zhong, Zeyan, *McGill University* (T6-05)

Undergraduate Student Award Competitors

Arbon, Jeremy, *Brigham Young University* (P2-245)
Byun, Suyeun, *U.S. Department of Agriculture* (P2-194)
Chen, Han, *Purdue University* (P1-90)
Cobar, Joshua, *Louisiana State University* (P3-177)
Craig, Jackson, *University of Tennessee* (P2-126)
Gomez, Carly, *Michigan State University* (P1-145)
Hodges, Jack, *University of Houston* (P2-135)
Johnson, Erica, *University of West Alabama* (P2-40)
Kelly, Alyssa, *University of Delaware* (P2-204)
Ladner, Taylor, *Mississippi State University* (P3-242)
Naden, Lauren, *Oklahoma State University* (P2-46)
Patregnani, Emma, *U.S. Food and Drug Administration – CFSAN, Office of Applied Research and Safety Assessment* (P3-100)
Walker, Kayla, *University of West Alabama* (P1-262)