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IAFP 2026 ABSTRACT BOOK

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GENERAL SESSIONS ABSTRACT



GREGORY ASTILL, Ph.D.

Economist

Provo, Utah



ELIZABETH A. BIHN, Ph.D.

Director, Produce Safety Alliance, Cornell University

Ithaca, New York

IVAN PARKIN LECTURE ABSTRACT:

An Engaging Exchange: Distilling Data for Impact

The systems and processes supporting food protection are complex and encompass a wide range of academic fields from chemistry, biology, physics, engineering, economics, sociology, statistics, and psychology. Data can be used to improve food safety systems and processes so that limited resources can be used efficiently to reduce food safety risks. Unfortunately, data can be distorted, misquoted, truncated, misunderstood, and misused. We all recognize the problem of GIGO: Garbage In, Garbage Out. Making food safety decisions in a world with so many data streams and opinions can be daunting. Working with colleagues who have different strengths allows for data assessment that leads to establishing meaningful food safety priorities while filling knowledge gaps.

For this year's Ivan Parkin Lecture, Dr. Greg Astill and Dr. Betsy Bihn will kick off the IAFP Annual Meeting by collecting real-time food safety views of the audience and analyzing this data from different perspectives to engage in a lively discussion of science, business, and policy. The goal is to interact with data that may inform your decisions at this year's Annual Meeting and impact your roles over the coming year. Perhaps it inspires you to do something new. Or to be more confident in how you experience IAFP 2026. Hopefully, this discussion gives you a moment to share with your colleagues, reacting to a live discussion which distills data to help you in your food safety journey.

JOHN H. SILLIKER LECTURE ABSTRACT:

From Fundamentals to Frontiers: A Consultant's Guide to Tackling Food Safety Risks

After many years working within the food industry, I transitioned to consulting with the goal of helping organizations strengthen their food safety practices while balancing science with the practical realities of manufacturing and operations. This presentation focuses on a snapshot of common "opportunities" that I've been asked about and how I've responded. There are lessons we all can learn from these experiences.



RUTH PETRAN

Principal & Founder, Ruth Petran Consulting, LLC
Senior Advisor, The Acheson Group

Minneapolis, Minnesota

We all know that in today's rapidly evolving food industry, organizations face a complex array of food safety challenges–from persistent lapses in basic practices to the emergence of new risks associated with novel products and processes. What I discovered was that a lot of issues stem from knowledge and implementation gaps in fundamental food microbiology and food safety practices. By focusing on core principles and adapting them as appropriate, we can improve our abilities in analyzing hazards and the accompanying management of risks to enable safer food production.

To keep food safe and companies thriving, it is imperative to maintain focus on these common concerns that are areas of highest risk. For example:

1. Persistent GMP violations including inadequate pest control, poor sanitary design, and inconsistent hygienic practices, continue to be reported by regulators, despite long standing requirements that they be implemented. We've seen instances where lapses in these basic programs can spiral quickly out of control.
2. The lack of understanding around controlling microbial growth, especially of toxin-producing and sporeforming organisms, is a recurring theme. Questions about shelf life, run time, and hold time often center on how to limit outgrowth and ensure product safety.
3. Identifying and controlling relevant hazards in raw materials brought into facilities has always been a challenge, but with the advent of FSMA, focus has been enhanced. Testing food products continues to be a popular technique to assess quality and/or safety, and it does have its place. But it also has limitations and should be used strategically as a verification method, rather than as a primary control. How can we best leverage testing in optimal ways as a verification technique to ensure that controls are properly implemented?
4. Advances in detection technologies now offer new opportunities to identify and address risks more effectively. By judiciously using these tools and focusing on robust hazard analyses, organizations can implement more robust risk management strategies.

We need to leverage the realities of these recurrent questions and issues to enable targeting controls where they will be most effective. This presentation will give you a clearer understanding of where and how to apply core principles using scenarios to bring this to life. My hope is that these reflections will inspire renewed focus on areas of highest risk and empower participants to drive safer food production in your own organizations.

Symposium Abstracts

S1 - OUTBREAK SYMPOSIUM: NOTABLE FOODBORNE DISEASE OUTBREAKS FROM 2025 – LESSONS FOR PUBLIC HEALTH AND INDUSTRY (PART 1)

Primary Author:
Laura Gieraltowski, Centers for Disease Control and Prevention

Persons:
Laura Gieraltowski, Centers for Disease Control and Prevention

Kari Irvin, USFDA

Ewen Todd, Ewen Todd Consulting LLC

Michael Batz, USFDA

Amanda Conrad, US CDC

Michael Vasser, US CDC

Brett Weed, USFDA

Matthew Wise, CDC

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

This symposium will gather experts from public health and regulatory agencies to discuss topics in outbreak investigations. A time slot will be held open for a late-breaking presentation highlighting a recent outbreak investigation. Since September of 2024, contaminated eggs were linked to three multistate outbreaks of *Salmonella* Enteritidis infections totaling more than 300 illnesses. Speakers will describe the recent outbreaks, sampling, and investigational challenges. Speakers will discuss an analysis of potential reasons for this recent increase in outbreaks linked to eggs. Once the contaminated food is identified and removed from the market, the occurrence of illness is expected to come to an end. Speakers will provide an overview of outbreak investigations and discuss potential causes of prolonged occurrence of illnesses involving FDA regulated foods after the implementation of recalls. In summer of 2025, investigators identified two outbreaks of

Listeria infections linked to ready-to-eat products. Speakers will discuss the epidemiology, traceback, in-plant findings, and investigation challenges. A signal for the source of an outbreak with a small number of cases should prompt further investigation and sample collection. Discussion with industry early on can be informative and help direct resources. A speaker will share the details of two examples of investigations of fewer than 20 cases and highlight methods for solving these challenging outbreaks. A speaker from the Public Health Agency of Canada will provide an overview of a multi-strain outbreak investigation of *Salmonella* infections in Canada linked to pistachios and pistachio-containing products. As of September 24, 2025, 105 cases have been reported across six provinces and over 30 recalls have been issued.

Primary PDG Sponsor: Committee on Control of Foodborne Illness

Primary Topic Area: Epidemiology

Secondary Topic Area: Communication, Outreach and Education

Keywords: Epidemiology,Outbreak,Public Health

S1 - OUTBREAK SYMPOSIUM: NOTABLE FOODBORNE DISEASE OUTBREAKS FROM 2025 – LESSONS FOR PUBLIC HEALTH AND INDUSTRY (PART 2)

Primary Author:
Laura Gieraltowski, Centers for Disease Control and Prevention

Persons:
Laura Gieraltowski, Centers for Disease Control and Prevention

Kari Irvin, USFDA

Ewen Todd, Ewen Todd Consulting LLC

Michael Batz, USFDA

Jaheon Koo, USFDA

Carlota Medus, Minnesota Dept. of Health

Katrina Mickanuck, Public Health Agency of Canada

Lalitha Kurupatham, Singapore Communicable Diseases Agency

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

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Listeria infections linked to ready-to-eat products. Speakers will discuss the epidemiology, traceback, in-plant findings, and investigation challenges. A signal for the source of an outbreak with a small number of cases should prompt further investigation and sample collection. Discussion with industry early on can be informative and help direct resources. A speaker will share the details of two examples of investigations of fewer than 20 cases and highlight methods for solving these challenging outbreaks. A speaker from the Public Health Agency of Canada will provide an overview of a multi-strain outbreak investigation of *Salmonella* infections in Canada linked to pistachios and pistachio-containing products. As of September 24, 2025, 105 cases have been reported across six provinces and over 30 recalls have been issued.

Primary PDG Sponsor: Committee on Control of Foodborne Illness

Primary Topic Area: Epidemiology

Secondary Topic Area: Communication, Outreach and Education

Keywords: Epidemiology,Outbreak,Public Health

S2 - SMARTER VALIDATION STRATEGIES FOR LOW-WATER ACTIVITY FOODS: PRODUCT GROUPING AND REVALIDATION IN PRACTICE (PART 1)

Primary Author:
Ruth Oni, USFDA

Persons:
Ruth Oni, USFDA

Jena Roberts, LogRed / Novolyze

Aparna Tatavarthy, USFDA

Laure Pujol, Novolyze

Shannen Gorman, ASTA

Grishma Prabhukhot, Elite Spice

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

With FDA's preventive controls rule in full enforcement, food companies, particularly in the low-water activity (a_w) sectors, face unique challenges validating pathogen-reduction processes across diverse product portfolios. Spices, grains, nut butters, chocolate, and similar products often require validation of thermal or other reduction steps to mitigate contamination risks. Yet, validating each product individually can be operationally and financially impractical. To address this, the industry has increasingly turned to Product Grouping as a science-based strategy to streamline validation. Early grouping methods like botanical categorization proved insufficient since similar plant parts could behave differently during processing. Advanced methodologies now incorporate multiple intrinsic factors such as carbohydrate, fat, moisture, a_w, density, particle size, as well as process variables like heat application mode, penetration patterns, and equipment design. While these approaches improve precision, they complicate identifying the true "worst-case" product for validation. Meanwhile, validation is dynamic—product, process, or regulatory changes may require revalidation for continued compliance and safety. This interplay between grouping strategies

and revalidation triggers underscores the need for practical, risk-based frameworks that balance scientific rigor with operational efficiency.

This symposium brings together industry, academic, and regulatory experts to explore the evolving practice of Product Grouping and Revalidation. Through case studies, technical guidance with a global approach, and regulatory insights, attendees will gain practical tools to design defensible validation programs while conserving resources and strengthening food safety controls.

Primary PDG Sponsor: Low Water Activity Foods PDG

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Food Safety Culture

Keywords: Low-water Activity Foods,Ready to Eat Foods,Risk Assessment,Validation

S2 - SMARTER VALIDATION STRATEGIES FOR LOW-WATER ACTIVITY FOODS: PRODUCT GROUPING AND REVALIDATION IN PRACTICE (PART 2)

Primary Author:
Ruth Oni, USFDA

Persons:
Ruth Oni, USFDA

Jena Roberts, LogRed / Novolyze

Aparna Tatavarthy, USFDA

Aaron Uesugi, Mondelez International

Ian Hildebrandt, Michigan State University

Elizabeth Grasso-Kelley, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

With FDA's preventive controls rule in full enforcement, food companies, particularly in the low-water activity (a_w) sectors, face unique challenges validating pathogen reduction processes across diverse product portfolios. Spices, grains, nut butters, chocolate, and similar products often require validation of thermal or other reduction steps to mitigate contamination risks. Yet, validating each product individually can be operationally and financially impractical. To address this, the industry has increasingly turned to Product Grouping as a science-based strategy to streamline validation. Early grouping methods like botanical categorization proved insufficient since similar plant parts could behave differently during processing. Advanced methodologies now incorporate multiple intrinsic factors such as carbohydrate, fat, moisture, a_w, density, particle size, as well as process variables like heat application mode, penetration patterns, and equipment design. While these approaches improve precision, they complicate identifying the true "worst-case" product for validation. Meanwhile, validation is dynamic—product, process, or regulatory changes may require revalidation for continued compliance and safety. This interplay between grouping strategies

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Primary PDG Sponsor: Low Water Activity Foods PDG

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Food Safety Culture

Keywords: Low-water Activity Foods,Ready to Eat Foods,Risk Assessment,Validation

S3 - THE UTILIZATION OF LARGE-SCALE SEQUENCE DATABASES FOR FOODBORNE PATHOGEN CHARACTERIZATION AND FUNCTIONAL GENOMICS (PART 1)

Primary Author:
Steven Foley, USFDA

Persons:
Maria Hoffmann, USFDA

Steven Foley, USFDA

Heather Carleton, US-CDC

Glenn Tillman, USDA-FSIS

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

With the continued advancement of high-throughput sequencing technologies and the democratization of data repositories, food safety experts have valuable data resources that can be utilized to address food safety challenges. Scientists have ready access to large-scale genomic sequence data in public repositories such as GenBank (NCBI), the European Nucleotide Archive (ENA), and the DNA Data Bank of Japan (DDBJ), which are providing extraordinary opportunities for advancing our understanding of foodborne pathogens. This symposium will explore key approaches to harness these big data resources for characterizing the genetic diversity, evolution, and functional capabilities of foodborne pathogens.

The session will begin with presentations describing an overview of the publicly available genomics resources available to inform public health knowledge through a series of speakers from the U.S. federal government public health agencies. These speakers will provide practical examples and experiences with the use of comparative genomics across genomic databases and how they can reveal serotype, virulence potential, antimicrobial-resistance mechanisms, and metabolic pathways that influence pathogen survival and

transmission through the food chain. The final set of speakers will represent academia and industry and approaches to pioneer the integration of large-scale genomic data into food safety applications. Altogether, the session will provide symposium attendees with key knowledge on leveraging large-scale genomic resources for enhanced foodborne pathogen surveillance, outbreak investigation, and risk assessment.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Data Management and Analytics

Additional Topic Area (Optional): Microbial Modelling and Risk Analysis

Keywords: Data,Genomics,Public Health

S3 - THE UTILIZATION OF LARGE-SCALE SEQUENCE DATABASES FOR FOODBORNE PATHOGEN CHARACTERIZATION AND FUNCTIONAL GENOMICS (PART 2)

Primary Author:
Steven Foley, USFDA

Persons:
Steven Foley, USFDA

Hendrik Den Bakker, Center for Food Safety, University of Georgia

Ryan Blaustein, University of Maryland

Celina To, bioMerieux

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

With the continued advancement of high-throughput sequencing technologies and the democratization of data repositories, food safety experts have valuable data resources that can be utilized to address food safety challenges. Scientists have ready access to large scale genomic sequence data in public repositories such as GenBank (NCBI), the European Nucleotide Archive (ENA), and the DNA Data Bank of Japan (DDBJ), which are providing extraordinary opportunities for advancing our understanding of foodborne pathogens. This symposium will explore key approaches to harness these big data resources for characterizing the genetic diversity, evolution, and functional capabilities of foodborne pathogens.

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Primary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Data Management and Analytics

Additional Topic Area (Optional): Microbial Modelling and Risk Analysis

Keywords: Data,Genomics,Public Health

S4 - FOOD SAFETY IN INDIGENOUS ARCTIC FOOD SYSTEMS IN FACE OF CLIMATE CHANGE

Primary Author:
Jeffrey LeJeune, FAO

Persons:
Jeffrey LeJeune, FAO

Amanda Boyd, Washington State University

Maura Di Martino, Food and Agriculture Organization of the United Nations (FAO)

Kristeen McTavish, Memorial University

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Many Indigenous Peoples live in remote areas and consume traditional foods that are produced outside of commercial markets, yet they hold an important place in Indigenous-led food systems. At the same time, the incidence of foodborne illnesses is often higher among Indigenous Peoples' than in the general population. Climate change — with its impact on diets, the prevalence of pathogens, toxins and chemical contaminants and traditional practices of food preparation, processing and storage — threatens to further widen this health disparity, especially in the Arctic which is particularly sensitive to rising temperatures. The objective of this symposium is to draw attention to this problem by discussing the traditional knowledge, perspectives, and thoughts on the subject as well as reviewing literature published on the topic. For example, two-thirds of published papers identified addressed chemical hazards, notably heavy metals and chemical contaminants. Twenty-five percent of papers focused on microbial hazards, including bacteria, viruses and parasites. Seven percent of publications were about radiological hazards. At the conclusion of the symposium, participants should have a greater understanding of the scope and magnitude of food safety problems in the Arctic, identifying research and knowledge gaps on the subject, how these are changing, and be encouraged to co-create solutions to this food safety challenge.

Primary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Non PDG Sponsor (Organization, Association, etc.): FAO

Primary Topic Area: Viral and Parasitic Foodborne Disease

Secondary Topic Area: Food Safety Culture

Additional Topic Area (Optional): Food Chemical Hazards and Food Allergy

Keywords: Chemical Contaminant,Climate change,Parasites

S5 - SURF & TURF SAFETY: INNOVATIONS IN DRY-AGING MUSCLE FOODS

Primary Author:
Katheryn Parraga-Escalante, Purdue University

Persons:
Tori Stivers, University of Georgia Marine Extension, Retired

Evelyn Watts, LSU AgCenter & Louisiana Sea Grant

Phillip Bass, University of Idaho

Katheryn Parraga-Escalante, Purdue University

Michael Nelson, GW Fins

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

The aging of muscle foods, particularly beef, has long been studied and optimized for both flavor enhancement and texture improvement. Dry and wet aging techniques are well-established in the beef industry, with the U.S. dry-aged beef market valued at $10.4 billion in 2015. The primary objective of aging—whether dry or wet—is to concentrate flavor and tenderize the muscle tissue under controlled environmental conditions.

In contrast, the concept of dry-aging seafood, especially fish, is a relatively new and emerging trend in high-end culinary settings. Despite its growing popularity among chefs in the U.S., there is limited scientific literature and regulatory guidance addressing the food safety implications of this practice. Dry-aged fish is increasingly featured in fine dining, yet the safety parameters, microbial risks, and environmental controls required for safe consumption remain underexplored.

This symposium will examine current aging methodologies in the beef industry and explore how these practices may inform the development of safe and effective dry-aging techniques for seafood. Key topics will include:

Comparative analysis of dry vs. wet aging in beef

Overview of dry-aging practices for fish in culinary settings
Identification of critical food safety parameters and environmental controls
Data gaps and research needs in seafood aging
Potential considerations for regulatory oversight

Attendees will gain a comprehensive understanding of the science and safety behind muscle food aging, with a focus on translating established beef aging protocols to the emerging practice of fish dry-aging. The session will highlight the urgent need for interdisciplinary research and regulatory clarity to ensure consumer safety as this trend continues to evolve.

Primary PDG Sponsor: Seafood Safety and Quality PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Seafood Safety and Quality

Secondary Topic Area: Meat and Poultry Safety and Quality

Keywords: Meat and Poultry,Seafood

S6 - PORK UNDER THE MICROSCOPE: ADVANCES IN MITIGATING OLD AND EMERGING RISKS

Primary Author:
Byron Chaves, Rutgers University

Persons:
Mick (Joseph) Bosilevac, USDA-ARS

Byron Chaves, Rutgers University

Patrick Mies, Tyson

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Salmonella is a recognized pathogen and Shiga toxin-producing *Escherichia coli* (STEC) is an emerging hazard linked to pork products worldwide. While much progress has been made in understanding their biology and public health impact, important gaps remain in connecting microbial behavior to quantitative risk frameworks and in applying this knowledge to improve decision-making in industry and regulatory settings. This symposium will provide an integrated perspective on *Salmonella* and STEC in pork. The first presentation will focus on molecular risk assessment approaches for both *Salmonella* and STEC, including the use of genomic data to inform management strategies. The second presentation will examine growth and inactivation dynamics of *Salmonella* and STEC in pork, emphasizing predictive models, environmental factors, and intervention effectiveness across the supply chain. The third presentation will explore how scientific findings on *Salmonella* and STEC are incorporated into industry practice, with emphasis on the integration of risk-based tools into decision-making, alignment with regulatory expectations, and communication of outcomes to stakeholders. The purpose of this session is to bridge research, modeling, and application by combining experimental microbiology, molecular risk assessment, and industry perspectives. Attendees will gain a deeper understanding of how pathogen behavior models inform risk analysis, while also learning how the pork industry uses these insights to strengthen preventive controls and regulatory compliance. The symposium will highlight critical knowledge gaps and promote dialogue across academia, government, and industry

aimed at advancing pork safety through risk-based decision-making.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Microbial Modelling and Risk Analysis

Keywords: Genomics,HACCP,Meat and Poultry,Modeling,Pathogens,Risk Analysis

S7 - “IS CIRCULAR SAFE?” — BALANCING SUSTAINABILITY WITH FOODBORNE VIRAL RISKS

Primary Author:
Vijay Juneja, USDA-ARS-ERRC

Persons:
Vijay Juneja, USDA-ARS-ERRC

Shalini Sehgal, Bhaskaracharya College of Applied Sciences University of Delhi

Payal Gupta, Graphic Era Deemed to be University

Ragini Reddyvari, Oregon State University

Matthew Moore, University of Massachusetts Amherst

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

As global food systems move toward circular models to enhance sustainability and climate resilience, new challenges are emerging at the intersection of food safety and environmental health. Circular food systems aim to minimize waste and recover valuable resources, such as water, nutrients, and organic matter, for reintegration into food production through practices like composting, wastewater reuse, and by-product valorization. However, this transformation also raises critical concerns around viral contamination. Enteric viruses such as norovirus, hepatitis A virus (HAV), and rotavirus are environmentally persistent and can survive standard treatment processes, increasing the risk of transmission through contaminated water, produce, or food contact surfaces. The growing use of treated wastewater and organic waste in agriculture creates potential pathways for these pathogens to re-enter the food chain, especially in the absence of robust monitoring and risk management systems. The COVID-19 pandemic further highlighted the role of environmental surveillance, such as wastewater-based epidemiology, in public health preparedness, but also exposed gaps in our understanding of how to safely manage biologically active waste in food systems.

This symposium session will explore the trade-offs between advancing circularity and

ensuring virological safety in food production, with focus on emerging risks, detection technologies, treatment innovations, and policy gaps. There is a need to discover interdisciplinary solutions to manage viral hazards in circular systems and ensure that sustainability does not come at the cost of public health. The way forward is evidence-based strategies along with regulatory harmonization so as to build trust in circular food systems.

Primary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Primary Topic Area: Viral and Parasitic Foodborne Disease

Secondary Topic Area: Water Safety and Quality

Keywords: Environmental monitoring,Viruses,Water

S8 - GRAS 2.0: WHAT'S NEW; NOT SO NEW AND ITS IMPACT TOWARD INDUSTRY COMPLIANCE

Primary Author:
Anthony Flood, IFIC

Persons:
Nakia Smith, The Coca-Cola Company

Anthony Flood, IFIC

Kristi Muldoon Jacobs, USFDA

Alex Eapen, Cargill

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Generally Recognized as Safe (GRAS) — the U.S. Food and Drug Administration's (FDA) process for assessing the safety of certain food additives — is expected to see significant changes in the coming year. In the U.S., food additives remain a leading consumer food safety concern, fueled by skepticism, social media misinformation, and advocacy rhetoric. These factors, coupled with inconsistent interpretations of regulations, are eroding public trust in the system designed to safeguard food ingredients. Further complicating matters is a patchwork of state laws, which create regulatory uncertainty and place added burdens on manufacturers, suppliers, retailers, and enforcement agencies. Addressing these issues requires closing the knowledge gap with clear, science-based information about existing laws, regulations, and the GRAS process. This includes fostering better communication, sharing best practices among industry leaders, and incorporating consumer insights to strengthen transparency and build public confidence in the regulatory framework. This session offers a unique opportunity to hear directly from FDA officials and food industry leaders. Attendees will gain deeper insight into recent and upcoming GRAS developments, learn strategies for navigating state regulations, and take away practical guidance to enhance compliance and advance food and ingredient safety in their organizations.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Secondary PDG Sponsor: Developing Food Safety Professionals PDG

Non PDG Sponsor (Organization, Association, etc.): IFIC

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Law

Additional Topic Area (Optional): Communication, Outreach and Education

Keywords: Chemical Contaminant,Education,Public Health

S9 - FROM RISK TO REGULATION: FAO/WHO SCIENTIFIC ADVICE ON LISTERIA MONOCYTOGENES AND CODEX APPLICATIONS

Primary Author:

Jeffrey LeJeune, FAO

Persons:

Jeffrey LeJeune, FAO

Martin Duplessis, Bureau of Microbial Hazards, Food and Nutrition Directorate, Health Canada

Ana Allende, CEBAS-CSIC

Bing Wang, University of Nebraska Lincoln

Benjamin Warren, USFDA-CFSAN, Office of Food Safety

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

This symposium will explore the latest scientific advice developed by FAO and WHO on *Listeria monocytogenes*, with a focus on its application by the Codex Committee on Food Hygiene (CCFH). The session will highlight outcomes from recent FAO/WHO Joint Expert Meetings on Microbiological Risk Assessment (JEMRA), which have advanced global understanding of *L. monocytogenes* risks in ready-to-eat foods and informed international food safety standards. The evolving risk landscape of *L. monocytogenes* in fresh produce, including cantaloupe and leafy greens and frozen vegetables will be discussed. The need for production-to-consumption modeling and consideration of virulence profiles are reshaping risk categorization and mitigation strategies. The development of updated risk-assessment models and scenario testing will be presented. Risk models need to incorporate new data on host susceptibility, climate change, and hygiene practices, and are now available as open-source tools for global use. The symposium will be concluded with perspectives from a risk manger's perspective – how the Codex Committee for Food Hygiene is using scientific advice to revise its guidance on *L. monocytogenes*, particularly the *Codex Guidelines on the Application of General Principles of Food Hygiene to the Control of* Listeria monocytogenes *in Foods (CXG*

61-2007). Together, these talks will provide insight into the interface between science (risk assessment and policy (risk management, and the role of Codex in translating expert advice into global standards, offering valuable insights for regulators, industry professionals, students, and researchers.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Secondary PDG Sponsor: Seafood Safety and Quality PDG

Non PDG Sponsor (Organization, Association, etc.): FAO

Primary Topic Area: Epidemiology

Secondary Topic Area: Fruit and Vegetable Safety and Quality

Keywords: Regulations,Risk Assessment,Risk Management

S10 - FSVP AT THE BORDER: RISING FDA DETENTIONS AND HOW TO PREPARE

Primary Author:
Taryn Horr, Ramboll

Persons:
Tom Sidebottom, Regulatory Science Consulting, LLC.

Taryn Horr, Ramboll

Christopher Snabes, The Acheson Group

Dan Solis, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

The Foreign Supplier Verification Program (FSVP) has become one of FDA's most important tools for ensuring the safety of imported foods. By requiring U.S. importers to verify that foreign suppliers meet the same standards as domestic producers, FSVP reduces the risk of unsafe products entering commerce and builds confidence in the global food supply.

Enforcement is accelerating: between FY 2017 and FY 2025, FDA inspections increased 257%, with more than half (53.7%) resulting in citations. The most common finding, cited nearly 4,000 times, is the absence of an FSVP program. Other frequent deficiencies include gaps in supplier evaluations, hazard analyses, and verification activities, along with procedural issues such as missing English translations. These weaknesses heighten risks for both public health and trade continuity.

FDA can place foreign suppliers on Import Alerts. Import Alert 99-41, issued in January 2022, significantly expanded enforcement by authorizing detention without physical examination for repeat FSVP noncompliance. Once listed, importers and their customers face ongoing trade disruptions until corrective actions are verified.

This symposium will provide current FDA enforcement data, case studies, and practical strategies. Panelists will cover: enforcement trends and priority areas; the most common

compliance pitfalls; navigating Import Alert 99-41; and concrete steps to build defensible programs, strengthen supplier evaluations, and maintain inspection readiness. With perspectives from FDA and consultants across global supply chains, attendees will gain actionable, data-driven insights to improve FSVP compliance, protect consumers, and avoid costly border disruptions.

Primary PDG Sponsor: Food Law PDG

Secondary PDG Sponsor: Food Safety Assessment, Audit and Inspection PDG

Primary Topic Area: Food Law

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Additional Topic Area (Optional): International Food Protection Issues

Keywords: Auditing,Regulations,Risk Assessment

S11 - CULTURE OR MOLECULAR? RETHINKING WHEN AND WHY WE CONFIRM PATHOGENS

Primary Author:
Season (Yicheng) Xie, Bio-Rad Laboratories

Persons:
Season (Yicheng) Xie, Bio-Rad Laboratories

Jesse Miller, Neogen

Pamela Wilger, Post Consumer Brands

Rachel Binet, USFDA

Robert Barlow, CSIRO

Amy Bosco, Cargill

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Pathogen confirmation is no longer a one-size-fits-all exercise. For decades, cultural methods have been the gold standard, delivering isolates essential for typing and regulatory defensibility. Yet as molecular tools provide speed, specificity, and actionable data, the critical question is not which method to use, but when to use which method and for what purpose.

This symposium pushes beyond “old versus new” to examine how confirmation strategies drive real-world food safety decisions. From a regulatory lens, we will explore how zero-tolerance policies and genus-level confirmations intersect with the realities of mixed-strain enrichments, and how risk assessment could reshape confirmation choices. From the industry side, we will tackle the operational consequences of molecular versus cultural positives, where minutes matter for product release, yet defensibility still requires live isolates. Internationally, the discussion will draw on Australian export-focused use cases to examine how diverse importing country requirements shape risk–reward decisions in testing strategies, and how the shift toward molecular and digital assurance systems is influencing speed, accuracy, and market access. Finally, an industry-wide risk assessment perspective will

show how pathogen history, plant-level data, and evolving technologies guide the choice of screening and confirmation methods, ensuring strategies remain compliant, data-driven, and adaptive.

At stake is more than method performance: it is the ability to make timely, risk-based, and defensible decisions in contexts ranging from outbreak prevention to global commerce. By reframing pathogen confirmation around purpose, consequence, and context, this session challenges entrenched practices and provokes a progressive dialogue on how to deploy both culture and molecular methods to strengthen food safety worldwide.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Non PDG Sponsor (Organization, Association, etc.): Meat and Poultry Safety and Quality PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Applied Laboratory Methods

Additional Topic Area (Optional): Meat and Poultry Safety and Quality

Keywords: Laboratory and Detection Methods,Pathogens,Public Health,Risk Assessment

S12 - KEEPING PARASITES OUT OF IMPORTED PRODUCE: LEARN HOW TO MITIGATE CYCLOSPORA CONTAMINATION IN FRESH HERBS

Primary Author:
Oscar Galagarza, USFDA

Persons:
Oscar Galagarza, USFDA

Samir Assar, USFDA

Alexandre Da Silva, USFDA

Mauricio Castelo, USFDA

Noemi Zuniga Thimeos, Inter-American Institute for Cooperation on Agriculture

Robert Mandrell, Produce Safety and Microbiology Research Unit, USDA-ARS, Western Regional Research Center (Retired)

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Imported fresh herbs such as basil, cilantro, and parsley from Latin America have been linked to multiple *Cyclospora cayetanensis* contamination events and foodborne illness outbreaks in the United States in recent years. In response, the U.S. Food and Drug Administration (FDA) has launched prevention initiatives focused on stakeholder engagement and collaboration across Latin America to support the goals and objectives of the FDA's *Cyclospora* Prevention, Response, and Research Action Plan. These international efforts foster a two-way exchange: local authorities and industry stakeholders gain critical knowledge about *C. cayetanensis* and its public health risks, while FDA deepens its understanding of regional agricultural practices to identify opportunities for safer herb production. This session will highlight education, outreach, and prevention activities carried out in Colombia and Mexico over the past two years, targeting herbs associated with recent outbreaks and microbiological findings. It will also explore progress made and ongoing challenges in working with growers—key partners in ensuring the safety of imported herbs. Attendees will gain valuable insight into the

collaborative efforts of the FDA and in-country partners to promote food safety culture and enhance safe production practices for herbs throughout Colombia and Mexico.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Secondary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Primary Topic Area: Fruit and Vegetable Safety and Quality

Secondary Topic Area: Viral and Parasitic Foodborne Disease

Additional Topic Area (Optional): Food Safety Culture

Keywords: Food Safety Culture,Parasites,Produce

S13 - SALMONELLA IN TAHINI AND SESAME PRODUCTS: FDA’S MULTI-PRONGED APPROACH THROUGH REGULATORY OVERSIGHT AND AI-TARGETED SAMPLING

Primary Author:
Aparna Tatavarthy, USFDA

Persons:
Aparna Tatavarthy, USFDA

Elizabeth Grasso-Kelley, USFDA

Chuck Hassenplug, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Tahini, made from sesame seeds, and related products including halva and hummus, has been linked to *Salmonella* outbreaks. Recent outbreaks involving tahini highlight food safety challenges with this ready-to-eat (RTE) product category. FDA's September 2025 Foodborne Outbreak Overview of Data (FOOD) Report documented four outbreaks from 2011–2019, resulting in 61 illnesses across 22 states, underscoring the ongoing public health concern. Tahini's low-water activity (0.18) and high fat content (58%) supports *Salmonella* survival for extended periods while providing thermal protection during processing. These characteristics, combined with the product's RTE nature and use in downstream products present significant public health risks. Most sesame seeds and tahini consumed in the U.S. are imported from countries with varying food safety infrastructure, further complicating control efforts.

Under FDA's Preventive Controls for Human Food Rule, tahini manufacturers must identify *Salmonella* as a hazard and implement validated preventive controls. However, inspection findings reveal inconsistent industry practices regarding hazard analysis, environmental monitoring, and process validation. Critical processing steps including soaking, drying, and roasting present challenges, as extended soaking periods can lead to significant *Salmonella* growth, while insufficient roasting may fail to achieve adequate pathogen reduction due to

increased thermal resistance in low-water activity conditions. To enhance regulatory oversight, FDA has developed machine learning models to improve targeting of tahini sampling. This data-driven approach, combined with enhanced inspection protocols represents a comprehensive strategy to address recurring contamination issues.

Speakers will discuss challenges posed by tahini processing, regulatory requirements, FDA's approach to evaluating preventive controls, and how FDA is leveraging advanced analytics to strengthen compliance and sampling strategies.

Primary PDG Sponsor: Low Water Activity Foods PDG

Secondary PDG Sponsor: Food Hygiene and Sanitation PDG

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Food Hygiene and Sanitation

Keywords: Low-water Activity Foods,Outbreak,Ready to Eat Foods

S14 - ANTIMICROBIALS FOR FOODBORNE PATHOGENS, SPOILAGE MICROORGANISMS, AND NAVIGATING FDA GRAS APPROVAL FOR NOVEL COMPOUNDS

Primary Author:
Joshua Gurtler, USDA-ARS

Persons:
Joshua Gurtler, USDA-ARS

Layal Karam, Qatar University

Rick Falkenberg, Food Safety and Process Technology Group, Inc.

Katie Overbey, FDA-HFP

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

One method for ameliorating problems with pathogenic or spoilage microorganisms in foods is applying bacteriostatic or bactericidal antimicrobials. Although various compounds are currently used, there are problems associated with (1) inappropriate application strategies, (2) a shift from synthetic additives (which are either no longer permitted or are not preferred by consumers) to clean-label additives, (3) developing new food-grade compounds, and (4) understanding FDA requirements for food ingredients, including new antimicrobials. The first speaker in this symposium will discuss advances in food antimicrobials and associated research, including synthetic vs. organic compounds, nanoencapsulation, effective application concentrations (based on food type), sensory thresholds for additives that affect organoleptic properties, and developing novel antimicrobials. The second talk will focus, more specifically, on compounds to inhibit spoilage bacteria and fungi in various food products (including cooked or raw meat and poultry, fresh produce, and processed or other ready-to-eat foods). Best practice recommendations will be provided, per the targeted food commodity, including antimicrobial selection, concentration efficacy (based on microorganisms of interest [e.g., bacteria, lactic acid bacteria, yeast, mold, etc.]), and recommended future directions for the food industry and researchers. Finally, the last speaker will discuss food ingredient regulation in the U.S., including an overview of FDA’s

notification process for GRAS ingredients. This symposium will be helpful for food industry personnel, to mitigate problems with foodborne pathogens and spoilage microorganisms, as well as for researchers conducting related studies and/or who are developing novel antimicrobials.

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Beverages and Acid/Acidified Foods

Keywords: Antimicrobials,Regulations,Spoilage

S15 - BEYOND DETECTION: HOW INDUSTRY IS USING NEXT GENERATION GENOMIC SEQUENCING TO DRIVE ROOT CAUSE ANALYSIS

Primary Author:
Benjamin Miller, The Acheson Group

Persons:
Benjamin Miller, The Acheson Group

Julie Haendiges, Theiagen Genomics

Joel Sevinsky, Theiagen Genomics

Jerri Lynn Pickett, Tyson Foods

Leslie Hintz, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

While whole-genome sequencing (WGS) and next-generation sequencing (NGS) have proven invaluable in outbreak investigations, many food manufacturers remain hesitant to incorporate these tools into their environmental monitoring and corrective action programs. The perceived liability of generating sequencing data, coupled with concerns about regulatory scrutiny, has created a paradox: manufacturers fear they cannot afford the risk of using these technologies, yet mounting evidence suggests they cannot afford NOT to use them.

This symposium moves beyond the traditional "why you should sequence" narrative to focus on practical implementation strategies currently used by the food industry, while drawing parallels to healthcare's adoption of genomic surveillance for outbreak prevention and infection control. Through real-world examples, presenters will show how companies use genomic technologies to strengthen root cause analyses, manage risk, and satisfy regulatory expectations. Once viewed as complex, costly, and potentially exposing operational vulnerabilities, these tools are now recognized in healthcare as essential to infection prevention and patient safety.

The session will contrast documented failures, where lack of pathogen differentiation led to major recalls or warning letters, with success stories where proactive sequencing operationally enhances food safety management systems. It will offer practical guidance on selecting sequencing strategies based on facility risk, product type, and resource availability, making these technologies accessible to operations of all sizes.

This evidence-based discussion will help quality assurance professionals, food safety managers, and consultants understand how to use next-generation genomic data as a key component of modern food safety management systems, ultimately demonstrating that strategic use of sequencing represents risk mitigation, not risk creation.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Additional Topic Area (Optional): Microbial Modelling and Risk Analysis

Keywords: Environmental monitoring,Genomics,Risk Management

S16 - ANTIMICROBIAL INTERVENTIONS FOR MEAT SAFETY AND MICROBIAL QUALITY: PRESENT AND THE FUTURE

Primary Author:
Xianqin Yang, Agriculture and Agri-Food Canada

Persons:
Xianqin Yang, Agriculture and Agri-Food Canada

Roopesh Syamaladevi, University of Alberta

Tyson Brown, Cargill

Hany Anany, Agriculture & Agri-Food Canada

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Livestock carries a large number of bacteria on their hide/skin and in their gastrointestinal tracts, most of which are harmless to humans. Some, however, may lead to premature food spoilage and others can be significant human pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella*. To reduce meat contamination with those significant pathogens, many meat plants have implemented HACCP-based systems. These often incorporate decontaminating treatments, including carcass pasteurization with hot water or steam, and washing carcasses with organic acids. The entire facility is cleaned and sanitized on a daily-basis, to further control microbial contaminants. A significant body of research has been produced investigating decontamination technologies and assessing the efficacy of in-use decontaminating treatments in commercial meat packing plants. These efforts have resulted in significant improvement in the microbial conditions of meat and fewer incidences of human infections. However, this is not without significant environmental cost reflected in e.g., chemical, water and energy usage. In recent years, a good number of studies have examined the potential of novel, chemical-free technologies such as cold plasma, UV-C LED, and ozone nanobubbles to reduce the microbial load on meat surface and equipment surface and controlling biofilms. The objective of this session is to provide a forum to put into perspective the current in-use of decontamination treatments in meat processing and recent/ongoing research findings demonstrating efficacy of those alternative novel

technologies and what is needed to move them from research labs to commercial facilities.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Food Hygiene and Sanitation

Keywords: Meat and Poultry,Pathogens,Water

S17 - HOW TO DECIDE? RISK-BASED DECISIONS FOR THE USE OF DRIED INGREDIENTS

Primary Author:
Heidy Den Besten, Wageningen University

Persons:
Heidy Den Besten, Wageningen University

Linda J. Harris, University of California-Davis

Rob Limburn, Campden BRI Group

Jennifer Acuff, University of Arkansas

Polly Courtney, General Mills, Inc.

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Low-water activity ingredients such as dried herbs, fruits, vegetables, and spices are widely incorporated into both ready-to-eat (RTE) and non-RTE foods. The diversity of these applications can lead to inconsistent understanding among food service and food processors regarding the microbiological risks and appropriate mitigation or intervention strategies. Some pathogens can survive in low-water activity foods for prolonged periods and depending on the infectivity and subsequent handling of the material in a finished food, can pose a foodborne disease risk. Frequent recalls and outbreaks linked to dried ingredients highlight the challenges of controlling the associated food safety risks. To address this issue, an international, multidisciplinary expert group recently convened under the aegis of the European Branch of the International Life Science Institute, and developed a framework to support risk-based decisions for using dried ingredients in RTE and non-RTE foods. This symposium will review the microbiological hazards associated with dried ingredients and examine how drying conditions, storage, and post-drying treatments influence pathogens levels. A microbiological risk-based decision framework will be presented along with practical examples of how the decision tool can be used to rank risks and determine the best risk mitigation approaches for dried ingredients in RTE and non-RTE food applications.

Primary PDG Sponsor: Low Water Activity Foods PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Microbial Modelling and Risk Analysis

Additional Topic Area (Optional): International Food Protection Issues

Keywords: Intervention,Low-water Activity Foods,Risk Management,Validation

S18 - MANAGING THE INVISIBLE THREAT OF MYCOTOXINS: EMERGING REGULATORY FRONTIERS AND FUTURE DIRECTIONS FOR RISK MANAGEMENT

Primary Author:
Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Persons:
Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Amanda Jones, AFB International

Michele Sayles, Diamond Pet Food

Beilei Ge, USFDA

Christopher Archer, Nestlé Purina

Giannis Stoitsis, Agroknow

Marlynne Hopper, World Trade Organization

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Mycotoxins remain a global food safety challenge, impacting human and animal health, supply chains, and international trade. While aflatoxins, fumonisins, and DON are regulated, numerous emerging or emerging mycotoxins lack defined action levels, creating uncertainty for regulators and industry alike. This symposium will provide a global perspective on mycotoxin risk management, drawing on the Pet Food Institute (PFI) Mycotoxin Task Force Report, and comparing European (EFSA) and North American regulatory approaches. Speakers will highlight monitoring and testing program design, industry-driven strategies, and future directions for risk management of unregulated mycotoxins. The session will also showcase new tools—ranging from AI-driven predictive analytics to rapid detection technologies—that are reshaping mycotoxin monitoring. Attendees will gain insights into regulatory trends, scientific advancements, and practical approaches to improve safety while reducing trade and compliance challenges.

Primary PDG Sponsor: Animal and Pet Food Safety PDG

Secondary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Primary Topic Area: Animal and Pet Food Safety

Secondary Topic Area: Food Chemical Hazards and Food Allergy

Additional Topic Area (Optional): Data Management and Analytics

Keywords: Animal and Pet Food Safety,Chemical Contaminant,Data,Laboratory and Detection Methods,One Health,Regulations,Risk Management,Toxicology,Validation

S19 - BIOFILMS AND VIRUSES: UNVEILING HIDDEN RISKS IN THE FOOD CHAIN

Primary Author:
Irene Falcó, USDA-ARS, EMFSL

Persons:
Irene Falcó, USDA-ARS, EMFSL

Doris D'Souza, University of Tennessee

Hany Anany, Agriculture & Agri-Food Canada

Gloria Sánchez, Institute of Agrochemsitry and Food Technology

Julie Jean, Universite Laval

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Biofilms are well established as reservoirs for bacterial pathogens and spoilage organisms in the food industry, driving contamination, persistence, and hygiene failures. Yet, the role of biofilms in the ecology and transmission of foodborne viruses remains largely overlooked. Enteric viruses such as human norovirus, hepatitis A and E viruses, and rotavirus are leading causes of foodborne illness outbreaks, responsible for millions of cases annually and major public health and economic burdens. These viruses do not replicate in food, but their low infectious dose, high stability, and resistance to disinfectants make them persistent contaminants across the food chain. Emerging studies suggest that viruses may interact with bacterial biofilms and extracellular polymeric substances, enhancing their attachment, stability, and survival in food environments. Environmental and clinical data also indicate that virus–bacteria interactions can influence infectivity, yet direct evidence of infectious viral particles within food-related biofilms is lacking. This knowledge gap limits our ability to fully assess risks and design effective control strategies in retail food safety and public health contexts.

This symposium will bring together experts in virology, biofilm biology, and food safety to address the limited but growing evidence of virus–biofilm interactions. Discussions will cover

molecular insights into virus–bacteria binding, detection approaches for viruses in biofilm matrices, and implications for contamination control in retail food systems. By focusing on this underexplored dimension of viral persistence, the session aims to foster cross-disciplinary dialogue, set research priorities, and promote strategies to strengthen food protection and safeguard public health.

Primary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Secondary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Primary Topic Area: Viral and Parasitic Foodborne Disease

Secondary Topic Area: Fruit and Vegetable Safety and Quality

Keywords: Food Safety Culture,Produce,Viruses

S20 - AI-POWERED FOOD SAFETY: PREDICTIVE MODELING, PATHOGEN DETECTION, AND BEYOND

Primary Author:
Samet Ozturk, Department of Food Engineering, Gümüşhane University

Persons:
Joyjit Saha, Kerry

Samet Ozturk, Department of Food Engineering, Gümüşhane University

Surabhi Wason, Kerry

Nitin Nitin, Dept. of Food Science and Technology, University of California Davis

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Foodborne illnesses impact 1 in 6 Americans each year, leading to significant public health and economic burdens. As the food industry adapts to increasing complexity and demand, the convergence of Artificial Intelligence (AI), machine learning (ML), big data, and the Internet of Things (IoT) is driving a new era of innovation in food safety. These technologies enable enhanced monitoring, predictive modeling, and rapid detection capabilities across the agri-food system, from farm to fork.

AI and ML algorithms integrate statistical, probabilistic, and optimization approaches to reveal hidden patterns in complex datasets. Applications in the food sector range from real-time microbial growth modeling to predictive risk assessments and non-destructive pathogen identification. IoT networks and sensor systems generate continuous streams of data that, when analyzed using AI, improve agricultural monitoring, supply chain transparency, and food safety protocols. Advances in gene sequencing, spectral analysis, and blockchain traceability further demonstrate how AI can transform both preventive and responsive food safety strategies.

This symposium will showcase tools and strategies that enhance analytics, data integration, and predictive modeling across the food system. The first presentations will introduce AI concepts, with emphasis on the development of accurate microbial growth models and

predictive tools using regression and machine learning approaches. Subsequent talks will showcase research on AI-driven pathogen detection and its implications for both academia and industry. Finally, the session will address the evolving regulatory landscape, discussing the frameworks necessary to validate AI-driven tools and ensure they meet regulatory standards and safety compliance.

Primary PDG Sponsor: Data Management and Analytics PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: Microbial Modelling and Risk Analysis

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Additional Topic Area (Optional): Fruit and Vegetable Safety and Quality

Keywords: Environmental monitoring,Laboratory and Detection Methods,Meat and Poultry,Modeling,Pathogens,Produce,Risk Analysis

S21 - BEST PRACTICES IN SALMONELLA AND CRONOBACTER CONTROLS IN A LOW-MOISTURE FACILITY USING DAIRY EXAMPLES

Primary Author:
Tim Stubbs, Dairy Management Inc.

Persons:
Tim Stubbs, Dairy Management Inc.

Abigail B. Snyder, Cornell University

Karen McCarty, Innovation Center for U.S. Dairy

Montgomery Bohanan, Leprino Foods

Dan Belina, Land O'Lakes

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Controlling pathogens in low-moisture foods, including *Salmonella* and *Cronobacter* in dairy powders, has been a major goal of the dairy industry for decades with great progress driven by improved design, plant practices, sanitation, and environmental monitoring. That said, there has been extensive new learning across the industry driven by an unprecedented investment in new facilities throughout the U.S. Because food safety is not competitive, we have spent more than a year convening experts to share their best practices with the goal of aligning and then sharing our collective knowledge in presentations, webinars, and eventually as published guidance.

In this session, we will hear from three industry experts sharing our learnings sorted by focus area, but all woven around a single case study. This three part symposium will focus on 1) Best practices in plant & equipment design and operational GMPs, 2) Environmental Monitoring for enhanced *Salmonella* and *Cronobacter* controls, including the use of WGS, metagenomics, and other emerging tools, and 3) RCA/CAPA in a LM facility using EM data.

Primary PDG Sponsor: Dairy Quality and Safety PDG

Secondary PDG Sponsor: Low Water Activity Foods PDG

Non PDG Sponsor (Organization, Association, etc.): Innovation Center for US Dairy.

Primary Topic Area: Dairy Quality and Safety

Secondary Topic Area: Low Water Activity Foods

Additional Topic Area (Optional): Advanced Molecular Analytics

Keywords: Dairy,Pathogens,Production/Plant Design

S22 - UPGRADING ENVIRONMENTAL MONITORING THROUGH MICROBIOME MAPPING IN FOOD PRODUCTION PLANTS

Primary Author:
Luxin Wang, University of California Davis

Persons:
Luxin Wang, University of California Davis

Kalliopi Rantsiou, University of Turin

Jasna Kovac, The Pennsylvania State University

Jack Burnett, Vikan

Avelino Álvarez Ordóñez, University of León

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Environmental monitoring is widely recognized as a critical activity for verifying the effectiveness of cleaning and sanitation programs and for detecting potential microbial contamination that could compromise food safety. Moreover, contamination originating from the processing or packaging environment has often been identified as a contributing factor in spoilage incidents. For decades, environmental monitoring in the food industry has relied on a *presence/absence* model, yielding a simple *yes/no* result that frequently overlooks the ecological context. Key questions, such as *Why is the pathogen present?* and *Is it a transient, incidental finding, or part of a persistent, niche-adapted community?*, often remain unanswered. This “positive/negative” approach inevitably ignores the broader ecological dynamics and the diversity of the environmental microbiota. Adopting microbiome-oriented approach offers several advantages and is increasingly being explored as an integrative tool for profiling microbial communities associated with post-harvest food production environments. This symposium advocates for moving beyond purely targeted testing by incorporating both targeted and untargeted metagenomics. It addresses the question of *“Why switch, and why now?”* and discusses how advances in Next-Generation Sequencing

(NGS have made plant-scale microbiome analysis operationally feasible. By featuring contributions from academic and industry experts worldwide, the symposium will also examine the practical and business trade-offs between traditional Environmental Monitoring Programs (EMPs) and metagenomic approaches.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Food Hygiene and Sanitation

Keywords: Antimicrobial Resistance,Environmental monitoring,Microbiome

S23 - USING DATA TO CHARACTERIZE RISK AND PREVENT LISTERIA MONOCYTOGENES CONTAMINATION: CHALLENGES AND OPPORTUNITIES

Primary Author:
John Jarosh, USDA-FSIS

Persons:
John Jarosh, USDA-FSIS

Kristina Barlow, USDA-FSIS

Isabel Walls, USDA-FSIS

Sharon Beals, BeaconPoint Labs

Kathleen Glass, University of Wisconsin-Madison

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

In the USA, food processing establishments and government agencies use microbial testing of products, food contact surfaces, and the processing environment to verify sanitation and to identify food-safety risks that could lead to contamination. Recent outbreaks and recalls in Ready-to-Eat meat and poultry products, however, demonstrate the continued food safety challenge of predicting *Listeria monocytogenes* (*L. monocytogenes*) contamination.

In response to these outbreaks and recalls, USDA Food Safety and Inspection Service has been collecting data on specific *L. monocytogenes*-related risk factors, increased the number of in-person Food Safety Assessments (FSA), and expanded verification testing to include *Listeria* spp., in addition to increasing testing for *L. monocytogenes*. The goal of these efforts is to expand the types of data collected by the Agency, such as sanitation and processing records, results from FSAs, enforcement reports, and sanitation indicator results, to help better predict future contamination.

This symposium will focus on ways that industry, retailers, government agencies, and academia use data to create opportunities to identify food safety risks and keep foodborne

outbreaks from occurring.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Microbial Modelling and Risk Analysis

Keywords: Meat and Poultry,Pathogens,Ready to Eat Foods

S24 - MIXED SIGNALS: THE PREVALENCE AND RISKS OF MULTI-GENOTYPE PATHOGEN CONTAMINATION

Primary Author:
Cameron Parsons, Merieux NutriSciences

Persons:
Cameron Parsons, Merieux NutriSciences

Angela Nguyen, Merieux NutriSciences

Margaret Kirchner, FDA CORE

Catharine Carlin, IEH Laboratories and Consulting Group

Baback Gharizadeh, Chapter Diagnostics

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Surveillance and sequencing efforts routinely operate under the assumption that contaminated food samples harbor a single pathogen genotype. Increasingly, when positive samples are surveyed correctly, multiple genotypes of the contaminating pathogen are found. These multi-genotype positive samples complicate outbreak investigations, risk assessments, and downstream laboratory workflows. Traditional detection methods often underrepresent this diversity, masking variation that can drive virulence, antimicrobial resistance, illness and transmission dynamics. Recent studies indicate that multi-genotype contamination is more prevalent in the food supply than previously recognized, underscoring the urgent need for advanced tools and strategies to address it.

Innovations in metagenomics, targeted sequencing, and bioinformatics pipelines now allow for the separation and characterization of distinct pathogen genotypes from mixed samples. Complementary approaches, including improved enrichment protocols, selective culturing, and marker gene tracking, provide additional resolution for both regulatory monitoring and academic research. Together, these advances are transforming our ability to detect, quantify, and interpret the complexity of multi-genotype contamination events.

This presentation will highlight the public health implications of multi-genotype pathogen positives, summarize current knowledge on their prevalence across the food supply chain, and showcase cutting-edge methodologies being deployed to better detect and characterize them. By bringing attention to this underappreciated challenge, we aim to encourage broader adoption of innovative approaches that will strengthen outbreak response, improve source attribution, and ultimately safeguard the food supply.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Applied Laboratory Methods

Keywords: Genomics,Laboratory and Detection Methods,Pathogens

S25 - BUILDING A DATA-DRIVEN CULTURE TODAY FOR SAFER FOOD TOMORROW

Primary Author:
Barbara Kowalcyk, George Washington University

Persons:
Devin LaPolt, George Washington University

Naim Montazeri, University of Florida

Amani Babekir, Ecolab

Barbara Kowalcyk, George Washington University

Brendan Ring, Creme Global

Andrew Kennedy, iFoods DS

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Data are crucial to deriving actionable insights that can improve food safety. The true potential is only realized when organizations foster a strong data-driven culture. Unlocking this potential requires more than new tools or technologies — it calls for a fundamental change in how we think about, collect, analyze, and apply data in everyday decision-making. In other words, it requires a data culture that empowers people and organizations to leverage data to detect, respond to, anticipate, and prevent food safety issues before they occur. An effective data culture is a mindset where data are valued, trusted, and ethically used by everyone in the organization – leadership to front-line workers – to inform decision-making and prevent foodborne illness. Establishing such a culture is not easy – it requires visible, sustained leadership commitment, a workforce that is data literate, a strategy for securing fit-for-purpose quality data, clear data governance guidelines, timely access to quality data, and the ability to effectively communicate data to a broad group of stakeholders. This symposium will include presentations highlighting real-world challenges and strategies for embedding data culture into practice, including workforce development, trust-building, and effective data communication. Audience members will also be engaged in

a robust panel discussion on:

- What a strong data culture looks like in the food safety context
- The most common barriers to data culture in food safety, and how they can be addressed
- How cross-functional teams can collaborate to make data more actionable
- The role emerging technologies play in shaping the future of food safety data
- How professionals at all levels can contribute to building and sustaining a data-driven culture

This session aims to define practical steps to build and sustain strong data-driven cultures that not only enhance food safety outcomes but also deliver broader organizational and societal benefits.

Primary PDG Sponsor: Data Management and Analytics PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: Food Safety Culture

Additional Topic Area (Optional): Microbial Modelling and Risk Analysis

Keywords: Data,Food Safety Culture,Risk Analysis

S26 - NEW SEQUENCING TOOLS FOR IMPROVED PARASITE GENOTYPING AND SOURCE TRACKING IN FOOD

Primary Author:
Jenny Maloney, ARS-USDA

Persons:
Jenny Maloney, ARS-USDA

Sonia Almeria, USFDA

Monica Santin-Duran, USDA

Susan Leonard, USFDA

Guy Robinson, Cryptosporidium Reference Unit, Public Health Wales Microbiology

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Food safety regulatory agencies worldwide are increasingly concerned with the presence and prevalence of parasites in food and water. These concerns stem from the fact that outbreaks linked to food are consistently reported, yet contamination sources continue to be difficult to identify. One technical issue that may contribute to this knowledge gap is that traditional sequencing strategies are not well suited for use in complex matrixes such as food and water. Robust and accurate parasite genotyping is key to improving source tracking and enhancing food safety. Thus, research laboratories around the world are working to develop massively parallel high-throughput sequencing strategies for characterizing foodborne parasites. In this symposium, researchers from U.S. and international food safety institutions will provide updates on how new sequencing tools have been applied to develop genotyping strategies for parasites in food. Speakers will present results from targeted amplicon sequencing and whole genome sequencing with details on how these methods have improved genotyping and source tracking of foodborne parasites including *Cyclospora cayetanensis, Cryptosporidium parvum,* and *Giardia duodenalis*.

Primary PDG Sponsor: Viral and Parasitic Foodborne Disease PDG

Secondary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Viral and Parasitic Foodborne Disease

Secondary Topic Area: Advanced Molecular Analytics

Keywords: Parasites,Produce,Public Health

S27 - RISK, SYSTEM, CULTURE, AND BUSINESS STRATEGY IN FOOD SAFETY

Primary Author:
Lone Jespersen, Cultivate SA

Persons:
Shingai Nyarugwe, University of Central Lancashire

Lone Jespersen, Cultivate SA

Conrad Choiniere, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

This symposium will explore the evolving landscape of food safety, focusing on the convergence of risk, systems thinking, organizational culture, and business strategy. As the global food industry faces increasing complexity, traditional food safety approaches are being re-evaluated. Organizations are shifting toward integrated, predictive models that anticipate and prevent hazards. A food safety risk profile, a comprehensive data-driven tool, serves as a leading indicator of food safety performance.

The symposium will feature experts and practitioners who have led transitions from reactive to proactive food safety systems. They will present case studies, frameworks, and practical tools demonstrating how risk profiling can integrate food and people safety, guide strategic decisions, and improve operational outcomes.

A central theme is aligning food safety with enterprise risk management and broader business strategy, ensuring food safety is embedded in organizational governance and planning. A case study from a leading food company will illustrate how risk profiling can identify high-risk areas, engage cross-functional teams, and drive measurable improvements. The symposium will also explore the regulatory perspective, featuring insights from the Canadian Food Inspection Agency (CFIA). This reflects a shift in regulatory philosophy from uniform compliance checks to risk-based prioritization. In addition to technical and strategic insights, the symposium will emphasize the human dimension of food

safety, addressing how to engage frontline workers, foster accountability, and ensure leadership alignment. The role of organizational culture in sustaining food safety improvements will be explored through examples from global organizations.

Secondary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Keywords: Data,HACCP,Risk Analysis,Risk Management

S28 - MAPPING RISK TO REALITY: APPLYING BIOMAPPING AND QMRA TO STRENGTHEN POULTRY SAFETY

Primary Author:
Angélica Godínez Oviedo, Universidad Autónoma de Querétaro

Persons:
Francisco Garces-Vega, Consultant

Angélica Godínez Oviedo, Universidad Autónoma de Querétaro

Matthew Stasiewicz, University of Illinois

Surabhi Wason, Kerry

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

The rapid growth of poultry production continues to challenge industry and regulators to ensure product safety. During harvesting, processing, and handling, poultry is vulnerable to contamination by pathogenic bacteria, particularly *Salmonella*. Addressing these challenges requires data-driven approaches that improve visibility, control, and decision-making across the production chain.

Biomapping generates a quantitative profile of microbial loads, including indicators and pathogens, throughout live production and processing. These data provide valuable insights into contamination dynamics and serve as a foundation for Statistical Process Control (SPC), enabling benchmarking, intervention validation, and continuous improvement. Quantitative Microbial Risk Assessment (QMRA), in turn, applies prevalence and concentration data within dose–response frameworks to quantify potential health risks and prioritize interventions at both plant and regional levels.

By integrating biomapping into a QMRA framework, stakeholders can connect process-level monitoring with broader risk assessments, supporting both operational decision-making and public health strategies. This dual approach can improve antimicrobial and plant sanitation practices, enhance food safety interventions, and guide regulatory policies based on real-world data.

Symposium attendees will learn about:

The role of biomapping to track microbial load throughout different stages of poultry processing

How the integration of QMRA with pathogen genotypic and phenotypic characteristics enhances risk assessment

How biomapping and QMRA tools have been effectively applied to address contamination risks and optimize food safety practices within the poultry industry and consumption chain

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: Meat and Poultry Safety and Quality

Secondary Topic Area: Microbial Modelling and Risk Analysis

Keywords: Antimicrobial Resistance,Meat and Poultry,Modeling,Risk Assessment,Traceability

S29 - FROM DATA TO DECISION: PREDICTIVE MODELING, VALIDATION AND LISTERIA CONTROL IN RTE FOODS

Primary Author:
Gijs Lommerse, Kerry

Persons:
Eelco Heintz, Kerry

Gijs Lommerse, Kerry

Marcel Zwietering, Wageningen University

Samuel Hadden, Conagra Brands

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Predictive modeling is transforming food safety, offering tools that move beyond retrospective data to proactive decision-making. While *Listeria* remains a persistent threat in ready-to-eat (RTE) foods, predictive approaches are increasingly being applied across multiple foodborne pathogens, spoilage microorganisms and shelf-life challenges. A recent *Listeria monocytogenes* outbreak associated with RTE meat highlights the critical need for predictive modeling platforms that can proactively identify and control the outgrowth of *Listeria* contaminants in processed foods.

Speakers will discuss how models and data-driven approaches can predict microbial behavior in foods, how models can be validated and tailored for industrial application and potentially can be integrated with HACCP systems to minimize risks after processing. Case studies will demonstrate both successes and limitations from preventing outbreaks to improving food safety and industrial adoption.

This symposium will bring together thought leaders in the field of predictive microbiology to discuss how the application of models in both regulatory frameworks and industry practices can help prevent future incidents. Furthermore, it can be used in product formulation to create food safety by design. The novel model distinguishes itself from existing models by including propionates as parameter, extensive determination of cardinal parameters and

validation with over 500 food product data. This predictive capability is essential for helping small and large food businesses alike proactively manage risk of *Listeria* growth and avoid costly recalls.

Primary PDG Sponsor: Modelling and Risk Analysis PDG

Secondary PDG Sponsor: International Food Protection Issues PDG

Primary Topic Area: Microbial Modelling and Risk Analysis

Secondary Topic Area: International Food Protection Issues

Keywords: Antimicrobials,Meat and Poultry,Modeling,Pathogens,Ready to Eat Foods,Shelf-life,Validation

S30 - A FOOD SAFETY DATA STANDARD: UPDATE AND REPORT

Primary Author:
Joseph Heinzelman, Neogen

Persons:
Angela Anandappa, Alliance for Advanced Sanitation

Joseph Heinzelman, Neogen

James Doyle, Creme Global

Vikrant Dutta, bioMerieux Inc.

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Building on the momentum of the 2025 session on ‘Creating a Food Safety Data Standard”, this roundtable will deliver an update on the initiative’s progress, highlight critical milestones, and share the status of the data standard’s development. Leaders from a diverse coalition of companies, systems, and business sectors will collaboratively review the latest draft of the data standard.

Key Milestones: An overview of pivotal achievements and progress markers in the development of the data standard.
Draft Publication Review: In-depth examination of the core components within the current draft, emphasizing foundational elements and practical implications.
Strategic Positioning: Discussion of current strategies and the role of the data standard in driving industry-wide food safety improvements.
Governance & Feedback: Clear articulation of the feedback mechanisms, planned adjustments, and the evolving governance structure required to ensure transparency and broad stakeholder engagement.

This session marks the first update for the IAFP audience for the public release of the data standard and the initial round of feedback from industry stakeholders. The roundtable’s primary focus is to engage participants in a robust dialogue about the initial draft, outline

the current publication process, and introduce actionable strategies for the standard’s future dissemination. The session will conclude with a detailed overview of the governance framework, both as it exists today and as proposed for the next phase, ensuring ongoing relevance, adaptability, and industry alignment.

Primary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: Food Safety Culture

Keywords: Data,Environmental monitoring,Food Safety Culture

S31 - TECHNOLOGIES FOR IMPROVING RECALL MANAGEMENT IN RETAIL AND FOODSERVICE

Primary Author:

Tracie Sheehan, Mérieux NutriSciences

Persons:

Tracie Sheehan, Mérieux NutriSciences

Gale Prince, SAGE Food Safety Consultants, LLC

Brittany Holmes, Wegmans Food Markets

Tatiana Lorca, Canteen (Compass Group NA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2

Session Summary:

Industry experts would like to share new technologies to make recall communication and retrieval more effective for consumers. Examples will include leveraging the FDA/USDA database services directly to the consumer’s phone when the recall is announced tailored to their specific food allergies. Retailers will share current technologies to contact customers, the limitations of those current methods, and potential future improvements to reduce recall fatigue from over-communication. Technology is available to control access to specific foods in vending machines to prevent purchase of recalled foods. Preventing food recalls is the ultimate goal, but designing strategies and tools to prevent customers from consuming recalled products drives the industry forward.

Primary PDG Sponsor: Retail and Foodservice PDG

Secondary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Retail and Foodservice

Keywords: Food Service,Recalls,Retail

S32 - BREAKING THROUGH TO CONSUMERS WITH TECHNOLOGY-DRIVEN FOOD SAFETY EDUCATION

Primary Author:
Aaron Lavallee, USDA-FSIS-OPACE

Persons:
Aaron Lavallee, USDA-FSIS-OPACE

Sara Starck, Instacart

Ellen Thomas Shumaker, North Carolina State University

Jeremy Jackson, Meta

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Reaching consumers with food safety information has always been a challenge. Emerging digital technologies now present powerful opportunities to embed food safety education directly into the platforms where consumers make daily choices.

Recent trends highlight this shift. Nearly 63% of U.S. adults use mobile apps for health-related purposes, and 58% turn to the internet for health or medical information each year, signaling growing reliance on digital platforms for trusted guidance. Looking ahead, artificial intelligence, adaptive learning, and conversational tools such as chat-based platforms are reshaping how consumers learn. These technologies can deliver personalized, real-time safety education at the exact moment of decision—whether shopping online, cooking at home, or engaging with digital health apps.

This symposium will explore how AI-powered adaptive learning systems, digital assistants, and micro-targeted content can “break through” to consumers in ways that are dynamic, contextual, and engaging. Case studies will illustrate how innovative tools—already in use for health and nutrition—can be adapted for food safety, with perspectives from technology leaders, regulators, retail partners, public health agencies, and academics.

Primary PDG Sponsor: Food Safety Education PDG

Primary Topic Area: Food Safety Education

Secondary Topic Area: Communication, Outreach and Education

Keywords: Data,Education,Social Media

S33 - BEYOND THE USUAL SUSPECTS: RISK PERSPECTIVES ON EMERGING PATHOGENS IN MEAT AND POULTRY PRODUCTS

Primary Author:
Byron Chaves, Rutgers University

Persons:
Byron Chaves, Rutgers University

Thomas Taylor, Texas A&M University

Jennifer Auchtung, University of Nebraska Lincoln

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Food safety and public health have traditionally focused on well-characterized pathogens such as *Salmonella*, STEC, and *Listeria monocytogenes*. However, growing evidence suggests that additional bacterial species may contribute to unrecognized or underestimated foodborne illness, in part due to diagnostic limitations, sparse surveillance, and uncertainty in exposure pathways. This symposium examines three such pathogens, *Aliarcobacter butzleri*, *Escherichia albertii*, and *Clostridioides difficile*, with emphasis on their potential contribution to human illness and challenges in burden-of-disease estimation. The session will begin with *A. butzleri*, a zoonotic organism associated with gastroenteritis and increasingly detected in poultry. The presentation will connect growth and persistence in raw chicken products with exposure risk, detection challenges, and implications for underreporting in public health surveillance. The second talk will focus on *E. albertii*, an emerging enteropathogen frequently misidentified in routine diagnostics, highlighting how misclassification obscures incidence estimates and how quantitative microbial risk assessment can help evaluate its potential public health impact from raw meats. The final presentation will examine *C. difficile*, a major cause of community- and healthcare-associated disease, exploring evidence of its occurrence in meats, hypotheses surrounding possible zoonotic or foodborne transmission, and the resulting uncertainty in attributing illness burden. Collectively, this symposium highlights how data gaps, diagnostic barriers, and

limited attribution frameworks complicate assessment of illness burden for emerging and underappreciated pathogens.

Primary PDG Sponsor: Meat and Poultry Safety and Quality PDG
Primary Topic Area: Meat and Poultry Safety and Quality
Secondary Topic Area: Microbial Modelling and Risk Analysis
Keywords: Meat and Poultry,Modeling,One Health,Pathogens,Risk Analysis

S34 - LIFE OF AN OUTBREAK: FROM THE FIRST ILLNESS TO THE COURTHOUSE (PART 1)

Primary Author:
Laura Gieraltowski, Centers for Disease Control and Prevention

Persons:
Michael Batz, USFDA

Joy Dell'Aringa, BioMerieux

Laura Gieraltowski, Centers for Disease Control and Prevention

Margaret Kirchner, FDA CORE

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Foodborne illness outbreak investigations are complex and unfold across multiple sectors, from the first patient’s symptoms to courtroom proceedings. This unique symposium will guide attendees through the entire lifecycle of an outbreak scenario, highlighting the perspectives of patients, public health investigators, industry leaders, regulators, and legal experts. The session will open with a person sharing a powerful first-hand testimonial describing the personal impact of a foodborne infection, setting the stage for the urgency of rapid detection and coordinated action. Next, speakers from CDC and FDA will explain how outbreaks are detected and investigated. They will discuss cluster identification, coordination of data collection, and the process for issuing public notices. Speakers from retail and food production will provide insight from their perspective regarding enacting recalls, managing public relations, finding the root cause, and process changes. A speaker from FDA will provide insight into regulatory inspections, detailing what investigators look for and the potential enforcement actions that may follow. Finally, a legal expert will outline the civil litigation process, from filing of complaints to discovery, depositions, mediation, and trial, illustrating how evidence gathered during the outbreak investigation plays a central role in the courtroom. The session will conclude with an interactive discussion allowing the audience to ask questions of all panelists. By tracing the journey of an outbreak from the first illness to legal resolution, this symposium offers a rare view of the science, communication, and

consequences of foodborne disease outbreaks.

Primary PDG Sponsor: Committee on Control of Foodborne Illness

Secondary PDG Sponsor: Food Law PDG

Primary Topic Area: Epidemiology

Secondary Topic Area: Food Law

Keywords: Epidemiology,Outbreak,Public Health

S34 - LIFE OF AN OUTBREAK: FROM THE FIRST ILLNESS TO THE COURTHOUSE (PART 2)

Primary Author:
Laura Gieraltowski, Centers for Disease Control and Prevention

Persons:
Laura Gieraltowski, Centers for Disease Control and Prevention

Michael Batz, USFDA

Sean Wolfley, Costco Wholesale

Kelly Stevens, General Mills

Shawn Stevens, Food Industry Counsel, LLC

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Foodborne illness outbreak investigations are complex and unfold across multiple sectors, from the first patient's symptoms to courtroom proceedings. This unique symposium will guide attendees through the entire lifecycle of an outbreak scenario, highlighting the perspectives of patients, public health investigators, industry leaders, regulators, and legal experts. The session will open with a person sharing a powerful first-hand testimonial describing the personal impact of a foodborne infection, setting the stage for the urgency of rapid detection and coordinated action. Next, speakers from CDC and FDA will explain how outbreaks are detected and investigated. They will discuss cluster identification, coordination of data collection, and the process for issuing public notices. Speakers from retail and food production will provide insight from their perspective regarding enacting recalls, managing public relations, finding the root cause, and process changes. A speaker from FDA will provide insight into regulatory inspections, detailing what investigators look for and the potential enforcement actions that may follow. Finally, a legal expert will outline the civil litigation process, from filing of complaints to discovery, depositions, mediation, and trial, illustrating how evidence gathered during the outbreak investigation plays a central role in the courtroom. The session will conclude with an interactive discussion allowing the audience to ask questions of all panelists. By tracing the journey of an outbreak from the first illness to

legal resolution, this symposium offers a rare view of the science, communication, and consequences of foodborne disease outbreaks.

Primary PDG Sponsor: Committee on Control of Foodborne Illness

Secondary PDG Sponsor: Food Law PDG

Primary Topic Area: Epidemiology

Secondary Topic Area: Food Law

Keywords: Epidemiology,Outbreak,Public Health

S35 - GENOMIC ANALYSES TO RANK FOODBORNE PATHOGENS BY RISK

Primary Author:
John Jarosh, USDA-FSIS

Persons:
John Jarosh, USDA-FSIS

Isabel Walls, USDA-FSIS

Glenn Tillman, USDA-FSIS

Renato Orsi, Cornell University

Vikrant Dutta, bioMerieux Inc.

KatieRose McCullough, Meat Institute

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Whole genome sequencing (WGS) and genomics have revolutionized how the public health community and in particular food safety regulatory officials track and attribute illnesses by foodborne pathogenic bacteria. In 2019, the Centers for Disease Control and Prevention (CDC) PulseNet fully transitioned to using WGS to characterize foodborne pathogens. There have been many successes of using WGS data to detect outbreaks earlier, to predict antimicrobial resistance, to support attribution models, and to predict sequence types and serotypes. However, there is the potential to gain further insights into potential virulence based on the genomic content. Reports in the published literature have identified that certain foodborne microbial pathogen subtypes inherently have a higher risk of association with illness. For example, certain Shiga toxin-producing *Escherichia coli* (STEC) subtypes have genes for Shiga toxin that have been associated with hemolytic uremic syndrome in patients. Bringing the concept of virulence into a risk assessment approach can lead to a strategic vision of how to focus resources on the riskiest subtypes of pathogens.

This symposium will focus on collaborative efforts across academia, government, and

industry on how genomics can be used to identify pathogen subtypes of greater public health significance and better inform the future of food safety.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Applied Laboratory Methods

Keywords: Genomics,Meat and Poultry,Risk Management

S36 - THE KEY PERFORMANCE INDICATORS OF FOOD SAFETY TRAINING

Primary Author:
Neal Fredrickson, Cargill

Persons:
Alexander Evans, Franklin County Public Health

Laura Nelson, Culture Strategies Group

Allison Howell, The Ohio State University

Neal Fredrickson, Cargill

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Food safety training is a regulatory requirement and a foundational element of food protection programs. However, trainers and educators often struggle to answer a critical question: *Is our training actually working?* Training is only effective if the learner applies what they have learned into context. Researchers have shown that the factors that influence the application of the training include instructional design, individual characteristics of the learners, and most importantly, the real-world work environment (De Rijdt, et al., 2013; Blume et al., 2019). This symposium will explore how organizations can move beyond compliance checkboxes and satisfaction surveys to evaluate training effectiveness using meaningful, business-relevant metrics.

The symposium will attract broad interest by providing both pragmatic best practices for those responsible for managing training in the industry, and by providing academic insights for researchers seeking to deepen our understanding of how food safety training works. Presenters will share real-world examples and quantitative methods for assessing training outcomes, linking learning to operational performance, and identifying key performance indicators (KPIs) that reflect true impact. An example from the public sector will explore how a training certification program related to health inspection performance. An international example from academia will explore how the design of tests can improve the validity of

training efforts. An example from private industry will explore how real-world data can be structured to gain insights from regression analysis. Attendees will gain insights into how to design training programs that not only meet regulatory standards but also drive measurable improvements in food safety culture and results.

Primary PDG Sponsor: Food Safety Education PDG

Primary Topic Area: Communication Outreach and Education

Secondary Topic Area: Data Management and Analytics

Keywords: Data,Education,Food Safety Culture

S37 - FOOD CHEMICAL SAFETY ASSESSMENT: HOW AND WHY

Primary Author:

Neal Saab, IAFNS

Persons:

Neal Saab, IAFNS

Paul Hanlon, Abbott Nutrition

Norbert Kaminski, Michigan State University

Kristi Muldoon Jacobs, USFDA

Craig Llewellyn, J.S. Held

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Concerns about the safety of food ingredients have heightened, such as seen with FDA action and U.S. state legislative activities on food and color additives. Food additives and ingredients must undergo a stringent food chemical safety assessment before being allowed on the market. The Generally Recognized as Safe (GRAS) pathway for regulatory acceptance has been under increasing scrutiny in the public and private sectors. A fact rarely known is that the GRAS pathway requires the same food chemical safety assessment process as the FDA would undertake for a Food Additive or Color Additive Petition. This session will lead off with an introduction to the basic principles of food toxicology, insights into the FDA's Food Chemical Safety programs, and a practical presentation on how a GRAS determination is performed.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Secondary PDG Sponsor: Food Packaging PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Packaging

Keywords: Chemical Contaminant,Risk Assessment,Toxicology

S38 - SAMPLE POOLING: SINK OR SWIM

Primary Author:
Marcel Zwietering, Wageningen University

Persons:
Konrad Korzeniowski, ILSI Europe

Anett Winkler, Cargill

Marcel Zwietering, Wageningen University

Alvin Lee, Institute for Food Safety and Health

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Pooling microbiological samples can offer clear benefits in terms of efficiency, effort, waste reduction, and cost savings. However, pooling introduces a range of challenges related to practical working procedures, the microbial/ecological response, kinetics of growth, and test performance. This symposium will explore the impact of pooling on sampling procedures from all these different perspectives. Since there is no globally aligned definition, first different definitions and types of pooling and compositing are described. In that context, general advantages and disadvantages will also be illustrated. There can be impacts on the working procedures and effects on the ergonomic practices of the technician. Ultimately, there are effects on efficiency and costs. There can also be an impact of pooling on the test performance due to dilution effects, physiological conditions of microorganisms, or competition with background microbiota. These could also have an impact on the limit of detection, which could be missed when performing the method. Additionally, before pooling can be used, validation is required, and verification needs to be performed. The symposium will describe pooling from these different perspectives, the concept, definitions, statistics, quantitative ecology, test performance, practical implications and validation and verification, ultimately advising on when pooling could be used, also outlining cases, where it negatively impacts results, or cannot be used at all.

Primary PDG Sponsor: Modelling and Risk Analysis PDG

Secondary PDG Sponsor: International Food Protection Issues PDG

Non PDG Sponsor (Organization, Association, etc.): ILSI-Europe

Primary Topic Area: Microbial Modelling and Risk Analysis

Secondary Topic Area: International Food Protection Issues

Additional Topic Area (Optional): Applied Laboratory Methods

Keywords: Environmental monitoring,HACCP,Laboratory and Detection Methods,Modeling,Pathogens,Regulations,Sampling,Validation

S39 - ALL HANDS ON DECK: THE PIVOTAL ROLE OF FOOD AND AGRIFOOD SYSTEMS IN THE GLOBAL ANTIMICROBIAL RESISTANCE (AMR) PANDEMIC

Primary Author:

Jorge Pinto Ferreira., Food and Agriculture Organization of the United Nations (FAO)

Persons:

Jorge Pinto Ferreira., Food and Agriculture Organization of the United Nations (FAO)

Issmat Kassem, University of Georgia Athens

Rosalia Trias, Danone SA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Antimicrobial resistance (AMR) is one of the top 10 global challenges facing humanity.

Antimicrobial-resistant bacterial contributed to 4.95 million deaths in 2019. Efforts to address it have focused on reducing the use of antimicrobials in animal agriculture and human medicine. Recently, there has been a growing recognition of the potential role of biocides and other food production and processing practices in contributing to the emergence and/or co-selection of AMR. New data on emerging bacterial hosts, mobile genetic determinants, and robust fitness emphasize that lapses and/or poor practices can transmit foodborne AMR across borders via food trade.

Food security and socioeconomic wellbeing are other dimensions that need to be considered in the AMR pandemic. Quantifying foodborne AMR remains a big challenge and the role of co-selection and cross resistance caused by biocides or other practices remain poorly defined. To address these issues, Codex Alimentarius has developed and published guidelines and a code of practice that are currently being implemented worldwide.

Speakers will discuss AMR in food-related matrices (including seafood, meat, and produce) and sources, addressing:

The rise of critical antibiotic resistance in important food-associated matrices and sources – research findings that highlight the role of the food trade in disseminating AMR determinants between countries.

The role of antimicrobial stewardship in food safety and the efforts against AMR locally and globally, via the implementation of Codex standards in low- and middle-income countries.

Antimicrobial resistance co-selection and cross resistance potentially caused by biocides and other environmental pollutants.

Primary PDG Sponsor: Seafood Safety and Quality PDG

Secondary PDG Sponsor: Seafood Safety and Quality PDG

Primary Topic Area: Seafood Safety and Quality

Secondary Topic Area: International Food Protection Issues

Additional Topic Area (Optional): Pre Harvest Food Safety

Keywords: Antimicrobial Resistance,Antimicrobials,One Health,Public Health

S40 - FOOD FRAUD PREVENTION: FROM SPREADSHEETS TO AI

Primary Author:
Karen Everstine, FoodChain ID

Persons:
Karen Everstine, FoodChain ID

Elise Forward, Forward Food Solutions, LLC

John Spink, Food Fraud Prevention Think Tank LLC

Sabahnnur Demirci, Merieux NutriSciences

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

This session will present a review of the current state of food fraud prevention strategies from a range of perspectives. Small businesses may still be conducting vulnerability assessments and hazard identification using spreadsheets and relying on trust built from long-standing supplier relationships. Large multinational brands are adapting new technologies and approaches, including leveraging large amounts of data and machine learning/artificial intelligence (AI).

Our first speaker will discuss the basics of food fraud risk identification, including practical approaches that are used by small- and medium-sized businesses. Resource constraints may affect the ability of small businesses to monitor multiple data sources and conduct horizon scanning. The speaker will describe common gaps in food fraud programs and realistic approaches identified by working closely with food companies to help them meet auditing requirements. Our second speaker will focus on two key strategies employed by global food brands to increase efficiency and stay ahead of emerging risks: horizon scanning and confidential data sharing through a third-party data aggregation system. This allows companies to identify risks earlier and put their testing programs in context of testing strategies used by other companies. Finally, our last speaker will bring an academic perspective on supply chain management and the evolution of AI as a tool for protecting

supply chains. Is the potential of AI for protecting food supply chains overstated, or is it truly the future of food safety and food fraud prevention?

Primary PDG Sponsor: Food Fraud PDG
Primary Topic Area: Food Fraud
Secondary Topic Area: Food Chemical Hazards and Food Allergy
Keywords: Food Defense,Risk Assessment,Risk Management

S41 - THE GENOMICS FOR FOOD AND FEED SAFETY (GEN-FS) INTERAGENCY COLLABORATION: RECENT ADVANCES IN GENOMICS METHODS AND DATA STANDARDS

Primary Author:
Jessica Chen, US CDC

Persons:
Samantha E. Wirth, Wadsworth Center, New York State Dept. of Health

Heather Carleton, US-CDC

Jessica Chen, US CDC

Ruth Timme, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Gen-FS is the Interagency Collaboration on Genomics for Food and Feed Safety, with representatives from the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the National Institutes of Health (NIH) of the Department of Health and Human Services (DHHS), and the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), Animal and Plant Health Inspection Service (APHIS), and Agricultural Research Service (ARS). Gen-FS has a broad-reaching impact on the food safety community. Specifically, it advances food safety and public health through the coordination of genomics methods and data interpretation by consortium members and working groups that also include other federal agencies such as NOAA, along with academia and public health laboratories. Together, the consortium improves contextual data standards to support machine readability, coordinates validation of new sequencing methods and approaches, improves sequence data quality through coordinated quality assurance, and advances genomic characterization for functional genomics and serotyping.

This session will be of particular interest to users of the public genomic surveillance data generated by Gen-FS members and collaborators. These potential users include academia, third party testing laboratories, food production companies, and other government

scientists. This session will highlight how the Gen-FS federal consortium, in partnership with state public health laboratories and academic institutions, is advancing genomics to strengthen food safety research and surveillance, testing methods, and data interpretation.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Applied Laboratory Methods

Keywords: Data,Genomics,Laboratory and Detection Methods,Pathogens

S42 - OVERCOMING CHALLENGES OF WATER USE AND REUSE IN FOOD PROCESSING REQUIRES A HOLISTIC APPROACH

Primary Author:

Leon Gorris, Food Safety Futures

Persons:

Leon Gorris, Food Safety Futures

Dima Faour-Klingbeil, DFK for Safe Food Environment /University of Veterinary Medicine Hanover

Ellen Wemmenhove, Arla Foods Ingredients

Theresa Mazure, PepsiCo

Anett Winkler, Cargill

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Water is an increasingly precious resource for food production and processing in many geographies today and increasingly more around the globe due to global warming and shifting climatic patterns.

In recent annual meetings, IAFP featured the topic at the level of Codex Alimentarius guidance to governments and industry, as well as sector specific assessment and management of microbiological hazards or chemical hazards when using or reusing water in the food sector.

In practice, however, food industries need to manage multiple dimensions of risk when using or reusing water. Key additional risks include physical hazards, occupational hazards, environmental impacts, antimicrobial resistance, engineering and other operational capabilities. Beyond food safety, consumer acceptance, regulatory alignment and business economics all need to be adequately addressed in the context of a food industry wishing to implement water use and, especially, reuse scenarios.

Evidently, each individual food industry operator needs to adopt a holistic approach to water use and re-use that, for their specific circumstances, considers all the interlinked dimensions of risk in their day-to-day operation. While challenges are significant, there are also benefits in terms of reduced dependency on first-use water availability and quality, reduced discharge costs, improved business continuity and others.

The novelty to IAFP of this symposium is that it features real-life, holistic case studies from three different food processing industry sectors. For the first time, speakers will share how various food safety hazards were addressed alongside the important other dimensions of risk that are part of the holistic approaches they followed to successfully overcome operational challenges of water (re-)use.

Primary PDG Sponsor: Water Safety and Quality PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Non PDG Sponsor (Organization, Association, etc.): International Commission on Microbiological Specifications for Foods (ICMSF)

Primary Topic Area: Water Safety and Quality

Secondary Topic Area: Microbial Modelling and Risk Analysis

Additional Topic Area (Optional): International Food Protection Issues

Keywords: Risk Analysis,Toxicology,Water

S43 - BEYOND THE ALGORITHM: HOW FOOD SAFETY SYSTEMS AND CULTURE ADAPT IN THE AGE OF AI

Primary Author:
Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Persons:
Jennifer Turpin, Mars

Lily Yang, The Acheson Group

Aaron Pleitner, Certified Group

Campbell Mitchell, Kraft Heinz

Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Giannis Stoitsis, Agroknow

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

As advanced AI technologies emerge, many food companies have pursued bold digital strategies, yet too often these efforts sideline Quality and Food Safety (Q&FS). This symposium will challenge that approach, asking: What if Q&FS managers were equipped with AI-driven dashboards that integrate history, documents, and deviations to give senior leadership real-time snapshots of risks and priorities? Speakers will explore how AI can be governed and audited with the same rigor applied to suppliers and manufacturing sites, ensuring it drives preventive thinking rather than replacing human judgment. The session will highlight how Q&FS KPIs are evolving—from lagging and leading indicators to predictive, human-in-the-loop models—and how food safety culture must adapt to embrace digital transparency, predictive tools, and collaborative decision-making. Attendees will leave with a practical framework for embedding AI into Q&FS in ways that deliver safer products, stronger governance, and a culture aligned with human-first responsibility. Specifically, attendees will benefit from speakers who will: Explain why many AI strategies in Q&FS fail to deliver value and how AI can be embedded into Q&FS processes effectively; Apply governance and auditing principles to AI tools with rigor; Define evolving KPIs that integrate

predictive, human-in-the-loop models; and Recognize how food safety culture must adapt to AI integration.

Primary PDG Sponsor: Food Safety Culture PDG

Secondary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Primary Topic Area: Food Safety Culture

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Additional Topic Area (Optional): Data Management and Analytics

Keywords: Data,Education,Environmental monitoring,Food Safety Culture,HACCP,Modeling,Professional Development,Regulations,Risk Analysis,Risk Assessment,Risk Management

S44 - PRODUCE WASH WATER: MULTI-COMPANY VALIDATION OF CRITICAL PARAMETERS

Primary Author:
Jennifer McEntire, Food Safety Strategy, LLC

Persons:
Jennifer McEntire, Food Safety Strategy, LLC

Chris Valadez, Grower-Shipper Association

Natalie Krout Greenberg, California Department of Food and Agriculture

Bin Zhou, FQL, USDA ARS

German Rios, Fresh Express

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Many fresh produce items are washed with sanitized water. Because this is not a kill step, this process step can result in cross-contamination if not properly managed. But defining “properly managed” is easier said than done. Results from laboratory and pilot scale studies examining many commodities, antimicrobials, and wash water systems suggest which parameters should be controlled, but the level of control that is possible in commercial scale operations has not been established yet. This session will show pre-pilot summary data from over 3,000 product and water samples, with a total 13,000 data points collected over 20 different types of tests, by four leafy green processors. United States Department of Agriculture (USDA) - Agricultural Research Service (ARS) scientists analyzed the more than 13,000 data points as part of the 2024–2025 pre-pilot, which is now expanded to include 2 additional companies and several additional production lines representing a range of business sizes, wash systems, and fresh produce types. Based on a statistical analysis of the data, the number of data variables was refined and the protocol for data collection was improved. Session attendees will gain insight on how to conduct a similar in-plant validation of their wash system and better understand which variables do- and don’t- need to be measured and managed.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Non PDG Sponsor (Organization, Association, etc.): Grower-Shipper Association of Central California

Primary Topic Area: Fruit and Vegetable Safety and Quality

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Additional Topic Area (Optional): Water Safety and Quality

Keywords: Produce,Validation,Water

S45 - NATURAL MOLD INHIBITION IN BAKED GOODS: FERMENTATE SOLUTIONS FOR SUSTAINABILITY AND WASTE REDUCTION

Primary Author:
Shalini Sehgal, University of Delhi

Persons:
Arshpreet Kaur Khattra, Michigan State University

Samet Ozturk, USDA

Shalini Sehgal, University of Delhi

Surabhi Wason, Kerry

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Mold spoilage in baked goods is a critical concern within the food industry, directly impacting food safety, shelf-life stability, and economic sustainability. Conventionally, synthetic antifungal agents such as calcium propionate have been employed to inhibit mold growth; however, consumer-driven demand for clean-label formulations and concerns regarding chemical additives have prompted the development of natural preservation strategies.

Fermentates, derived from controlled microbial fermentation (e.g., *Lactobacillus*, *Pediococcus*, *Propionibacterium* spp.), present a promising alternative. These bio-preservatives are rich in antifungal metabolites, including organic acids, bacteriocins, and other low-molecular-weight antimicrobial peptides. Their multifactorial mechanisms of action—such as pH reduction, membrane disruption, and metabolic interference—effectively inhibit the proliferation of spoilage molds including *Aspergillus*, *Penicillium*, and *Rhizopus* species.

From a food safety perspective, the application of fermentates supports microbiological stability and reduces mycotoxin risks. For consumers, this translates into longer-lasting freshness, reduced in-home waste, and extended shelf life without reliance on synthetic additives. On the supply chain side, improved mold resistance supports ambient shipping

and storage, reducing cold-chain dependence and easing logistical constraints—particularly important for e-commerce, export markets, and remote distribution.

Furthermore, the integration of natural antimicrobials contributes to reduced food loss across the supply chain, supporting sustainable manufacturing practices. Given the rising emphasis on food system sustainability, natural antimicrobials like fermentates not only offer functional efficacy but also align with regulatory and environmental priorities. Their adoption represents a forward-looking approach to shelf-life extension, safety assurance, and waste reduction in the bakery sector.

Primary PDG Sponsor: Low Water Activity Foods PDG

Secondary PDG Sponsor: Retail and Foodservice PDG

Primary Topic Area: Low Water Activity Foods

Secondary Topic Area: Retail and Foodservice

Additional Topic Area (Optional): Retail and Foodservice

Keywords: Low-water Activity Foods,Ready to Eat Foods,Retail,Shelf-life,Spoilage,Sustainability

S46 - ENVIRONMENTAL MONITORING AND ZONE 1 SWABS: HARD LESSONS FROM THE TURKEY BACON RECALL

Primary Author:
Campbell Mitchell, Kraft Heinz

Persons:
Campbell Mitchell, Kraft Heinz

Emilio Esteban, Merieux NutriSciences

Jack van der Sanden, bioMerieux Pty. Ltd.

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

This session will examine the lessons learned from a major ready-to-eat meat recall linked to *Listeria* contamination, with a focus on environmental monitoring programs and regulatory decision-making. It will highlight the challenges posed when presumptive positive results from Zone 1 swabs do not trigger mandatory product holds under USDA oversight, creating gaps between scientific risk management and regulatory expectations.

The discussion will explore how environmental monitoring data can be used proactively to detect early signals of contamination, prevent escalation, and strengthen food safety systems before recalls occur. Special attention will be given to the critical role of Zone 1 swabs, the importance of rapid confirmation and decision-making, and the impact of organizational culture on how companies respond to presumptive positives.

Participants will gain practical insights into the balance between compliance requirements and best-practice risk management, particularly when regulatory policy lags behind scientific understanding. The session will also consider the broader implications for industry, including alignment of USDA and FDA approaches, integration of digital monitoring tools, and the need for clear crisis management protocols.

By sharing the key takeaways from this high-profile event, the session aims to help food safety professionals refine their environmental monitoring programs, close compliance gaps,

and strengthen preventive controls to better protect consumers and brands.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Non PDG Sponsor (Organization, Association, etc.): Kraft-Heinz

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Applied Laboratory Methods

Keywords: Environmental monitoring,Equipment,Hygiene,Laboratory and Detection Methods,Meat and Poultry,Pathogens,Production/Plant Design,Ready to Eat Foods,Recalls,Regulations,Risk Analysis,Risk Management,Sampling,Sanitation,Validation

S47 - ADVANCING AI IN FOOD SAFETY THROUGH STUDENT COMPETITION ON BENCHMARK DATASETS

Primary Author:
Chenhao Qian, Cornell University

Persons:
Chenhao Qian, Cornell University

Giannis Stoitsis, Agroknow

Jiyoon Yi, Michigan State University

First, second, third place winners, Cornell University

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Artificial intelligence (AI) holds enormous potential to transform food safety, but meaningful progress depends on shared resources and collaborative communities. This symposium will feature the top three finalist teams from a student competition that challenged participants to design improved algorithms for curated benchmark datasets representing real-world food safety problems, such as contamination detection, risk forecasting, and computer vision.

A central goal of the competition was the use of a public data repository, enabling transparency, reproducibility, and benchmarking across diverse approaches. By working with openly available datasets, students not only developed innovative algorithms but also contributed to building a community resource that can accelerate future research.

The symposium will highlight how collaboration between students from different academic and computational backgrounds fosters creative solutions. Each team will present their methodology, results, and reflections on interdisciplinary teamwork, dataset challenges, and how public repositories can serve as catalysts for long-term progress.

By showcasing these finalists, the session will highlight how student-driven innovation can contribute to publicly available models and tools that address pressing food safety challenges. Attendees will gain exposure to cutting-edge methods while seeing how open

datasets and benchmark-driven development can lay the groundwork for sustainable, shared infrastructures that advance food safety research and practice.

Primary PDG Sponsor: Data Management and Analytics PDG

Secondary PDG Sponsor: Student PDG

Non PDG Sponsor (Organization, Association, etc.): AI4Food, AI Institute for Next Generation Food Systems (AIFS)

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: Food Safety Education

Additional Topic Area (Optional): Communication, Outreach and Education

Keywords: Data,Education,Modeling

S48 - PFAS IN FOOD AND WATER: REGULATION, OCCURRENCE AND MITIGATION

Primary Author:
Xuetong Fan, USDA-ARS

Persons:
Xuetong Fan, USDA-ARS

Changqing Wu, University of Delaware

Juzhong Tan, University of Delaware

Lowri DeJager, USFDA

Emanuele Sozzi, RTI International

Dongxia Liu, University of Delaware

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Per- and polyfluoroalkyl substances (PFAS) are a large group of man-made "forever chemicals" that have entered the food supply through various pathways. One major source is food packaging, particularly grease-resistant paper used for items like fast food wrappers, pizza boxes, and microwave popcorn bags. Over time, PFAS can migrate from this packaging and contaminate the food itself. In addition, environmental contamination from industrial manufacturing, firefighting foam, and landfills can lead to PFAS entering the soil and water. This can cause chemicals to accumulate in crops, fish, shellfish, and livestock. In water, PFAS pose a significant contamination risk, with exposure mainly occurring through drinking contaminated municipal or well water. Consumption of food and water over time leads to the accumulation of the chemicals in the body over time, resulting in potential health problems, including increased cholesterol, liver damage, and thyroid disease, and they are a growing public health concern.

The symposium will address various aspects of PFAS-related topics. The first presentation will address recent developments in the regulatory aspects on PFAS in food and water including

recent requirement of the industry to phase-out of these substances in food packages. In addition, latest results from FDA-tested PFAS in foods, and estimation of human exposure to PFAS and potential long-term health impact will be addressed. The second presentation will review recent studies on the occurrence of PFAS in water and various foods including, seafood, meat and poultry, fruit and vegetable. Also discussed are possible sources of PFAS for different categories of foods, and developments in PFAS detection and quantification techniques. The last presentation will be on remedies, processes, and treatments that can reduce or eliminate the levels of PFAS in food and water. General strategies to reduce exposure and risk at the consumer level will also be discussed.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Secondary PDG Sponsor: Food Packaging PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Packaging

Additional Topic Area (Optional): Water Safety and Quality

Keywords: Produce,Public Health,Toxicology

S49 - ALIVE OR DEAD? INCORPORATION OF PATHOGEN VIABILITY ASSESSMENT INTO ‘OMICS FOOD SAFETY RESEARCH

Primary Author:
Joelle Salazar, FDA

Persons:
Joelle Salazar, FDA

Steven Ricke, University of Wisconsin- Meat Science & Animal Biologics Discovery Bldg.

Katrina Counihan, USDA

Megan Fay, USFDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Food safety research has been revolutionized by ‘omics technologies including genomics, metagenomics, transcriptomics, proteomics, and metabolomics. However, these techniques often fail to distinguish between viable and non-viable pathogens, which can potentially lead to overestimation of food safety risks and misinterpretation of intervention efficacy. While functional assessment of pathogen viability is possible with transcriptomics and metabolomics, these methods are costly and involve handling specific molecules that can be cumbersome. Traditional microbiome ‘omics techniques, which work with DNA, are not alone capable of viability determination. Currently, methods used to distinguish between live and dead cells with sequencing studies include pretreatment methods utilizing PMA and EMA, which selectively target membrane-compromised cells. While these are the most widely adopted methods, these photoreactive dyes display variable efficacy across different bacterial species and under certain conditions. This symposium will discuss novel opportunities for integrating pathogen viability assessment methods with ‘omics technologies to enhance food safety research. Specifically, presenters from academia and government will discuss the use of viability sequencing to identify live foodborne pathogens in meat, low-moisture foods, and produce processing environments. Incorporating viability assessment into 'omics research will enable more precise risk assessments, better

evaluations pertaining to food processing interventions, and improved understanding of pathogen ecology in foods. The marriage of viability determination with 'omics technologies represents a critical advancement in food safety, regulatory decision-making, and enhances consumer protection and public health.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Applied Laboratory Methods

Keywords: Genomics,Laboratory and Detection Methods,Microbiome

S50 - ALLERGEN METHOD VALIDATION AND VERIFICATION: GUIDELINES AND REAL-WORLD EXPERIENCES

Primary Author:
Melanie Downs, University of Nebraska Lincoln

Persons:
Jodi Nickerson, Eurofins Microbiology Laboratories

Jasmine Lacis-Lee, Mérieux NutriSciences AQ

Mario Gadanho, Mars

Markus Lacorn, R-Biopharm

Melanie Downs, University of Nebraska Lincoln

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

The ability to obtain accurate and precise quantitative results with food allergen methods is a critical capability to support implementation of risk-based approaches for food allergen management and precautionary allergen labeling. The measurement of complex analytes in a nearly infinite number of foods and ingredients is a substantial challenge, however, and many misconceptions persist among users of food allergen methods and analytical data. To provide guidance for different stakeholder groups, the AOAC International Gluten and Food Allergens Initiative has developed comprehensive guidance for both food allergen method validation and verification. The end goal of the developed guidance is to improve transparency, communication, and understanding of the performance of all types of food allergen methods (including immunoassays, PCR, and mass spectrometry), so that analytical data can be a reliable component of risk-based approaches for allergen management. This symposium will include presentations from method developers, laboratories, and food manufacturers who have experience with the relevant guidance. The audience will be able to learn from the approaches and outcomes of the speakers and have tangible takeaways for how the guidance can be deployed in their own operations, including how to design and implement verification studies for specific matrices, how to interpret method validation data from developers, and how to select methods and understand which methods are fit-for-

purpose for a given application.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Secondary PDG Sponsor: Applied Laboratory Methods PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Applied Laboratory Methods

Keywords: Allergens,Laboratory and Detection Methods

S51 - BREAKING BARRIERS: ENHANCING FOOD SAFETY CULTURE THROUGH INCLUSIVE COMMUNICATION

Primary Author:
Obabiyi Ajao, University of Central Lancashire

Persons:
Obabiyi Ajao, University of Central Lancashire

Lone Jespersen, Cultivate SA

Sophie Tongyu Wu, University Central Lancashire

John Boyce, Boyce Food Safety Consulting Ltd.

Jeremy Langford, Puris Proteins LLC

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Inadequate communication among food handlers is a major contributor to food safety incidences. In the United States, around 22% of outbreaks are linked to poor communication, while multilingual companies in the UK show at least 30% higher hygiene non-compliance rates. A current and ongoing study has been able to identify through quantifiable measures, variation in food-handler reading and comprehension levels. Food handlers with no fluency and lowest proficiency level were up to 18% and 21%, respectively, in one food company. These employees may struggle to comprehend and integrate instructions, thus impairing their ability to comply with food safety requirements.

Food safety communication tools are often developed without consideration for language diversity and proficiency, particularly among shopfloor workers. This challenge poses risks to effective food safety systems and needs to be approached with the end goal of meeting the employee’s communication needs in mind. It will require tailored and targeted interventions using a variety of effective and inclusive communication strategies.

This symposium will explore the role and importance of communication as it affects food safety performance, and how communication challenges and language diversity impact

organizational and food safety culture in the food industry.

Speakers will highlight real-world examples of how organizations are addressing multilingual-communication barriers, adapting tools for diverse workforces, and fostering inclusive engagement to strengthen food safety culture. The session will also consider innovative approaches to developing communication strategies that respect cultural and linguistic diversity while maintaining rigorous food safety standards.

Primary PDG Sponsor: Food Safety Culture PDG

Primary Topic Area: Food Safety Culture

Secondary Topic Area: Communication, Outreach and Education

Keywords: Food Safety Culture,Intervention,Risk Management

S52 - ONE PATHOGEN, MANY LANDSCAPES: SALMONELLA DYNAMICS IN THE AMERICAS

Primary Author:
Byron Chaves, Rutgers University

Persons:
Byron Chaves, Rutgers University

Angélica Godínez Oviedo, Universidad Autónoma de Querétaro

Andrea Moreno Switt, Catholic University of Chile

Rachel Cheng, Virginia Tech

Montserrat Hernández Iturriaga, Universidad Autónoma de Querétaro

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Despite decades of surveillance and control efforts, cross-country comparisons of *Salmonella* prevalence, serovar diversity, virulence traits, and antimicrobial resistance (AMR) remain limited by differences in food systems, commodities, and monitoring frameworks. This symposium brings together experts from Chile, Mexico, and the United States to provide a coordinated regional perspective on *Salmonella* epidemiology using whole-genome sequencing (WGS) and national surveillance data. Presentations will highlight country-specific case studies illustrating how *Salmonella* burdens and genomic profiles vary across production systems and food categories. A focused examination of *S*. Infantis in Chile will showcase genomic lineages, multidrug-resistance patterns, and applied risk mitigation strategies. A U.S. perspective will present the genomic diversity and distribution of *Salmonella* across major commodities using large-scale surveillance datasets. A third contribution from Mexico will compare meat and non-meat sources, emphasizing differences in serovar distribution, genomic characteristics, and AMR profiles across food matrices. Collectively, the session will examine dominant and emerging serovars, AMR trends, and genomic markers relevant to risk assessment and management. Speakers will discuss how data availability and regulatory context influence interpretation and comparability across countries. The session will focus on key questions: How does *Salmonella* epidemiology differ across the Americas?

How are AMR and genomic diversity evolving regionally? What data gaps limit harmonized assessment? The symposium aims to foster dialogue on coordinated surveillance, risk-based interventions, and collaborative research opportunities across the region.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Data Management and Analytics

Keywords: Antimicrobial Resistance,Data,Epidemiology,Genomics,Pathogens

S53 - CAN MICROBIAL QUANTITATION CATCH UP WITH DETECTION METHODS? RECENT ADVANCES

Primary Author:
David Legan, Eurofins Scientific Inc.

Persons:
David Legan, Eurofins Scientific Inc.

Laurie Post, Deibel Laboratories

Amanda Brookhouser-Sisney, Midwest Laboratories

Suzanne Jordan, Campden BRI

Andrzej Benkowski, Eurofins Microbiology Laboratories

Daniel Smieszek, Nestle

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Rapid methods have been the workhorse of pathogen detection for decades allowing critical safety decisions to be taken quickly. However, plate counts and MPNs remain the primary means of enumerating microbes linked to quality and spoilage concerns. Chromogenic and fluorogenic media have made the target microorganism easier to see and film-based methods have reduced media volumes, but there has been little improvement in time to result. Recent advances in technology, and revision of AOAC's method validation guidelines create a unique and timely opportunity to more broadly adopt rapid quantitative methods, potentially improving food quality and freshness. Speakers will examine progress in validation of quantitative microbiological methods and how this can build confidence in quantitative technologies leading to regulatory and industry acceptance. Flow cytometry and digital PCR are two of the most developed rapid quantitation technologies and will be used to illustrate the capability expected of rapid enumeration methods. Finally, the need for rapid quantitation will be explored including case studies showing benefits and challenges. Attendees will learn about recent progress in rapid quantitative methods and their potential to support faster decision-making in real applications.

Primary PDG Sponsor: Applied Laboratory Methods PDG

Secondary PDG Sponsor: Dairy Quality and Safety PDG

Primary Topic Area: Applied Laboratory Methods

Secondary Topic Area: Dairy Quality and Safety

Additional Topic Area (Optional): Beverages and Acid/Acidified Foods

Keywords: Laboratory and Detection Methods,Ready to Eat Foods,Sampling,Shelf-life,Spoilage

S54 - THE DOLLARS AND SENSE OF FOOD SAFETY: ECONOMETRICS OF FOOD SAFETY INTERVENTIONS

Primary Author:

Jeffrey LeJeune, FAO

Persons:

Jeffrey LeJeune, FAO

Amyaz Moledina, The College of Wooster

Diletta Topazio, FAO

Marc Allard, FDA

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

The true economic impact of unsafe food, and the cost to control it, is unknown. Building on roundtables held at recent annual meetings (’21 and ’25) that addressed need for incentives and the investment in food safety, this symposium takes an econometric approach to explore the balance of economic costs and the expected return on financial investments of food safety control measures, using outcomes from academic research studies and empirical data from implemented government (USFDA) policy. The objective of this session is to provide examples of both how the cost and the return on investments in food safety prevention can be studied and what we can learn from the outputs, using three case studies: 1) the cost to food producers to invest in safer food production practices, 2) the willingness of consumers to pay for such food safety practices, 3) and the financial return on investment in deploying whole genome sequencing in tracking foodborne *Salmonella*, *E. coli* O157, and *Listeria monocytogenes* cases in the USA.

Non PDG Sponsor (Organization, Association, etc.): FAO

Primary Topic Area: Advanced Molecular Analytics
Secondary Topic Area: Data Management and Analytics

Keywords: Genomics,One Health,Risk Management

S55 - BEYOND THE KILL STEP: MANAGING RISKS OF TOXIN-PRODUCING MICROORGANISMS IN HIGH-MOISTURE IN-PROCESS FOODS

Primary Author:
Balasubrahmanyam (Bala) Kottapalli, The Marzetti Company

Persons:
Balasubrahmanyam (Bala) Kottapalli, The Marzetti Company

Aaron Uesugi, Mondelez International

Yvonne Masters, John B. Sanfilippo & Son, Inc.

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Food manufacturers often rely on validated kill steps as their primary safeguard, but biological hazards do not disappear simply because a lethality step is present. In-process matrices such as batters, doughs, slurries, sauces, or blanched vegetables may spend residence time at warm temperatures prior to cooking, baking, freezing, or post-lethality. Under these conditions, toxin-producing microorganisms such as *Staphylococcus aureus* and *Bacillus cereus* can grow and form heat-stable toxins not destroyed by subsequent processing. Recent outbreaks and recalls show toxin formation in work-in-process (WIP) materials is an under-recognized but critical hazard.

Even low-moisture foods, often considered low risk, can be affected when high-moisture slurries or other WIP components are introduced. These transient environments create conditions conducive to microbial proliferation and toxin formation, posing risks not fully mitigated by lethality or drying steps. This underscores the need for a nuanced understanding of moisture dynamics in process safety.

This symposium will explore scientific, regulatory, and practical aspects of controlling high-moisture WIP matrices. Speakers will address risk factors (pH, water activity, salt concentration, competing microflora), predictive modeling, and validation of residence times. Management strategies will include sanitation, equipment design, and monitoring

unplanned downtime. Discussion will also incorporate regulatory expectations and relevant academic research to connect foundational science with practical implementation. Emphasis will be placed on maintaining food safety during holding to prevent adulteration.

By sharing cutting-edge science, case studies, and regulatory expectations, this session will help manufacturers strengthen hazard analyses, minimize risk, reduce recalls, and build a stronger scientific basis for preventive controls.

Primary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: HACCP Utilization and Food Safety Systems

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Additional Topic Area (Optional): Low Water Activity Foods

Keywords: HACCP,Regulations,Risk Management

S56 - BEST PRACTICES FOR MANAGING ALLERGENS AND GLUTEN IN RETAIL AND FOOD SERVICE ENVIRONMENTS

Primary Author:
Lauren Jackson, FDA/HFP

Persons:
Lauren Jackson, FDA/HFP

Laurie Williams, USFDA

Xingyi Jiang, USFDA

Betsy Craig, CFF by MenuTrinfo, LLC

Session Type: Short Symposium - 1.5 hours (Full symposium - submit as Part 1 and Part 2)

Session Summary:

Dining out accounted for the second most common location for a food allergic reaction, after one’s home and most food anaphylactic reactions occur outside the home, with roughly 25% of them occurring while dining at restaurants. The risk factors for food-allergic reactions occurring in restaurants include failure to disclose or communicate the allergy to the restaurant or wait staff, inconsistent or incomplete food labeling or disclosure on the menu, hidden or undeclared allergens, and cross-contact. Similarly, consumers with celiac disease experience health challenges caused by potential for hidden gluten in foods due to labeling errors or inadequate disclosure on menus or due to lack of awareness regarding the potential for gluten cross-contact when shared kitchens and food-contact surfaces are involved in producing gluten-free items. In retail and food-service operations, effective allergen and gluten management is particularly critical due to the frequent handling of diverse products, the complexity of supply chains, and direct consumer interaction. This symposium will address these pressing challenges and best practices for allergen and gluten management in retail and food-service operations. Presentations will cover the FDA Food Code and its impact on protecting consumers with food allergies, best practices for managing ingredients, and a consumer perspective on best practices for reducing allergen and gluten exposure. The session will highlight incidents where inadequate control measures can result in exposure and compromise consumer safety and examples of successful

interventions that reduce risk. Attendees will gain scientific insights and practical tools on how to successfully manage allergens and gluten in retail and service operations, as well as actionable approaches to mitigate risks. The participants will also be equipped with knowledge to strengthen allergen control systems, reduce liability, and foster safer, more transparent consumer experiences.

Primary PDG Sponsor: Retail and Foodservice PDG

Secondary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Primary Topic Area: Retail and Foodservice

Secondary Topic Area: Food Chemical Hazards and Food Allergy

Additional Topic Area (Optional): Food Hygiene and Sanitation

Keywords: Allergens,Food Service,Risk Management

S57 - ALLERGEN LABELING AND RISK MANAGEMENT UPDATES FROM FDA

Primary Author:
Carol D'lima, USFDA

Persons:
Yinqing Ma, USFDA

Carol D'lima, USFDA

Stefano Luccioli, USFDA/CFSAN

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

This symposium provides insights into FDA's recent activities on food allergens, with a focus on two 2025 guidances, case study examples highlighting their application, and discussions on food allergen thresholds. The 5th Edition of *Questions and Answers: Guidance for Industry (Allergen Q&A Guidance)* touches on food allergen labeling requirements including the labeling of sesame, incidental additives, highly refined oils, dietary supplement products, and certain specific packing and labeling situations, such as individual units within a multi-unit package. The guidance also includes notable changes to FDA's interpretation of "tree nuts," "milk," and "eggs" as major food allergens. Some of these changes were initiated, in part, using criteria outlined in *FDA's Guidance for FDA Staff and Interested Parties: Evaluating the Public Health Importance of Food Allergens Other than the Major Food Allergens Listed in the FDC Act* (Allergen Framework Guidance). This guidance outlines the scientific factors and approaches FDA generally intends to take when evaluating the public health importance of food allergens that are not one of the nine major food allergens identified by law in the United States. The guidance also details how the available clinical and scientific information on factors relevant to the public health importance of major food allergens will be systematically reviewed. Application of the Allergen Framework Guidance will be highlighted by case studies that involved the body of evidence on allergies to tree nuts, milk, and eggs and were used by FDA to update information in the Allergen Q&A Guidance regarding the definition of tree nuts, milk, and eggs that FDA considers as major food allergens. The

symposium with also cover other recent FDA food allergen activities.

Primary PDG Sponsor: Food Chemical Hazards and Food Allergy PDG

Secondary PDG Sponsor: Food Law PDG

Primary Topic Area: Food Chemical Hazards and Food Allergy

Secondary Topic Area: Food Law

Keywords: Allergens,Labeling,Regulations,Risk Analysis

S58 - USING METAGENOMICS TO SOLVE REAL-WORLD FOOD SAFETY AND QUALITY CHALLENGES

Primary Author:
Celina To, bioMerieux

Persons:
Celina To, bioMerieux

Samoa Asigau, bioMerieux, Inc.

Paul Cotter, Teagasc

Hannes Pouseele, bioMerieux, Inc.

Marcos Sanchez, Texas Tech University

Matthew Henderson, Land O' Frost

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

Metagenomics has emerged as a powerful tool to uncover microbial dynamics that are otherwise missed by culture-based methods. While its promise is widely discussed, practical applications that directly benefit food processors and manufacturers remain underrepresented in food safety conversations. This symposia will bridge that gap by highlighting real-world implementations of metagenomics in the food industry. Speakers will showcase examples where metagenomic data have been applied to inform decision-making and risk management strategies across the food manufacturing supply chain. Case studies include metagenomic profiling to assess shelf-life stability, microbial biomapping for facility baselining, and monitoring population stability in starter cultures—applications that move beyond academic exploration and provide actionable insights for food manufacturers. We will also explore how these new approaches can complement regulatory requirements, enrich the understanding of food manufacturing processes and thus lead to routine adoption. The symposia will bring together industry, technology, and academic perspectives to highlight challenges and successes in applying metagenomics to food safety and quality problems. Discussions will emphasize lessons learned, key takeaways for processors and

manufacturers, and strategies for implementation and scaling adoption within commercial food environments. Attendees will leave with a) a real-life understanding of how to implement metagenomics, and b) tangible, operational examples, including applications such as assessing microbial stability, detecting and mitigating contamination risks, evaluating the health of manufacturing processes, and enhancing overall food system resilience.

Primary PDG Sponsor: Advanced Molecular Analytics PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Non PDG Sponsor (Organization, Association, etc.): NGS Food Microbiology Forum (pending approval)

Primary Topic Area: Advanced Molecular Analytics

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Microbial Modelling and Risk Analysis

Keywords: Data,Environmental monitoring,Genomics,Laboratory and Detection Methods,Meat and Poultry,Microbial stress,Microbiome,Modeling,One Health,Pathogens,Public Health,Risk Assessment,Risk Management,Sanitation,Spoilage

S59 - DATA-DRIVEN INSIGHTS IN FRESH PRODUCE SAFETY – PUTTING LEARNINGS INTO ACTIONS

Primary Author:
Kristin Butler, US Food and Drug Administration

Persons:
Kristin Butler, US Food and Drug Administration

Samir Assar, USFDA

Kurt Nolte, Food and Drug Administration

Audrey Draper, USDA

Greg Komar, California Leafy Greens Marketing Agreement

Laura Strawn, Virginia Tech

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

Ensuring the safety of fresh produce remains a critical public health priority, requiring collaboration across government, academia, and industry to identify contributing factors and implement effective interventions. This symposium will highlight how data-driven insights can shape prevention, strengthen industry practices, and translate scientific findings into actionable solutions.

The session will explore three interconnected themes. First, speakers will discuss the FDA's key insights into outbreaks from the past 10 years, focusing on inspection citations, main contributing factors, and microbiological findings. Second, industry representatives will share lessons learned from ongoing surveillance and monitoring programs, including how data has informed best practices and advanced food safety culture. Finally, the symposium will focus on bridging science and practice, highlighting approaches for turning research findings into practical, actionable strategies that reduce risks and protect public health.

By bringing together diverse perspectives, this symposium will foster discussion on how to optimize the use of data to prevent produce-related illnesses, strengthen food safety

systems, and drive continuous improvement across the produce supply chain.

Primary PDG Sponsor: Fruit and Vegetable Safety and Quality PDG

Primary Topic Area: Fruit and Vegetable Safety and Quality

Secondary Topic Area: Pre Harvest Food Safety

Keywords: Intervention,Pre-harvest,Produce,Risk Management

S60 - WHEN CLEANING TOOLS BECOME INOCULATORS: A FOOD SAFETY BLIND SPOT

Primary Author:
Vickie Lewandowski, Barry Callebaut

Persons:
Vickie Lewandowski, Barry Callebaut

Deb Smith, Vikan

Neil Bogart, Post Consumer Brands

David Buckley, Diversey, Inc.

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

An effective Cleaning Tool Management Program is not merely a best practice—it is a regulatory mandate, a Global Food Safety Initiative (GFSI) scheme certification requirement, and a vital control point in preventing foodborne illness. Despite its importance, cleaning tool management is frequently overlooked and remains a recurring root cause of contamination events.

This symposium will explore foundational components of a robust Cleaning Tool Management Program:

Tool Selection:
Selecting the right cleaning tools—whether manual or automated—is essential for effective sanitation of both surfaces and the tools themselves. Key selection criteria should include soil type, surface characteristics, and hygienic design to ensure optimal cleaning performance and minimize contamination risks.

Tool Management:

A structured program is necessary to prevent cleaning tools from becoming both sources and vectors of contamination. Key elements include:

Documented procedures for post-use cleaning e.g., risk-based cleaning frequencies,

validation, and assigned responsibilities
Providing the right space for cleaning
Considerations for automated systems such as internal cleaning cycles, filter and hose maintenance, floor scrubber cleaning

Tool Maintenance:

Regular inspection and upkeep ensure tools remain safe and effective including:
Replacement of worn or damaged tools
Inventory control to maintain tool availability
Proper storage to prevent cross-contamination
Calibration, software updates, preventive maintenance, mechanical inspections, etc. for automated systems

This session will provide actionable insights and practical strategies for implementing and sustaining an effective cleaning tool management system to reduce risk and comply with legal and food safety standard requirements.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Sanitary Equipment and Facility Design

Keywords: Equipment,Hygiene,Sanitation

S61 - DIGITAL INNOVATIONS FOR PRODUCE SAFETY: ENHANCING FSMA-PSR COMPLIANCE ABROAD TO PROTECT CONSUMERS

Primary Author:

Noemi Zuniga Thimeos, Inter-American Institute for Cooperation on Agriculture

Persons:

Ana Cordero, Inter-American Institute for Cooperation on Agriculture

Clare Narrod, USDA

Noemi Zuniga Thimeos, Inter-American Institute for Cooperation on Agriculture

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

This symposium will explore how digital innovations are strengthening grower compliance with the Food Safety Modernization Act's Produce Safety Rule (PSR), focusing on collaborative FDA–IICA efforts in Latin America. As the U.S. imports over 60% of its vegetables and nearly half of its fruits from Mexico and the wider region, improving compliance capacity abroad is critical for safeguarding U.S. consumers. Fresh produce remains a leading cause of foodborne illness in the U.S., with annual costs estimated at $17.5 billion for the most common pathogens and up to $51 billion for all foodborne infections. The PSR, introduced in 2015, mandates training and science-based standards for all growers supplying the U.S. market, yet international adoption has faced unique challenges.

Speakers will highlight multiple dimensions of the FDA–IICA collaboration to enhance produce safety in Latin America. Presentations will cover the regulatory importance of digital tools for international FSMA/PSR compliance, the design and rollout of the Growing Safe Produce (GSP) platform, and lessons from validation studies, including a randomized trial in Mexico, Chile, and Costa Rica that demonstrated significant knowledge gains among growers. Outreach experiences from across the region will illustrate how the platform is being adopted in practice, with success stories from industry and regulators.

By integrating scientific evidence, regulatory perspectives, and on-the-ground outreach, this

symposium will provide a comprehensive view of how digital tools can bridge science, regulation, and practice to strengthen global produce safety systems. It will highlight opportunities for scaling these solutions globally, reinforcing the value of international collaboration for advancing food safety.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Non PDG Sponsor (Organization, Association, etc.): Inter-American Institute for Cooperation on Agriculture (IICA)

Primary Topic Area: Food Safety Education

Secondary Topic Area: International Food Protection Issues

Additional Topic Area (Optional): Fruit and Vegetable Safety and Quality

Keywords: Education,Food Safety Culture,Produce

S62 - FOOD SAFETY MANAGEMENT IN FERMENTED FOOD PRODUCTION

Primary Author:
Caitlin Karolenko, Institute for the Advancement of Food and Nutrition Sciences

Persons:
Caitlin Karolenko, Institute for the Advancement of Food and Nutrition Sciences

Kathleen Glass, University of Wisconsin-Madison

Laurie Post, Deibel Laboratories

Maria Marco, Univerisity of California Davis

David Bean, Federation University Australia

Samuel Alcaine, IDFA

Sarah Griggs, Tyson Foods, Inc.

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

Fermentation has long been used to preserve foods, but products once considered niche have become mainstream, driven by demand for minimally processed, clean label, “gut health” foods. Items such as kimchi, kombucha, miso, and fermented sausages are now widely distributed in global markets, bringing greater attention to food safety challenges.

Many traditional fermented products lack a defined lethality step and are consumed without cooking. Instead, they rely on hurdles such as microbial competition, salt, acidification, or extended fermentations to limit pathogen growth. While effective in many cases, these approaches create challenges for processors tasked with demonstrating microbial safety. Auditors and regulators expect HACCP-style controls, yet defining a “kill step” is difficult when safety depends on hurdle methods. Recent outbreaks and recalls linked to products like kimchi and tempeh further underscore the need for clarity and practical guidance.

This session will open with an overview of fermentation processes and food safety risks,

identifying data gaps and priority concerns. Subsequent talks will address commodity-specific issues in fermented vegetables, dairy and plant-based analogs, and fermented meats. Presentations will examine validation and shelf-life studies, corrective actions when processes fail, and case studies that highlight both problems and solutions.

With an applied science perspective, this session will provide actionable strategies and practical pathways for improved risk management in the expanding world of traditionally fermented products.

Primary PDG Sponsor: Plant-Based Alternative Products Quality and Food Safety PDG

Non PDG Sponsor (Organization, Association, etc.): Institute for the Advancement of Food and Nutrition Sciences

Primary Topic Area: HACCP Utilization and Food Safety Systems

Secondary Topic Area: Plant-Based Alternative Products

Additional Topic Area (Optional): Dairy Quality and Safety

Keywords: Dairy,HACCP,Plant-based foods

S63 - BUBBLY, BREWED, AND PLANT-BASED: MICROBIAL FOOD SAFETY AND SPOILAGE CONSIDERATIONS FOR ALTERNATIVE BEVERAGES

Primary Author:
Martha Kimber, Eurofins

Persons:
Martha Kimber, Eurofins

Sun Ferreira, University of Arkansas - Food Science

Ann Vegdahl, Cornell University

Catherine Felice (Rolfe), USFDA

Andrew Maust, University of Arkansas

Florence Wu, AEMTEK, Inc.

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

Demand for alternative beverages has surged in recent years as consumers seek healthier, less-processed, non-alcoholic, higher-protein, and/or plant-based options over traditional beverages. This evolving market has introduced many innovative products that challenge conventional food safety and quality practices. This symposium will explore the microbiological safety and spoilage concerns associated with emerging alternative beverage categories. Unlike traditional beverages, many of these products lack formulation or processing hurdles that have historically mitigated microbial risks, often due to clean-label positioning, reduced processing, or alcohol removal. As a result, they may be more susceptible to contamination, spoilage, or reduced shelf life.

RTD beverages covered in this symposium include cold-brewed coffee; low- and non-alcoholic beverages; fermented beverages; and plant-based alternatives such as non-dairy milks, creamers, and high-protein drinks. Many of these products have been associated with recalls in recent years, and in the case of plant-based beverages, a foodborne illness outbreak

occurred in 2024. Consequently, microbiological safety and quality have become top-of-mind concerns for the beverage industry, particularly for manufacturers navigating rapid innovation, co-manufacturing challenges, and evolving regulatory expectations.

This symposium will highlight current challenges and future directions for ensuring food safety and quality in the alternative beverage sector. By bringing together experts from regulatory agencies, academia, and the beverage testing industry, the session will deliver practical, industry-relevant insights to help manufacturers proactively manage microbial risk while continuing to innovate.

Primary PDG Sponsor: Beverages and Acid/Acidified Foods PDG

Secondary PDG Sponsor: Plant-Based Alternative Products Quality and Food Safety PDG

Primary Topic Area: Beverages and Acid/Acidified Foods

Secondary Topic Area: Plant-Based Alternative Products

Keywords: Beverages,Pathogens,Plant-based foods

S64 - DE-BUGGING PET FOOD SAFETY: TACKLING RISKS IN INSECT PROTEINS AND NOVEL INGREDIENTS

Primary Author:
Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Persons:
Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Beilei Ge, USFDA

Aaron Pleitner, Certified Group

Celina To, bioMerieux

Maura Di Martino, Food and Agriculture Organization of the United Nations (FAO)

Ian Banks, Enviroflight

Lauren Howell, USFDA CVM Division of Animal Food Ingredients

Maria Velissariou, Food Security Solutions Americas

Session Type: Short Symposium - 2.0 hours (Limited slots on Wednesday 1:30pm-3:30pm)

Session Summary:

The pet food sector has undergone a rapid transformation driven by consumer demand for sustainability, novel proteins, and functional nutrition. Insect proteins, alternative ingredients, and new product formats are emerging key innovations with potential to reduce environmental impacts while meeting evolving market needs. However, these innovations also raise new questions for food safety, regulatory compliance, and consumer trust. This symposium will bring together global experts from academia, industry, and regulatory bodies to examine and address critical safety considerations including allergenic peptides, heavy metals and environmental contaminants, microbial hazards associated with novel ingredients in pet food and food authenticity and integrity testing strategies to verify ingredient identity, prevent adulteration, and ensure consumer confidence. Through case studies, risk assessment data, and practical mitigation strategies, speakers will highlight current science, regulatory perspectives, and emerging analytical tools to ensure that

innovation in pet food remains aligned with robust food safety principles for both the pets and pet food handlers. These insights will equip participants with improved understanding of the unique safety challenges posed by novel ingredients and practical approaches for de-risking these innovations across the supply chain.

Primary PDG Sponsor: Animal and Pet Food Safety PDG

Secondary PDG Sponsor: Plant-Based Alternative Products Quality and Food Safety PDG

Primary Topic Area: Animal and Pet Food Safety

Secondary Topic Area: HACCP Utilization and Food Safety Systems

Additional Topic Area (Optional): Plant-Based Alternative Products

Keywords: Allergens,Animal and Pet Food Safety,Chemical Contaminant,HACCP,One Health,Pathogens,Pet Food,Public Health,Risk Management,Sustainability,Traceability,Validation

Roundtable Abstracts

RT1 - THE CHANGING LANDSCAPE OF SCIENTIFIC PUBLISHING: PREDATORY JOURNALS, AI, AND COMPROMISING SCIENTIFIC PEER REVIEW

Primary Author:
Joshua Gurtler, USDA-ARS

Persons:
Joshua Gurtler, USDA-ARS

Luca Cocolin, University of Torino

Catherine Nettles Cutter, Penn State University

Doris D'Souza, University of Tennessee Knoxville

Panagiotis Skandamis, Agricultural University of Athens

Keith Warriner, University of Guelph

Marcel Zwietering, Wageningen University

Session Type: Roundtable - 1.5 hours

Session Summary:

Scientific publishing has changed dramatically. Although modern electronic submission and peer review have expedited things, other aspects are troubling. Some of these problems include AI in manuscript writing, predatory journals, and increasing pressure for rapid review. Another issue, compromised peer review, upends the >50-year anonymized peer-review process, which removes personal bias, preventing open discourse for fear of backlash. Some journals now opt for a "collaborative review process," allowing author-reviewer interaction online. Although there may be some advantages to this latter format, it makes rejecting manuscripts difficult and compromises the science. More troubling are newer publishing platforms, regulated by non-scientists, involving (1) no scientific editors, (2) *non*-anonymized reviewer comments, and (3) manuscripts published prior to peer review (only reviewed after publishing, and then subsequent changes are made online). In this case, unreviewed scientific publications are frequently cited, not having gone through a rigorous peer review. These practices introduce bias and prevent open critique, further compromising

science. The eight panelists will include four editors from IAFP's *Journal of Food Protection,* Dr. Cathy Cutter of IAFP's *Food Protection Trends*, Dr. Keith Warriner (editor-in-chief of *Food Microbiology*), and two former 15-year editors for the *International Journal of Food Microbiology* (IJFM), including Drs. Marcel Zwietering and Luca Cocolin (former IJFM editor-in-chief). Dr. Cocolin wrote his final IJFM editorial in early 2025, discussing many of the topics to be covered in this session. This roundtable will be highly informative for students, career scientists, editors, and reviewers, providing insight and advice on how to navigate this changing landscape for publishing their scientific research.

Primary PDG Sponsor: Developing Food Safety Professionals PDG

Primary Topic Area: Communication Outreach and Education

Secondary Topic Area: Food Safety Education

Additional Topic Area (Optional): Food Safety Culture

Keywords: Education,Professional Development,Social Media

RT2 - ENHANCING HAZARD ANALYSIS: SEPARATING INGREDIENT AND PROCESS STEP EVALUATIONS FOR IMPROVED FOOD SAFETY

Primary Author:
Balasubrahmanyam (Bala) Kottapalli, The Marzetti Company

Persons:
Balasubrahmanyam (Bala) Kottapalli, The Marzetti Company

Yvonne Masters, John B. Sanfilippo & Son, Inc.

Byron Chaves, Rutgers University

Timothy Jackson, FDA-CFSAN

Loralyn Ledenbach, Ledenbach Food Safety, LLC

Sara Mortimore, Sara Mortimore LLC

Ruth Petran, Ruth Petran Consulting, LLC

Aaron Uesugi, Mondelez International

Session Type: Roundtable - 1.5 hours

Session Summary:

Hazard analysis is the cornerstone of HACCP and Preventive Controls for Human Food (PCHF) plans, yet many food safety teams struggle to capture the full range of biological, chemical (including allergens & radiological), and physical hazards. An emerging practice to address this challenge is conducting separate hazard analyses for ingredients and for process steps. This roundtable will examine how this approach improves completeness, minimizes overlooked risks, and strengthens preventive controls.

Discussion will emphasize scientific methods for hazard identification and evaluation, including assessment of inherent ingredient hazards, hazards introduced or enhanced during processing (including from the environment), and systematic evaluation of severity and likelihood. Case studies involving multi-ingredient product lines and complex

manufacturing steps will demonstrate how separating analyses helps prevent gaps often seen in combined formats. This approach is also reflected in Codex guidance and in the FSPCA PCHF version 2.0 training, underscoring industry recognition of its value.

Key discussion points include:

- Advantages and challenges of separating ingredient versus process-step analyses
- Practical methods to capture hazards introduced, controlled, or enhanced during processing
- Using scientific data, regulatory guidance, and outbreak history to support hazard decisions
- Strategies to maintain documentation and demonstrate effectiveness to auditors and regulators
- Collaborative approaches among industry, academia, and regulators to strengthen hazard identification and preventive control implementation

This session will provide practical, science-based guidance for manufacturers of all sizes, regulatory agencies, and academic researchers. By adopting structured ingredient and process hazard analyses, food safety teams can improve the rigor and credibility of food safety plans, enhance audit readiness, and ultimately strengthen consumer protection and public health.

Primary PDG Sponsor: HACCP Utilization and Food Safety Systems PDG

Primary Topic Area: HACCP Utilization and Food Safety Systems

Secondary Topic Area: Food Safety Education

Additional Topic Area (Optional): Food Safety Assessment, Audit and Inspection

Keywords: HACCP,Public Health,Risk Assessment

RT3 - BALANCING INNOVATION AND CONSUMER EXPECTATIONS: ALIGNING FOOD SAFETY AND BUILDING TRUST

Primary Author:
Caitlin Karolenko, Institute for the Advancement of Food and Nutrition Sciences

Persons:
Caitlin Karolenko, Institute for the Advancement of Food and Nutrition Sciences

Kristen Hunt, Deibel Laboratories, Inc.

Abigail B. Snyder, Cornell University

Polly Courtney, General Mills, Inc.

Meredith Carothers, USDA-FSIS

Ben Chapman, North Carolina State University

Matt Herrick, International Dairy Foods Association

Jason Jacobs, Beech-Nut

Mark Moorman, Retired, USFDA

Session Type: Roundtable - 1.5 hours

Session Summary:

The global food system is rapidly evolving as consumer expectations for sustainability, transparency, and simplicity reshape how food is produced and processed. Regenerative agriculture emphasizes soil health, biodiversity, and reduced synthetic inputs, offering climate-positive benefits but also introducing new food safety considerations. Integrated livestock systems, water reuse, and shifts in soil pathogen ecology create emerging microbial risks that require careful oversight.

At the same time, debate around ultra-processed foods is driving reformulation efforts to reduce additives, simplify ingredient lists, and limit processing steps. While “ultra-processed” lacks a regulatory definition, consumer demand for foods perceived as clean, wholesome,

and safe continues to grow. Reformulation may improve perception but can also alter hazard profiles, impact shelf-life stability, and necessitate new preventive control strategies.

For food safety professionals, the convergence of sustainability goals and shifting consumer expectations requires proactive adaptation of hazard analyses, verification methods, and communication approaches to address these changes. This roundtable will provide an interactive forum to explore emerging risks, share perspectives, and identify strategies to build trust while advancing food safety.

Learning Objectives
Participants will be able to:
• Recognize safety concerns in regenerative and reformulated foods.
• Integrate risk management into evolving practices.
• Assess applicability of current regulatory and verification frameworks.
• Understand how consumer perceptions shape safety priorities.
• Develop empathetic messages that strengthen trust in safe, resilient food systems.

Key Takeaway
Innovation must be matched with proactive food safety. Empathetic communication, paired with risk-based approaches, is essential to aligning regenerative and reformulation practices with consumer trust in resilient food systems.

Primary PDG Sponsor: Food Safety Culture PDG

Non PDG Sponsor (Organization, Association, etc.): Institute for the Advancement of Food and Nutrition Sciences

Primary Topic Area: Food Safety Culture

Secondary Topic Area: Communication, Outreach and Education

Keywords: Food Safety Culture,One Health,Pathogens

RT4 - LEARNING IN SECONDS: RETHINKING FOOD SAFETY EDUCATION FOR THE FRONTLINE

Primary Author:
Chip Manuel, Chipotle

Persons:
Chip Manuel, Chipotle

Sara Starck, Instacart

Akhila Vasan, Uber

Julie Abouchar, Amazon and Whole Foods Market Worldwide Grocery Stores

Barbara Chamberlin, New Mexico State University

Jessica Leatherman, Steritech

Rachel Scanlan, Chick-fil-A

Jerry Wojtala, International Food Protection Training Institute

Session Type: Roundtable - 1.5 hours

Session Summary:

Traditional food safety education approaches are often long and text-heavy, and struggle to be effective in today’s fast-paced food environments. Evidence from clinical and workforce settings shows that micro-learning—short, focused learning moments—can improve engagement, retention, and compliance. For food safety professionals worldwide, the challenge is clear: how do we deliver meaningful education when we may only have minutes—or even seconds—with people? This roundtable will feature international perspectives from academia, retail, foodservice, and regulatory sectors on innovative, practical approaches to providing food safety education in time-short situations. Panelists will discuss their experience with developing food safety education that strengthens audit outcomes, improves frontline execution, and equips regulators with better tools to assess learning outcomes in diverse cultural and operational contexts. Attendees will explore how to capture attention quickly, deliver messages that stick, and drive buy-in. The session will provide

actionable insights into building scalable education programs that improve food safety culture globally.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Retail and Foodservice PDG

Primary Topic Area: Food Safety Education

Secondary Topic Area: Retail and Foodservice

Keywords: Education,Food Service,Retail

RT5 - WHAT COMES NEXT: BUILDING FOOD SAFETY STRATEGIES AFTER NACMCF

Primary Author:
Abigail B. Snyder, Cornell University

Persons:
Abigail B. Snyder, Cornell University

Elisabetta Lambertini, GAIN

Ben Chapman, North Carolina State University

Sandra Eskin, STOP

David Goldman, Groundswell Strategy

Jennifer McEntire, Food Safety Strategy LLC

Session Type: Roundtable - 1.5 hours

Session Summary:

The safety of powdered infant formula continues to draw attention due to the risk posed by *Cronobacter* spp. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) was charged in 2023 with evaluating risk factors for consumer populations, identifying strategies manufacturers can use to reduce contamination, and considering approaches for effective risk communication.

NACMCF was terminated via executive order in 2025, so no report or final recommendations from NACMCF will be published. The goal of this session is to share key insights on *Cronobacter* spp. control because the topic continues to be important to the health of infants.

Additionally, the session will then consider how to continue development of scientific consensus in the absence of NACMCF as a collaborative process. Panelists will explore options for collectively synthesizing science, policy, and industry practice—whether through professional societies, public-private partnerships, or other multi-stakeholder platforms.

Panelists will be invited to share perspectives on maintaining scientific rigor, transparency,

and actionable outputs in future collaborative efforts. By combining technical findings with forward-looking discussion, this roundtable aims to ensure continued progress in infant formula safety and the protection of infant health.

Primary Topic Area: Food Law

Secondary Topic Area: Dairy Quality and Safety

Additional Topic Area (Optional): Food Safety Education

Keywords: Dairy,Education,Food Safety Culture

RT6 - HYGIENIC RESTORATION: RETHINKING RISK, INVESTMENT, AND CONTINUITY IN FOOD FACILITIES

Primary Author:
Josie Greve-Peterson, Fortrex

Persons:
Josie Greve-Peterson, Fortrex

Deb Smith, Vikan

James Davis, OSI Group, LLC

Scott King, Fortrex

Nathan Mirdamadi, Kerry

Eric Schweitzer, 3-A Sanitary Standards, Inc.

Jaime Vaca, Boar's Head Brand

Adwy Van Den Berg, EHEDG

Session Type: Roundtable - 1.5 hours

Session Summary:

Yesterday’s cutting-edge is today’s compromise. Many legacy food facilities and equipment lines were built for performance, not necessarily for hygiene. As industry standards evolve and consumer expectations rise, the absence of hygienic design is no longer just a liability, it’s a potential food safety crisis waiting to unfold.

So, what do you do when your facility isn’t built with modern hygienic principles in mind? Before reaching for the bulldozer, let’s ask the harder questions: Can we restore rather than replace? What does "good enough" look like and when is that not enough? How do we align operational needs with capital limitations?

The expert panel will explore hygienic restoration through the lens of legacy facility challenges, crisis recovery, infrastructure modernization, long-term asset management and new GSFI standards. Panelists will challenge traditional thinking around infrastructure and

equipment upgrades, exploring "good, better, and best" solutions and how to weigh them through the lens of total cost of ownership not just upfront expense. We'll also delve into the often overlooked human element: How do you build the case for investment? What persuades decision-makers to fund hygienic restoration over other capital priorities? Finally, once the green light is given the real work begins. Panelists will highlight effective strategies to manage major construction events by balancing project execution with hygiene, safety and production continuity.

This isn’t just about designing equipment and facilities to be easily cleaned and sanitized. Hygienic restoration is a strategic commitment to future-proofing your facility, protecting your brand, preventing contamination and minimizing the risk of foodborne illnesses. Whether you're restoring a legacy plant or planning capital investments with hygiene in mind, this panel will provide actionable insights, field-tested strategies and innovative solutions for navigating the complexities of hygienic restoration.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Secondary PDG Sponsor: Sanitary Equipment and Facility Design PDG

Primary Topic Area: Sanitary Equipment and Facility Design

Secondary Topic Area: Food Hygiene and Sanitation

Keywords: Equipment,Hygiene,Production/Plant Design,Risk Management,Sanitation

RT7 - FULL STRENGTH, FULLY UNDERSTOOD: BRIDGING LANGUAGE GAPS WITHOUT CUTTING CONTENT

Primary Author:

Angela Walla, International Ctr. for Food Industry Excellence, Dept. of Animal and Food Science, Texas Tech University

Persons:

Angela Walla, International Ctr. for Food Industry Excellence, Dept. of Animal and Food Science, Texas Tech University

Neal Fredrickson, Cargill

Jose Guerrero, Genista Biosciences

Karla Acosta, University of Houston (Health Inspector)

Laura Acuna-Maldonado, Produce Safety Alliance, Cornell University

Richelle Beverly, WK Kellogg Co.

Dina Scott, Wendys

Session Type: Roundtable - 1.5 hours

Session Summary:

Delivering food safety education in the workplace is a complex task, made even more challenging in multilingual environments where 10 to 40 languages may be spoken within a single facility. While translation tools and AI-powered platforms offer promising solutions, effective training requires more than direct translation—it demands clarity, cultural sensitivity, and thoughtful instructional design. Simplifying materials to a fourth-grade reading level is a common best practice, but this raises a critical question: how can organizations preserve the full strength of technical content like hazard analysis and risk management without losing meaning in translation?

This roundtable brings together global experts from industry, academia, and the private sector to explore how companies can bridge language gaps without compromising content integrity. Attendees will gain practical strategies for assessing organizational language

needs, designing inclusive training materials, and validating translated content for accuracy and clarity. The session will also highlight instructional design methodologies that support multilingual learning and foster engagement across diverse teams. The panelists have experience with training a global workforce in many languages (i.e., Spanish, French, Chinese (Mandarin, Simplified), Indonesian, Portuguese, Polish, Tagalog (Filipino), Thai, and German).

Participants will leave with actionable tools to tailor food safety messaging to multilingual workforces, improve regulatory compliance, and build a more inclusive workplace culture. This session is designed for professionals seeking to elevate their food safety programs through communication strategies that are linguistically precise, culturally relevant, and operationally effective.

Primary PDG Sponsor: Food Safety Education PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Non PDG Sponsor (Organization, Association, etc.): Welcome Committee and DEI Council

Primary Topic Area: Food Safety Education

Secondary Topic Area: Food Safety Culture

Keywords: Education,Food Safety Culture,Inclusion

RT8 - IS EXACT COLONY COUNTING JUST RIGHT, OR IS ONE-LOG RESOLUTION ENOUGH?

Primary Author:
Daniele Sohier, Hygiena

Persons:
Daniele Sohier, Hygiena

Anett Winkler, Cargill

Amanda Brookhouser-Sisney, Midwest Laboratories

Caio Carvalho, Cargill

William K. Shaw, Jr., USDA-FSIS

Deb Smith, Vikan

Pamela Wilger, Post Consumer Brands

Session Type: Roundtable - 1.5 hours

Session Summary:

Reliable microbiological testing underpins food safety, guiding both regulatory compliance and industry practice. Regulations set contamination thresholds (e.g., <1000 CFU/g) and require scientifically validated and verified methods. This raises a central debate: Should compliance rely on exact colony counts, or is a one-log (10×) resolution sufficient to show contamination levels fall within acceptable limits?

Exact counts provide value for quality management, trend analysis, and risk assessment. They can reveal subtle contamination patterns, detect emerging issues, and support process optimization. However, obtaining exact colony counts often extends the time to results, which can delay decision-making and downstream processes. Yet for food business operators (FBOs), precision comes at a cost: colony counting is labor-intensive, resource-heavy, and often unnecessary when the goal is simply to demonstrate results below a regulatory cut-off.

By contrast, one-log methods can offer a more practical, cost-effective option for routine monitoring and hygiene checks, as long as they are scientifically validated and measurement uncertainty is properly managed. Challenges arise when results sit near the threshold: variability and standard deviation may blur the line between “compliant” and “non-compliant,” with potential for disputes or recalls.

This roundtable will bring together regulators, FBOs, contract laboratories, and academics to consider:

- Whether exact counting is essential for compliance decisions
- How a one-log approach affects risk assessment and regulatory confidence
- The scientific validity of different testing approaches
- How regulatory acceptance shapes method choice
- The role of uncertainty and variability in interpreting results
- Practical strategies, including cost-benefit analysis, to balance efficiency, accuracy, and compliance

Primary PDG Sponsor: Applied Laboratory Methods PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: Applied Laboratory Methods

Secondary Topic Area: Microbial Modelling and Risk Analysis

Additional Topic Area (Optional): Food Hygiene and Sanitation

Keywords: Environmental monitoring,Laboratory and Detection Methods,Regulations,Risk Management

RT9 - BEST PRACTICES FOR GOVERNING DATA IN THE AI ERA

Primary Author:
James Doyle, Creme Global

Persons:
James Doyle, Creme Global

David Monk, Chick-fil-A, Inc

Sarah Murphy, USFDA

Kristin Butler, US Food and Drug Administration

Renata Ivanek, Cornell University

Cronan McNamara, Creme

Gustavo Reyes, Western Growers Science

Suzy Sawyer, Cargill

Jiyoon Yi, Michigan State University

Session Type: Roundtable - 1.5 hours

Session Summary:

As artificial intelligence (AI) and advanced analytics become embedded in prevention-focused, food safety practice, data governance is more important than ever. Importantly, data governance allows for fully harnessing the capabilities of predictive analytics (e.g., by detecting commonalities among standardized data) and AI (e.g., by reducing the potential to hallucinate), and is especially useful for data sharing. Without robust governance, validation, and harmonization, AI outputs risk being seen as opaque or unreliable, limiting their value in protecting public health. This roundtable will explore the shift from compliance-focused data practices toward a culture of confidence and prevention, where trusted, well-governed data empowers regulators, industry, and researchers to make better, faster prevention decisions.

Panelists from regulatory agencies, academia, producer associations, retail, and data science will discuss the role of data governance in enabling voluntary data sharing that supports innovation while protecting privacy and maintaining trust. Real-world case studies will be

used to illustrate how diverse stakeholders have addressed barriers to sharing, defined clear rules for data use and access, and implemented governance approaches that work in practice.

This roundtable will also step back to consider the broader implications: why governance is essential, which features matter most, and how leadership and collaboration across the food chain can help build lasting confidence in AI-enabled food safety. Audience participants will be invited into the discussion to share perspectives and shape this vision for the future.

Primary PDG Sponsor: Data Management and Analytics PDG

Secondary PDG Sponsor: Modelling and Risk Analysis PDG

Primary Topic Area: Data Management and Analytics

Secondary Topic Area: Microbial Modelling and Risk Analysis

Additional Topic Area (Optional): Food Safety Culture

Keywords: Cyber,Data,Risk Analysis,Risk Management,Validation

RT10 - FROM CONFUSION TO COMPLIANCE: NAVIGATING THE FDA FOOD TRACEABILITY RULE FOR RETAILERS AND FOODSERVICE ESTABLISHMENTS

Primary Author:
Savana Everhart Nunn, North Carolina State University

Persons:
Savana Everhart Nunn, North Carolina State University

Carrie Rigdon, Association of Food and Drug Officials

Melissa Vaccaro, National Environmental Health Association (NEHA)

Patrick Guzzle, National Restaurant Association

Kathleen O'Donnell-Cahill, Wegmans

Hilary Thesmar, FMI - The Food Industry Association

Ellen Thomas Shumaker, North Carolina State University

Chris Waldrop, USFDA

Carlos Zapata, McDonald’s USA, LLC

Session Type: Roundtable - 1.5 hours

Session Summary:

FDA recently published the Food Traceability Rule (FTR) as part of the Food Safety Modernization Act (FSMA) which requires additional recordkeeping for entities that manufacture, process, pack, or hold foods that are part of FDA’s Food Traceability List (FTL). Adherence to the FTR highlights a need among retail food establishments, many who have not yet been subject to FSMA, for a rapid solution to learn about the requirements for this traceability rule and the best way to implement practices to adhere to the newly applicable legislation. These needs relate to knowing which foods require traceability, working with suppliers to ensure compliance, and requirements for data management. Although compliance has been extended to 2028, continued support and time to develop systems are needed to meet the FTR requirements. This session will explore the current retail and

restaurant needs for FSMA 204 compliance based on survey research and hearing directly from those in the field. Roundtable panelists will include industry members, academia, and associations representing the industry and environmental health professionals.

Primary PDG Sponsor: Retail and Foodservice PDG

Secondary PDG Sponsor: Food Safety Education PDG

Primary Topic Area: Retail and Foodservice

Secondary Topic Area: Communication, Outreach and Education

Additional Topic Area (Optional): Data Management and Analytics

Keywords: Food Service,Retail,Traceability

RT11 - EVERY DROP COUNTS: ADVANCING SAFE WATER REUSE IN DAIRY AND FOOD PROCESSING

Primary Author:
Preetha Biswas, Neogen Corporation

Persons:
Preetha Biswas, Neogen Corporation

Brendan Higgins, Auburn University

Raquel Medeiros, Nestlé

Chantal Nde, PepsiCo, Frito-Lay

Allen Sayler, Center for Food Safety & Regulatory Solutions (CFSRS)

Anett Winkler, Cargill

Session Type: Roundtable - 1.5 hours

Session Summary:

Water reuse is rapidly becoming a cornerstone of sustainable food production. As freshwater resources face increasing pressure from climate variability, industrial demand, and population growth, food processors are exploring reclaimed water as a viable solution for cleaning, cooling, irrigation, and even ingredient-level applications. However, this shift introduces complex challenges in **microbiological safety**, **regulatory compliance**, and **public perception**.

In the U.S., food and dairy processors are increasingly implementing **multi-barrier treatment systems** to reclaim water for non-potable uses. Some facilities report **up to 40% reductions in freshwater use** through reuse strategies. Globally, other countries have adopted **fit-for-purpose frameworks** that allow safe reuse based on intended application rather than blanket potable standards.

This roundtable will convene experts from regulatory, academic, and industrial sectors to examine the current state and future potential of water reuse in food processing. The session will focus on: **Microbial risk assessment and control strategies** and **Regulatory**

frameworks and standards guiding safe reuse practices; **Technological innovations** and **Fit-for-purpose water quality approaches** tailored to specific uses in food production; and **Case studies and lessons learned** from diverse regions and sectors. Participants will explore how water reuse can be safely integrated into food safety systems, including HACCP and environmental monitoring programs. The discussion will also address barriers to adoption, such as regulatory gaps, infrastructure limitations, and consumer concerns.

As global water stress intensifies, this session offers a timely and actionable forum for advancing safe, responsible water reuse in food and dairy processing.

Primary PDG Sponsor: Dairy Quality and Safety PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Dairy Quality and Safety

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Beverages and Acid/Acidified Foods

Keywords: Beverages,Controlled Environment,Dairy,Sustainability,Water

RT12 - PUBLIC DATA AGGREGATION TO SUPPORT WATER USE DECISIONS IN FOOD PRODUCTION

Primary Author:
Don Stoeckel, Cornell University

Persons:
Don Stoeckel, Cornell University

Jacquelina Woods, USFDA Gulf Coast

Jessica Jones, FDA Human Foods Program

Beatriz Martinez-Lopez, University of California Davis

Drew McDonald, Taylor Fresh Foods

Zach Nickerson, National Ecological Observatories Network (NEON)

Daniel Weller, US-CDC

Session Type: Roundtable - 1.5 hours

Session Summary:

Water quality, when used to grow food, is managed differently than other federally recognized designations like public water supply, industrial, and recreational use water. Currently, there is no numeric risk-based standard for agricultural water quality. This is due, in part, to the diversity of agricultural-use settings and variability in the dynamics of pathogens and fecal-indicator organisms. This variability is pronounced in feces sources and environmental fate-and-transport.

Many water resources have multiple users who make use decisions based on the same available data and guidance. Information tends to be either static (e.g., a single sanitary survey, total maximum daily load report, or agricultural water assessment that governs decisions for a full harvest season) or delayed (e.g., fecal-indicator organism monitoring with a multi-day lag between sample collection and results delivery). Aggregation of geospatial landscape data and continuously updated meteorological and other monitoring data, coupled with appropriate analytics and user interface, could result in a powerful decision

support tool analogous to the NowCast models used to assist with recreational swimming beach closure decisions in some areas.

The process of developing such a decision support tool has hurdles; panelists convened for this roundtable represent key users: primary production of fresh produce, shellfish harvesting, water resource management; potential sources: animal production; technical processes: large-scale data aggregation platforms, data analytics. Attendees will hear each perspective and have the opportunity to participate in a dynamic discussion of food system needs, data availability, and technical capabilities required to create a decision support tool for water quality management.

Primary PDG Sponsor: Water Safety and Quality PDG

Secondary PDG Sponsor: Data Management and Analytics PDG

Primary Topic Area: Water Safety and Quality

Secondary Topic Area: Data Management and Analytics

Additional Topic Area (Optional): Food Safety Assessment, Audit and Inspection

Keywords: Data,Environmental monitoring,Water

RT13 - INTERNAL ASSESSMENT: CLOSING THE GAP BETWEEN AUDITS AND FOOD SAFETY PERFORMANCE

Primary Author:
Shahram Ajamian, McCormick and Company

Persons:
Shahram Ajamian, McCormick and Company

Taryn Horr, Ramboll

James Doyle, Creme Global

Lone Jespersen, Cultivate SA

Judi Lazaro, AIB International

Emily Moyer, Chabaso Bakery

Session Type: Roundtable - 1.5 hours

Session Summary:

The food industry has long relied on third-party external audits, particularly GFSI-benchmarked certifications as a primary indicator of food safety preparedness and site performance. Companies often feel significant pressure to prepare for and pass these audits, since certification can be tied to market access, customer requirements, and brand reputation. Yet, history shows that many facilities successfully pass these external audits while still facing recalls, enforcement actions, and cultural breakdowns. This dynamic creates a disconnect, where the drive to pass audits overshadows the deeper internal assessments and the daily practices that sustain food safety. External audits alone cannot capture the true maturity of a site’s food safety culture or its operational readiness.

This roundtable will examine how internal assessments can fill that void. When designed and executed effectively, internal assessments provide organizations with insightful data and proactive mechanisms to identify vulnerabilities, uncover cultural blind spots, and strengthen accountability before risks escalate into crises. Panelists from industry, certification bodies, and consulting firms will share practical approaches, best practices, and lessons learned in

building robust internal assessment frameworks and data governance that complement, rather than duplicate, external audits.

The discussion will also explore how data management supports auditors to improve internal assessments, and why the robustness of these programs often serves as a leading indicator of long-term success. Participants will gain actionable insights into creating internal assessments that are not box-checking exercises but strategic tools for resilience, accountability, continuous improvement, and cultural transformation.

Primary PDG Sponsor: Food Safety Assessment, Audit and Inspection PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Primary Topic Area: Food Safety Assessment Audit and Inspection

Secondary Topic Area: Food Safety Culture

Keywords: Auditing,Data,Education,Food Safety Culture,HACCP,Recalls,Regulations

RT14 - FSMA AT 15: PROMISE, PROGRESS, AND PRIORITIES FOR THE FUTURE

Primary Author:
Jennifer McEntire, Food Safety Strategy, LLC

Persons:
Jennifer McEntire, Food Safety Strategy, LLC

Derek Hladki, Meijer, Inc.

Steven Mandernach, Association of Food and Drug Officials (AFDO)

Sandra Eskin, STOP

Donna Garren, American Frozen Food Institute

Erik Mettler, USFDA

Michael Taylor, STOP Foodborne Illness

Hilary Thesmar, FMI - The Food Industry Association

Session Type: Roundtable - 1.5 hours

Session Summary:

Fifteen years ago, the Food Safety Modernization Act (FSMA) promised to transform food safety in the United States. Since then, industry, regulators, and public health professionals have invested significant resources to implementing the Preventive Controls Rules, Produce Safety Rule, Foreign Supplier Verification Program, and other FSMA regulations. Yet, a key question remains: has FSMA delivered on its promise and truly moved the needle on food safety?

This roundtable convenes the architects and implementers who were instrumental in the development and execution of FSMA regulations, offering a unique opportunity to reflect on the Act's intent, implementation, outcomes, and impact. The session will open with a brief overview of the major outbreaks and challenges that drove FSMA's creation, followed by a moderated discussion on whether its rules, as written and implemented, have delivered meaningful progress in food safety. Panelists will examine whether FSMA's vision has been

realized, discuss the remaining gaps in food safety systems, and identify the metrics needed to evaluate true effectiveness.

Regulators, industry, academia, and other attendees will be part of a rare, candid conversation and gain practical insights from diverse perspectives—legal, regulatory, public health, and industry—on lessons learned, best practices, and opportunities for innovation in preventive controls, data-driven metrics, and cross-sector collaboration. Unlike prior sessions focused on a single rule, this roundtable will provide a comprehensive, forward-looking, multi-rule, multi-stakeholder assessment of FSMA's impact and the work still ahead.

Primary PDG Sponsor: Food Law PDG

Primary Topic Area: Food Law

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Keywords: Outbreak,Public Health,Regulations

RT15 - PRODUCT LABELING MISTAKES: WHEN HUMAN FAILURE LEADS TO A FOOD RECALL; STRATEGIES AND IMPACTS

Primary Author:
Sarah Smith-Simpson, Nestle Nutrition

Persons:
Sarah Smith-Simpson, Nestle Nutrition

Anthony Flood, IFIC

Ben Chapman, North Carolina State University

Conrad Choiniere, USFDA

Melanie Downs, University of Nebraska Lincoln

Sarah Kozak-Weaver, Wegmans Food Markets Inc.

Session Type: Roundtable - 1.5 hours

Session Summary:

Food recalls play an important role in protecting public health. When implemented properly to reduce the risk of a potential micro, chemical, foreign matter, biological, or any otherwise deemed “unsafe” food, these strategies are carefully choreographed alongside a well-rehearsed medley industry and federal food agency collaboration. According to IFIC Consumer Research, approximately 75% of Americans believe that “food recalls are on the rise", with a majority hearing about a recall first from traditional or social media.

In IFIC’s *American Perceptions of Food Allergens, Intolerances and Sensitivities*, “unclear labeling is a top concern among Americans that know someone with a food allergy". The majority of food allergen recalls are caused by preventable labeling errors, according to a recent analysis of recall data for U.S. Food and Drug Administration (FDA)-regulated products. Of the 1,471 total recalls, 1,415 were due to major food allergens, 34 were due to gluten-free labeling violations, and 23 involved other allergens. In general, recalls due to major food allergens increased during the study period, with a peak incidence rate in fiscal year 2017. The 2015

Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food Rule (21 Code of Federal Regulations (CFR) Part 117) defines preventive allergen controls including labeling requirements. In comparison to *21 CFR 117*, 76.8 percent of major food allergen recalls with a known root cause were likely due to inadequate label controls, the study found.

This discussion will examine consumer response to food recalls, allergen labeling, and will peel the onion on food safety culture to better assess the root cause of inadequate label management controls within our food system.

Primary PDG Sponsor: Physical Hazards and Foreign Material PDG

Secondary PDG Sponsor: Retail and Foodservice PDG

Non PDG Sponsor (Organization, Association, etc.): International Food Information Council (IFIC)

Primary Topic Area: Retail and Foodservice

Secondary Topic Area: Communication, Outreach and Education

Additional Topic Area (Optional): Food Safety Culture

Keywords: Allergens,Education,Food Safety Culture,Food Service,Labeling,Public Health,Recalls,Regulations,Retail,Traceability

RT16 - HOW LGBTQIA+ FOOD SAFETY PROFESSIONALS CAN THRIVE DURING LESS-AFFIRMING TIMES

Primary Author:
Tori Stivers, University of Georgia Marine Extension, Retired

Persons:
Tori Stivers, University of Georgia Marine Extension, Retired

John Boyce, Boyce Food Safety Consulting Ltd.

Andrew Clarke, Loblaw Companies Limited

James Jeffrey, University of Maryland

Chris Jordan, Diversey

Lisa Robinson, Ecolab

Erin Wade, Olam Food Ingredients

Session Type: Roundtable - 1.5 hours

Session Summary:

In 2023, IAFP hosted its first session focused on helping LGBTQIA+ (queer) food safety professionals. It was well attended and received highly positive feedback reflecting the need for such spaces. Since then, broad societal shifts have created new challenges for queer individuals and allies across many sectors, including food safety. Recent changes in cultural and political attitudes have left many queer professionals—and students—facing heightened uncertainty about their place in the workforce. For students and developing professionals, questions about career pathways and supportive employers have become increasingly pressing. For those already established, concerns about belonging, recognition and safety in work environments continue to evolve. These dynamics underscore the importance of building resilient, inclusive networks and creating professional opportunities where queer food safety professionals can thrive regardless of external shifts.

This session aims to help attendees adapt to elimination of diversity, equity, and inclusion efforts within the U.S. federal government, universities, and some food companies. Panelists

and attendees will give insight on employers and countries who welcome diversity. Attendees will learn how queer food safety experts have dealt with and overcome discrimination. Audience participation is strongly encouraged; a means to anonymously provide comments and ask questions will be offered to protect those who feel susceptible to recrimination.

Objectives are: 1) foster both a short and long-term sense of community among queer professionals and their allies at IAFP meetings, 2) exchange information on companies, organizations, and countries that affirm diversity, and 3) empower diverse engagement in IAFP's worldwide forum to advance food safety.

Primary PDG Sponsor: Developing Food Safety Professionals PDG

Secondary PDG Sponsor: Student PDG

Primary Topic Area: Developing Food Safety Professionals

Secondary Topic Area: Communication, Outreach and Education

Additional Topic Area (Optional): Food Safety Culture

Keywords: Inclusion,Professional Development,Sustainability

RT17 - HYDRATION PRIOR TO PROCESSING: CONTROLLING MICROBIAL CONTAMINANTS IN DRY INGREDIENTS

Primary Author:
Manoj Sawale, PepsiCo

Persons:
Manoj Sawale, PepsiCo

Kavita Patil, University of Arkansas

Nathan Anderson, USFDA

Pablo Coronel, CRB Group

Yuqian Lou, PepsiCo

Dharmendra Mishra, Purdue University

Wilfredo Ocasio, IEH Laboratories and Consulting Group

Aikansh Singh, Tetra Pak Inc.

Session Type: Roundtable - 1.5 hours

Session Summary:

Hydration is a critical step in processing dry ingredients such as beans, cocoa, and plant-based products, influencing thermal efficiency, nutrition, safety, and quality. Traditional methods remain effective but are time- and energy-intensive, often causing bean splitting, texture variation, sterility issues, and nutrient loss. Modern approaches improve efficiency and product consistency while also supporting microbial control and nutritional quality.

Controlled slurry preparation with temperature management, homogenization, agitation, and stabilizers improves wetting, minimizes dry zones, and supports spore inactivation while maintaining sensory properties. In beans, optimized hydration reduces cooking time, improves texture and digestibility, and ensures cold spot consistent sterilization. Similarly, plant-based beverages and foods face hydration and protein fouling challenges, complicating microbial control and sanitation.

This roundtable will address challenges in dry ingredient and cocoa processing, including microbial contamination, uniform sterilization, and product integrity. Cocoa powder poses unique risks since its hydrophobic nature hinders hydration, leaving dry pockets where bacterial spores may persist even after UHT treatment. Mycotoxins further complicate safety. The session will also examine contamination sources across the cocoa value chain from harvesting and fermentation to drying, roasting, and powder handling and explore mitigation strategies such as optimized roasting, steam and infrared treatments, hygienic equipment design, antimicrobial packaging, environmental monitoring, and HACCP-based controls.

The roundtable will emphasize next-generation solutions, including advanced hydration, strain-specific resistance studies, predictive microbiology, and industry-academia collaborations, to deliver safer, higher quality, plant-based and dry-ingredient products worldwide.

Primary PDG Sponsor: Beverages and Acid/Acidified Foods PDG

Secondary PDG Sponsor: Plant-Based Alternative Products Quality and Food Safety PDG

Primary Topic Area: Beverages and Acid/Acidified Foods

Secondary Topic Area: Plant-Based Alternative Products

Additional Topic Area (Optional): Food Safety Assessment, Audit and Inspection

Keywords: Beverages,Plant-based foods,Risk Assessment

RT18 - ADDRESSING KNOWLEDGE GAPS AND RESEARCH PRIORITIES FOR THE IMPACT AND CONTROL OF EMERGING DRY BIOFILMS IN FOOD SAFETY

Primary Author:
Dale Grinstead, Mountain Top Microbiology

Persons:
Dale Grinstead, Mountain Top Microbiology

David Blomquist, EAS Consulting Group

Juan Goncalves, P&G PRO Commercial Solutions

Neil Bogart, Post Consumer Brands

Jeffrey Kornacki, Kornacki Microbiology Solutions, Inc.

Jean-Yves Maillard, Cardiff University

Mark Moorman, Retired, USFDA

Haley Oliver, Purdue University

Melissa Vaccaro, National Environmental Health Association (NEHA)

Session Type: Roundtable - 1.5 hours

Session Summary:

The risk, nature, and ways to control wet surface biofilms in food settings have been studied for decades. However, dry surface biofilms are a relatively new phenomenon that have only recently begun to be studied and understood. Dry biofilms have been identified as a risk in health care settings but the risk that they pose in food settings is not well characterized. There have been presentations on dry biofilms at previous IAFP conferences, and those sessions have indicated that there is a general interest in this topic among many IAFP Members. However, those sessions have also shown that there is a paucity of knowledge available about dry biofilms and the significance (if any) of dry biofilms in food settings. Rather than simply repeat what little is understood about dry biofilms, this session will focus on discussing the gaps in our understanding of dry biofilms and attempt to identify the most

critical questions about dry biofilms that need to be addressed. Among those gaps are: How do dry biofilms form and how quickly do they grow? What differentiates a dry biofilm from a wet biofilm? Can biofilms change between wet and dry? Are dry biofilms present in food facilities, and if so, where and how often? What kind of food facilities are they present in? What is the impact of dry biofilms on environmental monitoring programs? How do you control dry surface biofilms? What is their risk and how do you control them specifically in dry clean food facilities? Clearly there are many basic research questions pertaining to dry biofilms. Unfortunately, we have very few answers to most of these questions. The panel will also discuss dry biofilm research needs and priorities. Attendees of this roundtable will gain an awareness of the current understanding of dry biofilms. They will also have an opportunity to engage in discussion with the panel and other attendees about dry biofilm research needs that they consider most critical.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Food Safety Assessment, Audit and Inspection

Additional Topic Area (Optional): Applied Laboratory Methods

Keywords: Hygiene,Laboratory and Detection Methods,Microbiome

RT19 - FLEXITARIANS - NEW PRODUCT OFFERINGS BLENDING THE BEST OF BOTH WORLDS

Primary Author:
Aaron Pleitner, Certified Group

Persons:
Deepa Thiagarajan, Independent Consultant, Food Safety & Regulatory Compliance Services

Lily Yang, The Acheson Group

Melanie Abley, USDA-FSIS

Aaron Asmus, Hormel Foods

Aaron Pleitner, Certified Group

Joyjit Saha, Kerry

Session Type: Roundtable - 1.5 hours

Session Summary:

The Flexitarian diet approach is an increasingly common trend in which consumers reduce their meat intake without cutting it from their diets completely. What started out as a flexible vegetarian diet approach has led to plant-based innovation opportunity for alternative options to consumers. With global populations exceeding 8 billion people and the demands for protein content on the rise, alternative sources of protein is ever paramount. The next stage of innovation will include exploring the blending of high-quality animal meat with texturized plant protein in order to fulfill demand and nutritional expectations. This is not just a consideration for the human foods commodity base but also applicable for the pet food sector as well. Not only are nutritional needs, sensory and alternative product offerings in consideration, but also supply chain constraints. Expanding out from the health and nutrition benefits of alternative protein sources is the sustainability impact. The World Resources Institute has estimated that the replacement of 30% of meat used in the currently consumed 10 billion burgers a year would cut greenhouse gas emissions by 10.5 million tons. It is paramount to understand the microbial community and considerations around these novel blended products. Alteration of substrates and base formulation components

from different sources (e.g., animal slaughter and soy protein processing) will result in unique spoilage flora and food safety risks.

The goal of this roundtable is to discuss the microbial considerations and regulatory space that these products could thrive on the shelves.

Roundtable attendees will learn about:

1. Assessing the risks of blended (meat/meat alternative protein) products, and risk mitigation during development

2. Pathogen presence and persistence in a variety of blended products

3. Microbiological concerns related to refrigerated shelf life

4. Regulatory space for ingredient inclusion and governing bodies for these products

Primary PDG Sponsor: Plant-Based Alternative Products Quality and Food Safety PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Primary Topic Area: Plant-Based Alternative Products

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Animal and Pet Food Safety

Keywords: Meat and Poultry,Plant-based foods,Sustainability

RT20 - TRANSLATING 100 YEARS OF HYGIENIC DESIGN FROM FOOD PROCESSING ENVIRONMENTS INTO FOOD SERVICE, FOOD RETAIL EQUIPMENT, AND FACILITY DESIGN

Primary Author:
Dale Grinstead, Mountain Top Microbiology

Persons:
Dale Grinstead, Mountain Top Microbiology

David Rasmussen, The Kroger Company

Nicolas Vivanco, Avenire

Mark Moorman, Retired, USFDA

Haley Oliver, Purdue University

Howard Popoola, The Kroger Co.

Eric Schweitzer, 3-A Sanitary Standards, Inc.

Hein Timmerman, Diversey, a Solenis Company

Angela Valadez, Publix Super Markets, Inc.

Session Type: Roundtable - 1.5 hours

Session Summary:

Hygienic or sanitary design as practiced today, started in the 1920s with the 3-A Sanitary Standards focused on the dairy processors. In 1989, the European Hygienic Engineering & Design Group (EHEDG) was founded, dedicated to promoting hygienic design in food processing and packaging more broadly. The hygienic design concept spread throughout the food processing industry since then. The food processing industry has had decades to come to grips with the idea of hygienic design and has significant experience with how to successfully (and sometimes unsuccessfully) implement these principles in the food processing environment. However, food service and food retail environments have not yet fully embraced hygienic design concepts. In part, this is due to the fundamentally different nature of food service and retail facilities which are, by design, open and inviting

environments to attract shoppers and patrons. This leads to design decisions that must then be different from those made for a food processor facility where appearance and esthetics are not as important. However, hygienic design is a largely underestimated prerequisite in food safety that should be applied to facility and equipment design in food service and retail operations. This roundtable will bring together a group of experts to discuss how learning and experience from food processing hygienic design implementation can flow to food service and retail. Questions that will be discussed include: What has worked in food processing and can those successes be repeated in food service and retail? How can hygienic design failures be avoided in non-food processing environments? What are the unique requirements for retail and food service equipment and facilities? How do those requirements impact hygienic design implementation? What preventive actions can the industry take in terms of hygiene and sanitation? And how can we anticipate the risks of not properly implementing hygiene and sanitation measures?

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Secondary PDG Sponsor: Retail and Foodservice PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Retail and Foodservice

Keywords: Food Service,Hygiene,Retail

RT21 - DRY CLEAN ONLY: ESTABLISHING DRY SANITATION SOLUTIONS FOR LOW-MOISTURE, READY-TO-EAT FOODS

Primary Author:
Christina Allingham, Bigelow Tea

Persons:
Christina Allingham, Bigelow Tea

Shihyu Chuang, University of Massachusetts Amherst

David Buckley, Diversey, Inc.

Andres Rodriguez Lozano, Commercial Food Sanitation L.L.C.

Deb Smith, Vikan

Abigail B. Snyder, Cornell University

Benjamin Warren, USFDA-CFSAN, Office of Food Safety

Session Type: Roundtable - 1.5 hours

Session Summary:

Sanitary operations in low-moisture food facilities are especially challenging because excess moisture can promote the growth, survival, and spread of environmental pathogens such as *Salmonella* spp. Because of this, outbreaks linked to dry processing environments continue to occur. In fact, several recent high profile salmonellosis outbreaks have been associated with low-moisture foods, reinforcing the need for more effective sanitation approaches in these settings.

The 2025 FDA *Draft Guidance for Low-Moisture, Ready-to-Eat Foods* further underscores the importance of implementing effective cleaning and sanitation practices. At the same time, many manufacturers still face significant gaps in the strategies, education, and technologies needed to build strong dry sanitation programs. These unmet needs create challenges for processors working to control pathogens while protecting product safety and quality.

This roundtable will examine best practices, highlight new and emerging research, and

discuss the limitations of dry cleaning and sanitation from the perspectives of industry, academia, and government. Speakers will include internationally recognized food safety and sanitation experts who will share practical dry sanitation solutions to help mitigate pathogen risks in food facilities; a postdoctoral research associate presenting academic research challenges and emerging technologies; and a global hygiene specialist discussing unmet industry needs, evolving regulatory expectations, and tools for overcoming dry cleaning limitations.

This session will benefit food safety professionals at all levels, along with educators seeking to strengthen training on dry sanitation programs. Panelists will provide actionable strategies and case studies to help participants improve sanitation effectiveness in processing facilities, prepare for upcoming regulatory guidance, produce safer low-moisture foods, and reduce recall risk.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Secondary PDG Sponsor: Low Water Activity Foods PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Low Water Activity Foods

Keywords: Low-water Activity Foods,Regulations,Sanitation

RT22 - ONE CULTURE, MANY VOICES: EMBEDDING DEI INTO FOOD SAFETY

Primary Author:
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Persons:
Lola Oyedokun, MPS Egg Farms

Jill Stuber, Catalyst Food Leaders

Moazzam Chaudhry, Richelieu Foods

Ayo Dahunsi, N/A

Tia Glave, Catalyst Food Leaders

Jay Mayr, JTMayr Food Safety and Quality Consulting, LLC

Adeniyi Odugbemi, Archer Daniels Midland Co (ADM)

Session Type: Roundtable - 1.5 hours

Session Summary:

Food processing and packaging facilities operate within multicultural, ethnically diverse, and multiracial environments. This session explores how integrating Diversity, Equity, and Inclusion (DEI) principles into food safety culture can strengthen performance, enhance employee engagement, and support public health. With a highly diverse workforce, the goal is to highlight the importance of inclusive practices in building resilient food safety systems. The session will show how DEI can be incorporated into daily food safety operations and increase awareness of workforce diversity across ethnic and cultural backgrounds.

Abstract:
Food factories often employ both local and migrant workers, creating a wide range of cultural perspectives. Food safety culture is central to public health, yet it is often shaped by norms that may unintentionally overlook diverse viewpoints. This session examines the connection between DEI and food safety culture, emphasizing how inclusive environments improve hazard awareness, risk communication, and compliance.

Using case studies, research insights, and real-world examples, presenters will demonstrate how DEI initiatives—such as multilingual training, culturally responsive communication, and equitable access to resources—can strengthen food safety outcomes. The session will also address challenges including unconscious bias, representation gaps, and the need for inclusive leadership within quality and safety roles.

Key Discussion Points:

- The Role of DEI in Food Safety Culture
- Equity in Training and Access
- Inclusive Leadership and Accountability
- Case Studies and Industry Examples
- Metrics and Measurement
- Actionable Takeaways
  - Practical steps for integrating DEI into food safety culture
  - Templates for inclusive training and policy development

Primary PDG Sponsor: Developing Food Safety Professionals PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Non PDG Sponsor (Organization, Association, etc.): DEI Council

Primary Topic Area: Food Safety Culture

Secondary Topic Area: Developing Food Safety Professionals

Additional Topic Area (Optional): Food Safety Education

Keywords: Education

RT23 - EXPLORING TOMORROW'S FOOD, TODAY, UNDER HIGH PRESSURE: LATEST ADVANCES AND CHALLENGES

Primary Author:
Mary-Grace Danao, University of Nebraska Lincoln

Persons:
Mary-Grace Danao, University of Nebraska Lincoln

Ann Vegdahl, Cornell University

Erdogan Ceylan, Merieux NutriSciences

Frank Jimenez, Universal Pure

Alvin Lee, Illinois Institute of Technology

Joyce Longfield, Good Foods Group

Carole Tonello, Hiperbaric

Session Type: Roundtable - 1.5 hours

Session Summary:

High Pressure Processing (HPP) has grown from a novel preservation method into a globally accepted technology for producing microbiologically safe, high-quality, clean-label foods. This roundtable will explore HPP's scientific, regulatory, and operational progress and future directions.

Key topics include industry advances in microbial inactivation (pathogens, viruses, spore-formers), shelf-life extension, packaging innovations, and combining HPP with hurdle technologies. The session will also address challenges such as process validation costs, limitations with complex food matrices (e.g., ready-to-eat meals, pet foods, low-acid products like cold brew coffee and coconut juice), risks of post-HPP contamination, regulatory variability, and sustainability concerns.

Panelists will discuss emerging issues like HPP's role in controlling novel pathogens (e.g., highly pathogenic avian influenza in raw pet diets) and the need for cross-sector

collaboration to generate safety and shelf-life data for predictive modeling. Future applications in plant-based, functional, and pet foods—as well as potential uses in nutraceuticals, cosmetics, and pharmaceuticals—will also be explored.

This interactive session invites scientists, technologists, regulators, and industry professionals to share insights and help shape a coordinated research and innovation agenda.

Primary PDG Sponsor: Beverages and Acid/Acidified Foods PDG

Secondary PDG Sponsor: Meat and Poultry Safety and Quality PDG

Non PDG Sponsor (Organization, Association, etc.): Cold Pressure Council

Primary Topic Area: Beverages and Acid/Acidified Foods

Secondary Topic Area: Meat and Poultry Safety and Quality

Additional Topic Area (Optional): Animal and Pet Food Safety

Keywords: Pathogens,Shelf-life,Validation

RT24 - THEORY TO PRACTICE: APPLYING FOOD SAFETY CULTURE RESEARCH TO DYNAMIC AND DIVERSE FOOD BUSINESSES

Primary Author:
Autumn Stoll, University of Minnesota Extension

Persons:
Autumn Stoll, University of Minnesota Extension

Shauna Henley, University of Maryland Extension

Amanda Kinchla, Univeristy of Massachusetts

Christina Allingham, Bigelow Tea

Tia Glave, Catalyst Food Leaders

Kenisha Gordon, Tuskegee University

Armitra Jackson-Davis, Alabama A&M University

Lone Jespersen, Cultivate SA

Lauryn Strong, Alabama A&M University

Session Type: Roundtable - 1.5 hours

Session Summary:

Food safety compliance goes far beyond regulations and checklists. While a growing body of research exists on food safety culture, ambiguity persists regarding its practical application, particularly in dynamic and evolving food businesses. This roundtable will explore the barriers and opportunities in implementing food safety culture frameworks across diverse, evolving food businesses. We will discuss approaches taken to adapt food safety culture education to various audiences, featuring case studies that examine food safety culture from differing perspectives.

This panel will feature a dynamic roundtable discussion, bringing together interdisciplinary perspectives from academia, industry, and government/auditing sectors on implementing

food safety culture in practice. A food safety culture consultant will share experiences translating academic research into practical applications, highlighting successes and challenges of fostering cultural change across companies of varying sizes. An industry representative will provide firsthand insights into cultivating food safety culture in the workplace. A food safety auditor will discuss how culture is assessed during audits and how methods are evolving to reflect organizational complexity. A Diversity, Equity, and Inclusion (DEI) professional will contribute expertise in engaging learners and communicating food safety principles through a DEI lens. An international researcher will discuss application of food safety culture research in global contexts. A food safety educator working with small-scale farmers will share experiences as farms expand and evolve. Finally, a student researcher will offer a fresh perspective on the benefits and challenges of food safety culture based on their research and outreach.

Designed for food safety professionals, quality assurance leaders, and operations managers, this session aims to move beyond theory and foster meaningful, practical improvements in food safety culture across sectors.

Primary PDG Sponsor: Food Safety Culture PDG

Secondary PDG Sponsor: Food Safety Education PDG

Primary Topic Area: Food Safety Culture

Secondary Topic Area: Food Safety Education

Additional Topic Area (Optional): Communication, Outreach and Education

Keywords: Education,Food Safety Culture,Professional Development

RT25 - REVISITING THE FDA FOOD CODE DEFINITION OF TIME/TEMPERATURE CONTROL FOR SAFETY (TCS) FOODS

Primary Author:

Glenda Lewis, USFDA/HFP/Office of Integrated Food Safety Systems and Partnerships/Office of Retail Food Protection

Persons:

Glenda Lewis, USFDA/HFP/Office of Integrated Food Safety Systems and Partnerships/Office of Retail Food Protection

Tong-Jen Fu, USFDA

Courtney Mickiewicz, USFDA

Deon Mahoney, University of Queensland

Liz Reilley, Jonathan Sprouts, Inc

Donald Schaffner, Rutgers University

Brian Schaneberg, Illinois Tech Institute for Food Safety and Health

Mary Yavelak, Compass Group

Session Type: Roundtable - 1.5 hours

Session Summary:

Proper handling of Time/Temperature Control for Safety (TCS) foods represents a cornerstone of food safety inspections and training programs, making clear definition and practical application essential for retail food operators and inspectors. FDA periodically assesses key provisions in the Food Code for ensuring that the language, public health rationale, and interpretation is current, congruent, and applicable. Advances in food processing and preservation methods; new risk assessment studies; evolving product trends and retail practices; implementation challenges as well as multiple TCS-related issues (addressing production assessment, cold brew coffee, fresh-cut produce, dehydration processes, and non-TCS food definitions, etc.) being submitted to the Conference for Food Protection for evaluation have prompted FDA’s intention to comprehensively revisit the TCS definition in the Food Code.

FDA's holistic approach in revisiting the TCS food definition is to ensure the definition reflects current scientific knowledge, allows regulatory consistency, assists in gaining industry compliance, and strengthens public health protection. This session will provide attendees with an opportunity to interactively engage on issues and recent developments impacting the TCS food definition such as: Emerging food technologies; Research involving different food categories; Global perspectives on temperature-time relationships for food and application at retail; and Stakeholder implementation challenges.

Information presented and discussed in this session will allow attendees to: Gain knowledge of what prompted FDA to take a holistic review of the TCS food definition; Identify areas of science and data gaps/advances that may impact applying the definition in daily retail operations; Understand stakeholders' role in the regulatory development process; and identify ways for providing input to FDA's review.

Primary PDG Sponsor: Retail and Foodservice PDG

Primary Topic Area: Retail and Foodservice

Secondary Topic Area: Food Hygiene and Sanitation

Keywords: Food Service,Shelf-life

RT26 - EMERGING, REEMERGING AND REP PATHOGENS: FOOD SAFETY CHALLENGES

Primary Author:
Alvin Lee, Institute for Food Safety and Health

Persons:
Alvin Lee, Institute for Food Safety and Health

Eric Brown, USFDA–Human Foods Program

Craig Hedberg, UMN School of Public Health

Megin Nichols, US CDC

Annette Sansom, Campden BRI Group

Purnendu Vasavada, University of Wisconsin River Falls / PCV@Associates, LLC

Anett Winkler, Cargill

Session Type: Roundtable - 1.5 hours

Session Summary:

Over the last few years, the world has seen pathogens inflict their effects on the most unlikely hosts. Reported incidence of emerging and opportunistic bacterial and viral pathogens have been linked to outbreaks of food and waterborne illness globally. It is increasingly recognized that many otherwise commensal organisms can become pathogens under the right conditions, in a right host, and if exposed or consumed in sufficient quantities. *Campylobacter jejuni*, a common commensal of poultry and wild birds has been linked to shellfish outbreaks and new potential pathogenic species of *Vibrio* spp. are being identified. *Streptococcus* spp. has caused human infection via milk and cattle. Viral pathogens such as Hepatitis E viruses traditionally associated with pigs and pork products have been associated with fresh produce, RTE foods, milk and shellfish outbreaks and Highly Pathogenic Avian Influenza usually associated with poultry and wild birds have caused chaos with infections reported in dairy cattle and humans. With our food increasingly being made from ingredients sourced globally, the year-round availability of seasonal fresh produce, fruits, and vegetables due to new or modified farming practices along with climate changes,

the RTE and minimally processed foods have allowed the emergence of pathogens in novel food sources. Speakers with subject matter expertise will review less-recognized emerging, reemerging and REP pathogens and discuss their food safety implications and control strategies.

Panelists will discuss how these pathogens are adapting and spreading to new food vehicles; how they affect new populations; how agricultural practices, globalization, human behavior, and demographic changes pose food safety challenges; and what are the challenges posed to surveillance, both domestically and internationally; and potential control strategies.

Primary PDG Sponsor: International Food Protection Issues PDG

Primary Topic Area: International Food Protection Issues

Secondary Topic Area: Epidemiology

Keywords: Epidemiology,Pathogens,Public Health

RT27 - WE DON’T NEED NO STINKIN’ CULTURE: WE NEED A NEW GOLD STANDARD FOR CONFIRMATIONS!

Primary Author:
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Persons:
David Legan, Eurofins Scientific Inc.

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Erin Crowley, Neogen

Christopher Crowe, Eurofins

Julie Weller, Hygiena

Laura Bleichner, Gold Standard Diagnostics

Catharine Carlin, IEH Laboratories and Consulting Group

Jerome Combrisson, Mars

Joelle Salazar, FDA

Daniele Sohier, Hygiena

Shawn Stevens, Food Industry Counsel, LLC

Session Type: Roundtable - 1.5 hours

Session Summary:

Modern pathogen detection methods are fast, sensitive and used for screening large numbers of samples, most of which give “not detected” results allowing food plants to keep operating and product to be released into commerce. Since rapid methods were first released, the time to result has been reduced from around two days to around one day in most cases. For the small fraction of samples giving a presumptive detection, confirmation generally follows cultural approaches, causing waits of up to several days to isolate and identify a colony of the target pathogen, and much pain for all involved. To reduce this delay, one option is to adopt a rapid analytical confirmation without resorting to culture. Within the

food industry there seems to be much confusion about the acceptability of such an approach and whether a colony isolate is always required, even though cultural methods may be seen as less sensitive. In contrast, culture-independent methods are routinely used for decision-making in clinical diagnostics. AOAC International is revising the microbiological methods validation guidelines known as “Appendix J” and one element of the revision focuses on validation of analytical confirmations, including harmonization with global validation schemes. Also, ISO TC 34 / SC 9 Microbiology has an activity on analytical confirmations.

However, these alone cannot drive adoption. Beyond method performance and validation, stakeholders’ concerns include appropriately interpreting results, regulatory and customer acceptability, legal liability, risk perception, and even the training and experience of food safety leadership. Panelists representing various food safety-related industries will discuss these elements and answer audience questions–supplemented with interactive audience polling–to help identify a generally acceptable path forward.

Primary PDG Sponsor: Applied Laboratory Methods PDG

Secondary PDG Sponsor: Advanced Molecular Analytics PDG

Primary Topic Area: Applied Laboratory Methods

Secondary Topic Area: Advanced Molecular Analytics

Additional Topic Area (Optional): Food Safety Assessment, Audit and Inspection

Keywords: Environmental monitoring,Laboratory and Detection Methods,Pathogens,Validation

RT28 - KEEP IT SIMPLE: BUILDING ACHIEVABLE PATHWAYS TO IMPROVE FOOD SAFETY

Primary Author:

Ariel Garsow, The Global Alliance for Improved Nutrition

Persons:

Ariel Garsow, The Global Alliance for Improved Nutrition

Markus Lipp, FAO

Dima Faour-Klingbeil, DFK for Safe Food Environment /University of Veterinary Medicine Hanover

Jeffrey LeJeune, FAO

Caroline DeWaal, Center for Strategic and International Studies

Tia Glave, Catalyst Food Leaders

Deon Mahoney, University of Queensland

Rose Omari, CSIR-STEPRI

Matthew Stasiewicz, University of Illinois

Constanza Vergara Escobar, Undersecretariat of International Economic Affairs

Session Type: Roundtable - 1.5 hours

Session Summary:

Current global food safety standards and regulatory frameworks promote the adoption of the most stringent regulations, even in settings where resources are limited and these standards may not be realistic given constraints. In a recent (2024) FAO and WHO survey of Codex member states, it was found that more than 80% of respondents from low- and middle-income countries (LMIC) use Codex standards either “mostly” or “completely” in their national food legislation. Strict standards, whether Codex-based or private, often apply mainly to export sectors, while domestic and informal markets are often left outside of the regulatory enforcement framework. The Codex Guidelines for Food Hygiene Control Measures in Traditional Markets for Food (CXG 103-2024) adopted in 2024 helped to fill a part

of this gap, but challenges in commitment to and enforcement of food safety for domestic and informal markets in LMIC continue to exist. Progress toward addressing these challenges is also evident in the ongoing efforts to develop and implement risk-based food control systems and other innovative approaches, such as in Egypt, Ethiopia, and Ghana. Ensuring food safety is a pathway with multiple milestones. This roundtable will discuss the development of a more realistic pathway to ensuring food safety amongst the public and new and informal business owners who may have limited experience with food safety, particularly in low-resource settings where access to effective methods of mitigating food safety risks are limited.

Primary PDG Sponsor: International Food Protection Issues PDG

Secondary PDG Sponsor: Food Safety Culture PDG

Non PDG Sponsor (Organization, Association, etc.): Diversity, Equity, and Inclusion Council

Primary Topic Area: International Food Protection Issues

Secondary Topic Area: Food Safety Culture

Additional Topic Area (Optional): Food Law

Keywords: Food Safety Culture,Food Security,Risk Assessment

RT29 - ASSET LIFECYCLE MANAGEMENT IN FOOD MANUFACTURING: ENGINEERING PRINCIPLES FOR EQUIPMENT AND FACILITY LONGEVITY

Primary Author:
Mark Moorman, Retired, USFDA

Persons:
Mark Moorman, Retired, USFDA

Scott King, Fortrex

Gary Goessel, The Acheson Group

Timothy Jackson, FDA-CFSAN

Patrick Wouters, EHEDG

Session Summary:

Food manufacturing facilities face a critical challenge in determining optimal asset replacement timelines while maintaining food safety integrity. Too often industry practices emphasize reactive responses to facility failures rather than proactive asset management strategies. Food safety teams frequently encounter situations where aging infrastructure compromises product protection despite robust response protocols. Common issues include roof leaks, drainage failures, and water penetration events that exceed facility design capabilities. The prevailing capital investment mindset favors reactive spending over preventive infrastructure replacement, creating ongoing food safety risks. Industry and specifically food safety professionals often lack standardized frameworks for quantifying asset depreciation and establishing replacement criteria based on food safety considerations.

This multi-disciplinary roundtable will examine facility and equipment lifecycle management through four critical perspectives to address the fundamental question: "How long should equipment and facilities last?" The roundtable will explore important topics including

technical criteria for determining optimal asset lifespan based on structural integrity and performance degradation; accounting frameworks for evaluating cost-benefit ratios of preventive maintenance versus replacement; clarifying FDA and industry standards for facility performance focusing on contamination prevention; and inspection criteria for evaluating facility condition and replacement needs. This roundtable will include experts from engineering, accounting, auditing, and regulatory agencies.

Primary PDG Sponsor: Food Hygiene and Sanitation PDG

Secondary PDG Sponsor: Sanitary Equipment and Facility Design PDG

Primary Topic Area: Food Hygiene and Sanitation

Secondary Topic Area: Sanitary Equipment and Facility Design

Additional Topic Area (Optional): Low Water Activity Foods

Keywords: Auditing,Food Safety Culture,Production/Plant Design,Sanitation

Technical Abstracts

T1-01: ADAPTIVE ENVIRONMENTAL MONITORING IN LOW-MOISTURE FOOD PROCESSING SYSTEMS USING GRAPH CONVOLUTIONAL NETWORK–RECURRENT NEURAL NETWORK (GCN–RNN)

Primary Author:
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Persons:
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Bradley Marks, Michigan State University

Sanghyup Jeong, Michigan State University

Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: Environmental Monitoring Programs (EMPs) involve routine sampling of food processing environments to verify preventive controls and prevent product contamination. In low-moisture food (LMF) facilities, zone-based EMPs may overlook contamination risks due to changes in layout, equipment, airflow, dust transfer, or moisture events, allowing emerging hotspots to go undetected while resources are allocated to low-risk areas.

Purpose: This study aimed to (i) develop a graph convolutional network–recurrent neural network (GCN–RNN) framework to generate adaptive EMP risk maps from synthetic data, and (ii) translate predicted high-risk locations into robot-navigable sampling routes using a Robot Operating System (ROS)-compatible workflow.

Methods: A low-moisture food facility was modeled as a spatial graph with 40 sampling locations, where edges represented proximity and contamination-transfer pathways. Synthetic EMP data spanning 52 sampling periods were generated. A GCN–RNN model implemented in MATLAB captured spatial contamination diffusion and temporal risk evolution. Model performance was evaluated using hotspot precision, recall, F1-score, and

spatial overlap. High-risk locations were converted into robot-navigable waypoints and ordered using a nearest-neighbor distance-minimization heuristic.

Results: The GCN–RNN identified all persistent hotspots (recall = 1.0) while conservatively prioritizing additional locations (precision = 0.30; F1 = 0.46). Predicted risk showed moderate spatial agreement with true contamination patterns (spatial overlap = 0.30). Dynamic EMP maps reduced sampling locations by 75% compared to zone-based sampling. Robot-guided routes efficiently visited all high-risk locations, with a total path length of 27 spatial units.

Significance: These results demonstrate the potential of AI-driven adaptive EMPs as decision-support systems for predicting contamination risk and optimizing environmental sampling in LMF processing environments.

Award Application: Developing Scientist Award

T1-02: FARM WASTEWATER AS A TOOL FOR FOODBORNE PATHOGEN SURVEILLANCE: MICROBIAL AND ANTIMICROBIAL RESISTANCE INSIGHTS

Primary Author:
Gabriella Strocko, University of Delaware

Persons:
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Brenna DeRocili, University of Delaware

Mathilde Trudel-Ferland, University of Delaware

Jennifer Jones, University of Delaware

Kalmia Kniel, University of Delaware

Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Foodborne illness outbreaks linked to contaminated agricultural water (AGW) highlight the need for consistent surveillance of water used with raw agricultural commodities. Proactive farm wastewater monitoring has the potential to be vital in preventing outbreaks, as it is non-invasive and convenient.

Purpose: This study investigates the presence of foodborne pathogens, indicators, and AMR genes in AGW (on-farm) and rural wastewater sampled over the growing season with the aim of determining potential use of farm wastewater for rapid on-farm foodborne pathogen control.

Methods: AGW (n=30) and wastewater (n=24) samples were collected June–October from farms and adjacent effluent wastewater collection sites in Delaware. Samples were filtered, centrifuged, and nucleic acid extraction was performed. dPCR was performed for hepatitis A virus (HAV), norovirus GI&GII (NoV), *Cyclospora, Cryptosporidium parvum (Cp),* pepper mild

mottle virus (PMMoV), crAssphage, human & ruminant *Bacteroides* (BacH,BacR), and poultry parvovirus (PaV). Presence of antibiotic resistance genes *blaCTX-m* (beta-lactam) and *tetA* (tetracycline), were detected by qPCR.

Results: PMMoV was detected in 100% of wastewater samples and 73% of AGW samples, with a significantly different (student's *t* test, $p<.0001$) concentration of genomic copies/µL. *Bac*R and PaV were only detected in AGW. *Bac*H and crAssphage were both detected in 100% of wastewater but only 3% of AGW. NoVGI was found in 71% and GII in 88% of wastewater samples. *blaCTX-m* was present in 100% of wastewater samples and 97% of AGW samples while *tetA* was present in 100% of both wastewater and AGW samples, demonstrating the presence of widespread beta-lactam and tetracycline resistance. No *Cyclospora, Cp*, or HAV was detected.

Significance: Farm wastewater surveillance may allow for swift implementation of mitigation and control compared to conventional pathogen detection and can inform public health data on AMR presence in communities.

Award Application: Developing Scientist Award

T1-03: VALIDATION OF THE DYNAMIC MODEL FOR PREDICTING THE EFFECT OF TEMPERATURE HISTORY ON THE LAG PHASE DURATION (LPD) OF LISTERIA MONOCYTOGENES

Primary Author:
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Persons:
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Binita Goshali, University of Georgia

Aishani Tewari, University of Georgia

Govindaraj Dev Kumar, University of Georgia, Center for Food Safety

Abhinav Mishra, University of Georgia

Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Accurate estimation of LPD is essential for predicting microbial growth, food safety, and shelf life. However, most published predictive growth models do not account for the influence of the pre-inoculation environment, often resulting in poor estimation of LPD. It depends not only on current growth conditions but also on the previous growth environment.

Purpose: To validate a previously developed predictive model that quantified the effect of temperature history on the physiological parameter, h_0 in response to environmental change.

Methods: Validation experiments were conducted for three strains of *Listeria monocytogenes*, 390-2, MDD640, MDD641. A previously developed tertiary model integrated Baranyi's primary model with a secondary model for dependence of h_0 on temperature history was validated

using independent stepwise temperature upshift and downshift experiments. These included separate stepwise temperature downshift [20 (14.4h) – 15 (31h) – 10°C (83h); 35 (6.6h) – 30 (15.6h) – 25°C (20h)], upshift experiments [10 (60h) – 15 (31h) – 20°C (22h); 25 (9h) – 30 (12h) – 35°C (7.5h)]. The model performance was evaluated using accuracy factor (A_f), bias factor (B_f) and acceptable prediction zone (APZ) analysis.

Results: Predicted growth curves showed good agreement with observed data under dynamic temperature conditions. The model predicted new lag, termed as intermediate lag following temperature transitions, which varied with the magnitude and direction of temperature change and was more pronounced when fluctuations occurred at lower temperatures. The values for A_f and B_f for time to a 1-log increase ranged from 1.25 to 1.32, and □70% of prediction errors fell within the APZ limits.

Significance: These findings demonstrate that incorporating temperature history into predictive models enables the estimation of intermediate lag phases under fluctuating temperature conditions, thereby reducing the risk of overprediction of bacterial growth in foods.

Award Application: Developing Scientist Award

T1-04: AN IOT-ENABLED HYBRID PAPER/POLYMER-BASED DEVICE FOR RAPID AND ON-SITE DETECTION OF CAMPYLOBACTER JEJUNI IN POULTRY PRODUCTS

Primary Author:
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Persons:
Yihan He, McGill University

Tian Yang, McGill University

Xiaonan Lu, McGill University

Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Campylobacter jejuni* is the leading bacterial cause of foodborne gastroenteritis worldwide and is commonly linked to poultry products. Culture-based detection takes 1–2 weeks, while PCR methods rely on centralized laboratories and specialized equipment. Rapid, on-site detection tools are therefore needed to support effective poultry safety management.

Purpose: This study aimed to develop a rapid and field-deployable platform for *C. jejuni* that integrates isothermal amplification, CRISPR/Cas12a sensing, lateral flow readout, and an IoT-enabled data infrastructure to enable real-time surveillance.

Methods: An RPA-CRISPR/Cas12a assay targeting the *hipO* gene was optimized and integrated into a multilayer hybrid paper–polymer microfluidic device with lateral flow visualization. Analytical performance was evaluated using pure cultures and poultry meat samples contaminated with *C. jejuni*. An IoT-based workflow was established for digital result capture, centralized data storage, and remote data sharing.

Results: The platform showed 100% specificity for *C. jejuni*. The limit of detection reached 580 CFU/mL by lateral flow visualization, with an on-device sensitivity of 5,800 CFU/mL. In

chicken meat, *C. jejuni* was detected at 10^0–10^3 CFU/g after 6–12 h enrichment, while levels above 10^3 CFU/g were detected without enrichment. All measurements were performed in triplicate (n = 3).The complete workflow, from DNA extraction to visual readout, was completed within one hour. The device enabled sequential RPA and CRISPR reactions without complex instrumentation, and IoT integration enabled real-time digitization, centralized data management, and rapid data sharing across testing sites.

Significance: By combining rapid molecular detection with IoT-enabled data integration, this platform enables on-site testing, real-time monitoring, and coordinated decision-making, providing a scalable solution for next-generation poultry safety surveillance.

Award Application: Developing Scientist Award

T1-05: TWO-YEAR ASSESSMENT OF LISTERIA SPP. PREVALENCE ON FOOD CONTACT SURFACES IN CHERRY PACKINGHOUSES

Primary Author:

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Persons:

Swapnika Medikonda, Washington State University - Irrigated Agriculture Research and Extension Center

Miriam Ruiz, Washington State University

Claire Murphy, Washington State University

Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Tree fruits have been identified as a commodity of concern for *Listeria monocytogenes*, with recalls and outbreaks linked to cross-contamination from food contact surfaces (FCS) in packing environments.

Purpose: This study assessed the prevalence of *Listeria* spp. on FCS in Washington cherry packinghouses over two packing seasons to identify surfaces most likely to harbor *Listeria* spp.

Methods: A total of 40 FCS per packinghouse were selected for environmental monitoring across four commercial cherry packinghouses in Washington. Sampling was conducted during the 2024 and 2025 packing seasons. Sponge swab samples were collected (i) before daily packing operations and (ii) five hours into operation. Each packinghouse was visited three times during a 6–10 week season (beginning, middle, and end). The isolation, detection, and confirmation of *Listeria* spp. were conducted by following a modified *FDA Bacteriological Analytical Manual* method with PCR confirmation.

Results: Of the 1,920 samples collected, 2% (38) were positive for *Listeria* spp. The highest prevalence was observed on bristle flaps (9/144; 6.3%), solid conveyor belts (6/144; 4.2%), and singulators (5/132; 3.8%). In two cases (5.6%), *Listeria* spp. was detected at both sampling times during a single visit, 50% of positives were detected only before packing, while 44.4% were detected only during packing. Prevalence increased over the packing season in year 1, whereas in year 2 it was highest at the start of the season.

Significance: This study is the first to evaluate the prevalence of *Listeria* spp. on FCS in cherry packinghouses, offering valuable insights into harborage sites. The identified high-prevalence FCS are the critical areas for targeted sanitation and equipment adjustments. These findings are crucial for guiding the cherry industry in implementing science-based interventions to mitigate the risk of contamination.

Award Application: Developing Scientist Award

T1-06: MOISTURE MATTERS: UNINTENDED CONSEQUENCES OF PERFORMING WET SANITATION IN DRY ENVIRONMENTS

Primary Author:
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Devin Daeschel, Cornell University

Lynne McLandsborough, University of Massachusetts

Abigail B. Snyder, Cornell University

Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Cross-contamination of low-moisture foods (LMFs) with pathogens from surfaces during production is a persistent food safety concern. Wet sanitation is sometimes employed, but introducing moisture to dry environments can inadvertently promote pathogen growth.

Purpose: This study evaluates the risk of pathogen growth on surfaces following wet sanitation in LMF facilities.

Methods: Evaporation kinetics of hydrated powdered infant formula (PIF) soil on stainless steel coupons were measured in duplicate from 33–97% relative humidity (RH). RH was monitored in a low-moisture food (LMF) facility before, during, and after wet sanitation to characterize sanitation-driven moisture fluctuations. Growth dynamics of *Salmonella*, *Listeria monocytogenes*, *Cronobacter sakazakii*, and *Enterococcus faecium* were assessed over 120 h on PIF-soiled steel coupons under static RH conditions (33–97% RH; four biological replicates).

Salmonella growth was also recorded over 72 h under a dynamic RH profile simulating observed facility fluctuations (3 biological replicates).

Results: Higher RH slowed the drying of PIF-soiled surfaces, prolonging periods when soil water activity was high enough to supported microbial growth. All four organisms grew significantly at 81 and 97% RH within 120 h ($p<0.05$, Welch's *t*-test), while only *E. faecium* grew significantly below 81% RH ($p<0.05$, Welch's *t*-test). RH monitoring in a commercial facility showed spikes up to 100% during wet sanitation and sustained RH above 75% for more than 7 h in some locations. Under simulated facility RH conditions, *Salmonella* populations on PIF-soiled coupons increased by more than 3.5 log CFU/coupon within 66 h ($p<0.05$, Welch's *t*-test).

Significance: These findings demonstrate the potential for wet sanitation to unintentionally enable environmental pathogen growth and highlight the importance of moisture and RH control in LMF facilities.

Award Application: Developing Scientist Award

T1-07: AN INTEGRATED PACKAGING SYSTEM FOR HANDS-FREE DETECTION OF SALMONELLA IN WHOLE CHICKEN PRODUCTS

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: Conventional pathogen testing relies on destructive sampling and centralized analysis, limiting real-time detection within packaged foods.

Purpose: The purpose of this study was to develop and validate a food-safe packaging system for in situ detection of *Salmonella* in sealed poultry products.

Methods: A “Lab-in-a-Package” system was designed consisting of an inclined food tray (45°), a reagent-saturated membrane, and a surface-immobilized fluorescent nucleic acid probe specific to *Salmonella enterica*. Chicken samples were inoculated with 10^2 to 10^6 colony forming units (CFU)/g of *Salmonella*. Fluid localization, analyte diffusion, and sensor response were evaluated over 18 h at 37°C. Additional contamination routes were simulated using inoculated utensils, gloves, and food-contact surfaces. Fluorescence signals were measured using a fluorescence microscope and handheld scanner with smartphone connectivity.

Results: Sensors exposed to *Salmonella* contaminated chicken samples exhibited concentration-dependent linear fluorescence increases across 10^2 to 10^6 CFU g^{-1} ($R^2 = 0.98$, $p < 0.05$, n=6). At 10^6 CFU g^{-1}, mean fluorescence fold-change reached 2.83, significantly higher

than the 1.09 observed in uncontaminated controls ($p < 0.0001$). The overall limit of detection of the system as 10^3 CFU g^{-1}, with a mean fold change of 1.54 ($p < 0.0001$). Handling-based contamination simulations using inoculated knives, gloves, and surfaces resulted in significant detection responses with mean fold-changes of 4.0, 3.73 and 3.00 ($p < 0.0001$, 0.0001, 0.001, n=3), respectively.

Significance: This packaging-integrated sensing platform enables product-level pathogen detection without opening food packages, supporting improved food safety surveillance and reducing reliance on destructive testing.

Award Application: Developing Scientist Award

T1-08: DEVELOPMENT OF AN AGENT-BASED MODEL THAT PREDICTS RISK-BASED SET-BACK DISTANCES FOR FLOODED FIELDS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Flooding of produce fields poses a food safety risk by introducing and dispersing pathogens via floodwater; however, current standard for harvest set-back distances may not adequately account for differences in flooding events.

Purpose: To develop an agent-based model (ABM) that can simulate population dynamics and dispersal of bacterial pathogens and predict appropriate risk-based set-back distances.

Methods: The ABM was developed using the layout of a 60 ft x 90 ft experimental field. This model was used to simulate (i) water movement driven by rainfall, flooding, surface runoff, deep drainage, and evapotranspiration, (ii) *Salmonella* transport from a flood source, and (iii) *Salmonella* survival over a 70-day period. We accounted for dynamic variables that allow integration of site-specific inputs, such as daily temperature, precipitation, solar radiation, field slope, and water flow direction. The model procedures, including water movement, microbial transfer, and survival, were parametrized using data from published studies. A current limitation is that the model does not incorporate crop-related heterogeneity.

Results: Simulation results showed that the subsurface floodwater required 9 days to reach the farthest point of the field from the flood source. While subsurface floodwater moved across the entire field, *Salmonella* transport was limited to 20 ft from the flood edge (i.e., 30 ft away from the flood source), given an initial contamination level of 3 $\log_{10}$ CFU/g and a detection threshold of -1 $\log_{10}$ CFU/g. Furthermore, *Salmonella* concentrations at the flood edge declined below the detection limit within 40 days, whereas *Salmonella* in the central flooded zone (i.e., 30–50 ft away from the flood source) persisted for up to 70 days.

Significance: Our ABM can be used to enhance risk-based decision-making for managing food safety hazards from flooded fields.

Award Application: Developing Scientist Award

T1-09: PERSISTENCE OF E. COLI, SALMONELLA, AND LISTERIA FROM SEEDLING TO HARVEST IN TWO SOILLESS FARMING SYSTEMS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Limited data exist on the persistence of foodborne pathogens in soilless farming systems from seeding through harvest.

Purpose: To characterize the persistence of *Salmonella, E. coli,* and *Listeria* in a soilless farming system over a full production cycle.

Methods: We inoculated *Salmonella* Javiana and Thompson, *Escherichia coli* STEC O179:H8 and EPEC O13:H6, and two strains of *Listeria monocytogenes* at ~6 $\log_{10}$ CFU/mL in RO water and applied it to rockwool containing butterhead lettuce seeds (28 cubes per 3 system replicates). We assessed pathogen persistence during the 3-week nursery stage, as well as 4-week plant growth in deep water culture (DWC) or Kratky (KR) systems. We quantified pathogens in nutrient solution near plant roots and from the corners of each system throughout the growth stage, as well as in rockwool cubes and plant root surfaces at harvest.

Results: *E. coli* and *Listeria* concentrations declined in the nursery and plant growth stage (p<0.001). *Salmonella* counts increased during the nursery stage (p=0.029; 0.01 log units/day) but did not change during the plant growth stage in the systems (p=0.35). *E. coli* and

Salmonella concentrations were lower at system corners than near the plant roots during the plant growth stage (linear mixed model, p=0.047 and p=7.14 × 10^{-9}, respectively). Pathogen concentrations did not differ between the DWC and KR systems (p=0.7). At the end of the growth period, all three pathogens were detected on roots (*Salmonella* at 3.1 ± 0.4, *E. coli* at 1.6 ± 0.6, *Listeria* at 0.6 ± 0.4 CFU/g) and rockwool (*Salmonella* at 4.6 ± 0.3, *E. coli* at 3.1 ± 0.9, *Listeria* at 0.6 ± 0.3 CFU/g).

Significance: *Salmonella* concentration increased during the nursery stage and persisted through plant growth in the system. *E. coli* and *Salmonella* concentrations were higher near plant roots than at system corners, highlighting root-associated microenvironments as key hotspots for pathogen persistence independent of hydroponic system design.

Award Application: Developing Scientist Award

T1-10: IMPROVING NOROVIRUS DETECTION SENSITIVITY USING ENGINEERED BACTERIAL CONCENTRATION

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus is the leading cause of non-bacterial gastroenteritis worldwide. However, their detection is challenged by the lack of effective and practical virus concentration methods, as existing approaches are often costly and inefficient.

Purpose: This study aimed to develop a novel norovirus concentration method using engineered nonpathogenic *Escherichia coli* expressing norovirus-affinity peptides and to determine whether bacterial growth conditions influence virus capture efficiency.

Methods: Human norovirus affinity peptides were cloned at the C-terminus of ice nucleation protein(INP) and expressed in *E. coli* BL21. Capture efficiency was evaluated using nine independent samples (n = 9). Capture efficiency for human norovirus GI.1, GII.2, and GII.4 was assessed using a suspension assay and quantified by RT-qPCR. The effect of bacterial growth conditions was evaluated using TSB supplemented blood, TSB, and half-strength TSB. Additionally, interactions between the engineered *E. coli* and norovirus particles were

visualized by TEM.

Results: Capture efficiency for GII.4 norovirus ranged from 66% (SD ± 4.0) to 81% (SD ± 0.24) across engineered *E. coli* strains expressing different peptides (n = 9). For GII.2, capture efficiency ranged from 27.4% (SD ± 0.8) to 62.3% (SD ± 0.5) (n = 9), while GI.1 virus exhibited capture efficiencies ranging from 33% (SD ± 15.1) to 73% (SD ± 17.3) (n = 9). Capture efficiency was highest when bacteria were grown in TSB with blood, followed by half-strength TSB and TSB. Norovirus particles were also confirmed to interact with bacterial cells by TEM, with the binding observed on the bacterial cell surface.

Significance: This study presents a promising capture and concentration method targeting human noroviruses using engineered bacteria. Due to its simple, scalable, and cost-effectiveness, this approach shows strong potential for effectively concentrating norovirus from food and environmental samples.

Award Application: Developing Scientist Award

T1-11: CELL LINE INFECTIVITY ASSAY, MRNA VIABILITY ASSAY AND BULK RNA SEQUENCING REVEALED THAT HIGH PRESSURE PROCESSING MAY HAVE LIMITED EFFICACY AGAINST CRYPTOSPORIDIUM PARVUM IN APPLE JUICE USING INDUSTRY-RELEVANT PARAMETERS

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Juice Hazard Analysis Critical Control Point requires apple juice manufacturers to implement a kill step that achieves at least a 5-log reduction of *Cryptosporidium parvum*. There is no validation study on HPP against *C. parvum* in apple juice.

Purpose: The objectives of this study are to establish research methods, determine efficacy of HPP against *C. parvum*, and elucidate the structural and molecular inactivation mechanisms.

Methods: In the current study, we determined process efficacy of HPP using cell line infectivity method and mRNA viability method. We evaluated structural damage of HPP on *C. parvum* oocysts using scanning electron microscopy (SEM). Lastly, we investigated molecular responses of *C. parvum* oocysts to HPP using RNA sequencing. ANOVA was used to determine significant differences in *C. parvum* survivors after different HPP treatments. All experiments were performed in three independent biological replicates.

Results: Firstly, we established that mRNA viability method requires a 24-hr room temperature incubation to allow *hsp70* mRNA expression to return to a baseline level before quantification with RT-qPCR. Additional heat shock steps often used in mRNA viability method led to additional log reduction that represent inaccurate inactivation of injured *C. parvum* oocysts. HPP at 600 MPa and 5℃ for 3 minutes led to 1.24-log and 2.64-log reductions of viable and infectious oocysts determined using cell line infectivity method and mRNA viability method, respectively. SEM results showed severe and potentially irreversible damage to oocyst cell walls and RNA sequencing results revealed ribosomal damages.

Significance: Considering that parasites do not replicate in food matrices, we believe HPP is an effective process control for *C. parvum* in apple juice. However, to demonstrate a 5-log reduction for regulation compliance purposes, animal infectivity models may be necessary.

Award Application: Developing Scientist Award

T1-12: APPLICATION OF PLASMA NANOBUBBLES FOR SALMONELLA INACTIVATION AND MITIGATION OF CROSS-CONTAMINATION ON FRESH PRODUCE IN THE PRESENCE OF ORGANIC MATTER

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Plasma nanobubbles (PNB) are stable, high-surface-area carriers of plasma-generated reactive species that effectively inactivate pathogens and reduce cross-contamination during fresh produce washing, offering a chemical-free alternative to conventional sanitizers under high organic load.

Purpose: This study evaluated PNB for inactivating *Salmonella* and reducing wash water-mediated cross-contamination on fresh produce under different organic matter conditions.

Methods: PNB were generated using a rod-to-mesh dielectric barrier discharge plasma reactor coupled with a nanobubble generator using compressed air (5.78 kV, 1000 Hz, 5 SCFH). Cherry tomatoes (n=36) and mini cucumbers (n=36) were spot-inoculated with *Salmonella* Newport and washed in 3 L of PNB water, nanobubble water (NBW), or tap water (TW) for 1, 3, and 5 min. Uninoculated produce assessed cross-contamination. Synthetic wash water containing 4% and 7.5% produce extract with 0.1% SiO_2 simulated low and high

organic matter (turbidity ~32 and 60 NTU). *Salmonella* on produce surfaces and in wash water were enumerated on XLT4 agar, and effects were analyzed using one-way ANOVA (p□0.05).

Results: Initial *Salmonella* populations were 6.2 ± 0.1 log CFU/produce on cherry tomatoes and 6.9 ± 0.2 log CFU/produce on mini cucumbers. After 5 min, PNB achieved reductions of 5.6 ± 0.3 and 3.0 ± 0.3 log CFU/produce, respectively. *Salmonella* was below the detection limit (1 log CFU/produce) on uninoculated cherry tomatoes washed with PNB, even under high organic matter, whereas 1.8–2.6 and 3.1–4.5 log CFU/produce were recovered after NBW and TW treatments. PNB achieved reductions of 4.8 ± 0.3 and 3.2 ± 0.4 log CFU/produce under low and high organic matter, respectively. No significant changes in produce color or texture were observed.

Significance: PNB inactivated *Salmonella* and reduced cross-contamination under high organic load, demonstrating its potential as a chemical-free strategy to improve fresh produce safety.

Award Application: Developing Scientist Award

T2-01: EXPANSION OF PESI-LIKE PLASMIDS ACROSS SALMONELLA SEROVARS: FSIS DATA (2020–2025)

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The spread of pESI-like plasmids, associated with multidrug resistance and enhanced virulence, has expanded beyond *Salmonella* Infantis to include serovars Agona, Ouakam, Schwarzengrund, Senftenberg, and Alachua, indicating a broader spread than previously recognized.

Purpose: Summarize non-Infantis strains carrying the pESI-like plasmid, including serotype, commodity and geographic trends over time.

Methods: Isolates were screened for the presence of pESI-like plasmids using seven genetic markers previously described in the literature. Associated metadata were compiled to summarize key metrics of interest.

Results: Since 2020, a total of 62 non-Infantis strains across 14 establishments have been identified to carry the pESI-like plasmid; Agona (n=1), Alachua (n=1), Ouakam (n=1), Schwarzengrund (n=11), and Senftenberg (n=48). Geographically, most are from southeastern states (88.7%); however, since 2024, isolates have been identified from six

additional states nationwide. Of note, a single inspected-establishment has had four different serovars carrying the pESI-like plasmid. Except for one strain (Ouakam) from a cecal dairy cattle sample, all were from turkey. Excluding exploratory and follow-up sampling, counts rose from 3 in 2020 to 18 in 2025, showing a significant upward trend (Spearman's $\rho = 0.93$, $p = 0.0077$).

Significance: This analysis suggests an expanding presence of strains associated with pESI-like plasmids among different serotypes and across geographic locations, reinforcing the need for continued surveillance. The further expansion of the pESI-like plasmid to additional serotypes demonstrates a need to consider clinically relevant genotypes–not just serotypes–when considering *Salmonella* reduction approaches. This highlights an important research need to explore mechanisms or events driving the plasmid spread and potential fitness advantages it may confer.

T2-02: EFFICACY AND MODE OF ACTION OF A FOOD-SAFE ANTIMICROBIAL COATING AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Recent European Union regulations mandating >10% of food packaging be reusable by 2030 affects United States crop exporters by introducing additional food safety challenges. GS-2 (food-safe antimicrobial coating) has been developed to combat microbial persistence and cross-contamination on reusable packaging.

Purpose: Evaluate antimicrobial efficacy of GS-2 against bacterial (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*) and fungal (*Aspergillus niger* and *Aspergillus flavus*) pathogens and to elucidate mode of action against *L. monocytogenes*.

Methods: GS-2 (0.1–15.7%) was co-inoculated with bacterial and fungal strains at 7–9 log CFU/mL. Samples were stored at 22±1°C and populations enumerated on days 0–3 using Brain Heart Infusion agar + rifampicin (bacteria) and potato dextrose agar (fungi). For

transcriptomic analysis, *L. monocytogenes* was exposed to sublethal GS-2 concentrations (0.1–1.0%) for 30s prior to RNA extraction, library preparation, and sequencing. Differential gene expression was analyzed using discrete pairwise comparisons and continuous concentration-response modeling ($p<0.01$).

Results: GS-2 exhibited concentration-dependent activity against all bacterial pathogens, with *L. monocytogenes* showing the greatest sensitivity. At □2.1% GS-2, *L. monocytogenes* populations were reduced by >2 log CFU/mL by day 3. *E. coli* O157:H7 and *S. enterica* were reduced by ~0.7–1.0 log CFU/mL at □2.8% GS-2. GS-2 exhibited fungistatic activity against *A. flavus* (~0.8 log CFU/mL reduction at 3.0%) but limited efficacy against *A. niger*, even at 15.7%. Transcriptomic analysis revealed concentration-dependent induction of stress-response and metabolic pathways alongside repression of genes involved in DNA repair, translation, and core cellular functions, particularly at 0.5–1.0% GS-2.

Significance: GS-2 provides effective bacterial and selective fungal control and may be a sustainable antimicrobial intervention for reusable food packaging.

T2-03: IMPACT OF BRUSH BRISTLE COLOR ON THE EFFICACY OF ANTIMICROBIAL LIGHTS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Foodborne pathogens can attach to brushes in produce packinghouses, leading to cross-contamination.

Purpose: Brush bristle color was evaluated to enhance the efficacy of antimicrobial light-based interventions to reduce the contaminating pathogen population.

Methods: White, black, blue, green, and red polyethylene brush bristle stacks (2g) were dip-inoculated in cocktails (8 log CFU/ml) of antibiotic-resistant *Listeria monocytogenes, Salmonella enterica,* and *Escherichia coli*. The bristle stacks were air-dried and vertically stacked in a sterile petri-dish under 405 nm and 222 nm antimicrobial lights, respectively. Both light exposure and control treatments (fluorescent bulb light and darkness) were performed in a grow-tent. Brush bristles were exposed to light for 8h and then evaluated for a decrease in pathogen population. Three biological replications of each treatment were performed, and differences in pathogen reduction on brush bristles were compared using Analysis of Variance.

Results: The bacterial species (p□0.0001), bristle color (p□0.05), and type of light treatment

(p□0.05) significantly impacted pathogen reduction. Light intensity for the 405 nm and 222 nm lights were approximately 1000 J/cm^2 and 3000 mJ/cm^2. Both *L. monocytogenes* and *E. coli* were more sensitive to the antimicrobial lights than *S. enterica*. The highest decrease in pathogen population after 405 nm and 222 nm treatment was for *L. monocytogenes* (3.65±1.88 log CFU/g) on white bristles and *L. monocytogenes* (1.82±1.13 log CFU/g) on black bristles, respectively. Black and red bristles were the least effective in influencing population decay of the pathogens. *Salmonella* contaminated black brush bristles exposed to fluorescent bulb light or kept in the dark had the least decline in population with reductions of 0.38±0.2 log CFU/g and 0.36±0.2 log CFU/g, respectively.

Significance: Brush bristle color selection could be used as a strategy to improve the efficacy of antimicrobial lights.

T2-04: INACTIVATION OF SALMONELLA EXPOSED TO COPPER-BASED PESTICIDES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Pesticide application water may serve as a pathogen contamination route for produce. Copper (Cu)-based pesticides are widely used for plant disease management in organic produce production systems.

Purpose: The objective of this study was to evaluate the antimicrobial efficacy of Cu-based pesticides against *Salmonella*.

Methods: Cu-pesticides evaluated included cuprous oxide (CO), basic copper sulfate (CS), copper hydroxide (CH), and copper octanoate (COct). Minimum inhibitory concentrations (MICs) were determined using a microtiter plate assay. Wells were streaked onto tryptic soy agar-R, and the presence or absence of growth was used to determine MIC values (mg Cu/mL). *Salmonella* die-off in water under agitation was evaluated following exposure to Cu-based pesticides diluted in deionized water (0.38 mg Cu/mL) and inoculated at 5 log CFU/mL. Samples were collected at 0, 15, 30, 45, 60, 90, and 120 min and enumerated on non-

selective media (N = 6). If *Salmonella* counts fell below the limit of detection (LOD, <1 CFU/mL), samples were enriched using a modified FDA BAM protocol. Differences in *Salmonella* reduction were analyzed using Tukey's HSD test in RStudio (V4.5.2).

Results: MIC values (mg Cu/mL) ranged from 0.60–0.96 for COct, 20–25 for CS, 1.75–2.50 for CH, and 50 for CO. COct resulted in the greatest *Salmonella* reduction (4.49 ± 1.06 log CFU/mL) after 15 min, with 22 of 45 samples below the LOD, followed by CO (2.26 ± 0.75 log CFU/mL) ($p < 0.01$). No significant reductions were observed for CS or CH at 15 min ($p > 0.05$). After 120 min, *Salmonella* levels fell below the LOD for COct and CO and were reduced by 2.24 ± 0.77 and 0.95 ± 0.40 log CFU/mL for CS and CH, respectively ($p < 0.01$).

Significance: Differences in *Salmonella* survival among Cu-based pesticides suggest variable antimicrobial efficacy. These findings provide data to inform grower decisions regarding Cu-based pesticide selection.

Award Application: Developing Scientist Award

T2-05: SYNERGISTIC EFFECTS OF BIOACTIVE COMPOUNDS AND ELEVATED HYDROSTATIC PRESSURE FOR INACTIVATION OF LISTERIA MONOCYTOGENES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* is an important pathogen of public health concern that is ubiquitous in nature. The pathogen could multiply in an array of products and is of particular concern in ready-to-eat commodities and for susceptible individuals such as the elderly, the very young, pregnant women, and the immunocompromised.

Purpose: Inactivation of this epidemiologically important pathogen was investigated by using four bioactive compounds (Quercetin, Rutin, Berberine, Limonene) and various degrees of elevated hydrostatic pressure.

Methods: Experiments were randomized complete block designs consisting of two blocks (i.e., two biologically independent replications). Each block was further composed of three replications and two instrumental repetitions. The log-transformed data was statistically analyzed using Tukey- and Dunnett’s-adjusted analysis of variance at type I error level of 5%. Samples were treated by elevated hydrostatic pressure up to 650 MPa for up to 10 minutes. The temperature of the trials was precisely controlled using a circulating water bath connected to a steel jacket surrounding the chamber.

Results: Elevated hydrostatic pressure at levels of 650 MPa was able to reduce ($P < 0.05$) the microbial pathogen in an acidic environment by >99%. Efficacy of the treatment was further augmented by the addition of bioactive compounds with limonene at concentrations of 1.5 to 10%, exhibiting the greatest efficacy ($P < 0.05$).

Significance: Our results indicate that application of elevated hydrostatic pressure, synergized with novel antimicrobials, could be used as a practical solution for controlling this prevalent pathogen of public health concern. The addition of novel bioactive compounds could additionally provide residual protection of the product during the shelf life and protection against post-processing cross-contamination.

Award Application: Developing Scientist Award

T2-06: SURVEILLANCE OF ANTIBIOTIC-RESISTANT CAMPYLOBACTER IN CHICKEN AND TURKEY CARCASS RINSES ACROSS THE UNITED STATES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Given the high consumption of poultry in the U.S. and its link to foodborne outbreaks, the rise of antimicrobial resistance (AMR), driven by the overuse of antibiotics, has become a growing public health challenge due to exposure to resistant pathogens.

Purpose: This study aimed to evaluate the prevalence and characterize the AMR profiles of *Campylobacter* in poultry across the U.S.

Methods: Chicken and turkey carcass rinse isolation data obtained from the USDA-FSIS monitoring program (2015–2023) across processing plants in the South, West, Midwest, and Northeast regions of the U.S. were sorted by year, region, and species. *Campylobacter* prevalence was analyzed using generalized linear model in R ($p<0.05$).

Results: Among 80,282 chicken and 14,996 turkey carcass rinses analyzed, respective 14% (11,409) and 1% (158), were *Campylobacter*-positive. Of these, 46% chicken (5,241) and 71% turkey (112) was resistant to ≥1 antibiotic. AMR prevalence differed significantly by year and region ($p<0.05$) and was highest in the West (66%) and Midwest (52%) in chicken, West (93%) and Midwest (83%) in turkey. AMR peaked in 2019 for chicken (18%) and turkey (29%) and

was lowest in 2015–2017. Multidrug resistance was generally low, 2% in chicken (121) and 8% in turkey (9), with the highest levels in the Midwest (chicken) and Northeast (turkey). Species distribution varied regionally; in chicken, *C. coli* (48%) and *C. jejuni* (49%) were equally high and predominated the South, while *C. coli* (65%) was the highest and in the Midwest for turkey. Resistance to tetracyclines was most common across both poultry, followed by quinolones and macrolides, especially azithromycin often used for foodborne infection. Overall, the South (58%) and Midwest (52%) remained regional AMR hotspots for chicken and turkey, respectively.

Significance: The rising proportion of AMR strains in both poultry, especially with notable increases in certain regions, signals an evolving public health concern.

Award Application: Developing Scientist Award

T2-07: MICRONEEDLE-ENABLED DELIVERY OF BACTERIOPHAGES FOR INTERNAL DECONTAMINATION OF SOLID FOODS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Bacteriophages are promising food-safe antimicrobials, but their efficacy in solid foods is limited by poor diffusion beyond surface contamination.

Purpose: This study evaluated a microneedle-based delivery system for targeted bacteriophage decontamination within solid food matrices.

Methods: Microneedle arrays were fabricated from food-compatible polymers and loaded with lytic bacteriophages specific to *Escherichia coli* and *Salmonella enterica*. Mechanical integrity, penetration, and reusability were assessed across five food products (n=3 per product) at insertion forces from 2.5 to 7.5 N. Antimicrobial efficacy was evaluated in raw beef and ready-to-eat (RTE) chicken inoculated with $10^{2\text{-}5}$ colony forming units (CFU)/g of target bacteria. The infectivity of both phages, multiplexed into the same patch was also briefly confirmed.

Results: Microneedle delivery achieved nearly 2 log reductions in raw beef contaminated with 10^5 and 10^2 CFU/g of *E. coli* ($p < 0.05$ and 0.0001, n=3). With 10^5 and 10^2 CFU/g of *E. coli* contaminated RTE chicken, phage-loaded microneedles demonstrated log reductions of

3.40 and 2.00 compared to 0.57 and 0.08 for flat, phage-loaded patches ($p < 0.05$ and 0.01, n=3), confirming the validity of microneedle based phage delivery. Importantly, bacterial reduction met regulatory thresholds of 10^1 CFU/g for lower levels of contamination, providing successful decontamination. Finally, when both T7 and FelixO1 bacteriophage for both pathogens were loaded into the same microneedle patch, significant 2.20 and 1.46 log reductions for *E. coli* and *Salmonella* were observed ($p < 0.0001$ for both, n=3).

Significance: Microneedle-assisted phage delivery overcomes diffusion barriers in solid foods, providing a targeted, non-disruptive strategy for improving food safety and extending product shelf life.

Award Application: Developing Scientist Award

T2-08: EVALUATION OF ANTIMICROBIAL COATINGS FOR CONTROLLING LISTERIA MONOCYTOGENES AND PENICILLIUM EXPANSUM ON ORGANIC GALA APPLES DURING LONG-TERM COLD STORAGE

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: As organic apple production grows, packers face major challenges from postharvest fungal decay and concerns about foodborne pathogens.

Purpose: Assess the survival of *Listeria monocytogenes* (LM) and *Penicillium expansum* (PE) on organic apples during 11 months of controlled-atmosphere (CA) cold storage.

Methods: Organic Gala apples were spot-inoculated, then coated with sodium alginate containing 2% essential oils (EOs; v/v) [cinnamon bark (CBO), oregano (OO), coriander (CO), clove bud (ClBO), or a four-oil mixture (MO)]. A coating without EOs (C), and no-coating (NC) were included as controls for each inoculum [LM (~8.5 log CFU/apple) or PE (~6 log CFU/apple)]. Apples were stored at 3°C, (first 90 d) or in CA (3°C; 2% O_2, 1% CO_2) up to 330 d. LM and PE were quantified on selective agar at 1, 30, 60, 90, 150, 210, 270, and 330 d (n=9 apples per treatment). LM samples below the limit of detection (3.01 log CFU/apple) were enriched for presence/absence. Data was analyzed using two-way ANOVA with Tukey's HSD ($P < 0.05$), with separate LM survival kinetic model fitting for each treatment.

Results: OO, ClBO, and MO treatments caused the greatest early population reduction of LM compared to controls ($p < 0.0001$). All coated apples, except OO, followed segmented inactivation models, with early reductions (16–38 d/log) followed by slow tailing. After the first breakpoint, C showed no additional log reduction compared to the EOs treatments. OO followed a steady Weibull-type decline (~63 d/log) throughout storage. By 11-mon, CBO led to ~6-log reduction, and all treatments except NC achieved 4-log reduction ($p < 0.001$). PE remained at ~6 log CFU/apple for 90 d, then increased steadily, with no significant treatment differences ($p>0.05$).

Significance: EOs enhanced LM suppression, though long-term efficacy varied by oil and storage time. No treatment prevented PE decay and does not show promise under these conditions for preventing fungal decay during storage.

Award Application: Developing Scientist Award

T2-09: AN ANTIMICROBIAL PEPTIDE FROM PRIESTIA MEGATERIUM: ISOLATED FROM HYDROPONICS FOR THE MITIGATION OF SALMONELLA AND LISTERIA BIOFILMS FROM FOOD CONTACT SURFACES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Biofilms on hydroponic surfaces can promote cross-contamination and food safety risks, highlighting the need for effective alternatives to traditional sanitizers due to system constraints.

Purpose: This study investigated the antimicrobial peptide from *Priestia megaterium* (PM) against *Salmonella* and *Listeria* biofilms.

Methods: Bacterial microflora isolated from a hydroponic farm in Louisiana were identified by MALDI. The co-exclusion study revealed the predominance of PM. An antimicrobial peptide (AMP) was isolated from PM, and its efficacy was evaluated. For biofilm assay, hydroponic coupons were inoculated with *Listeria* and *Salmonella* (5-6 logCFU/cm^2) and stainless-steel coupons (SS) with *Salmonella* (Tennessee and Oranienburg), and *Listeria* (LCDC and Scott A; 6-7 logCFU/cm^2). After 72h, coupons were treated with AMP for 5min, and structural disruption was assessed using microscopy (SEM and TEM), while biofilm viability was quantified using confocal microscopy (CLSM).

Results: All strains were susceptible in agar well assay with *Listeria* Scott A exhibiting strong

inhibition (17±0.09mm). AMP treatment significantly reduced biofilms (P<0.05) to 2.98±0.01, 1.20±0.16, 1.48±0.09, and 1.98±0.20 log CFU/cm^2 for LCDC, Scott A, Tennessee, and Oranienburg, respectively, for SS, corresponding to about 2.0±0.08 and 2.8±0.15 log CFU/cm^2 for *Listeria* and *Salmonella* post-CFS treatment on hydroponic coupons. The AMP was characterized by gel electrophoresis. Glutamate was identified as the dominant residue, contributing to the AMP's stability and a key factor in enhancing biofilm disruption. SEM and TEM revealed biofilm disruption and intracellular component leakage, and CLSM indicated >60% biofilm cell death post-AMP treatment.

Significance: These findings underscore the efficacy of PM-derived AMPs in disrupting pathogenic biofilms, demonstrating their potential as a surface-compatible biocontrol strategy for enhancing hygiene on food-contact surfaces.

Award Application: Developing Scientist Award

T2-10: ELECTRON BEAM (EBEAM) TECHNOLOGY AS A KILL STEP FOR RAW PET FOOD PRODUCTS

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Presentation type/Format: Technical

Category: Animal and Pet Food Safety

Abstract:

Introduction: Raw pet foods are increasingly associated with foodborne illnesses, caused by *Listeria monocytogenes* and *Salmonella* spp. There is a need to evaluate novel technologies to eliminate this route of infections in pets.

Purpose: To assess the effectiveness of eBeam technology in eliminating *Salmonella* sp. and *L. monocytogenes* in raw freeze dried and frozen pet food.

Methods: Freeze-dried and frozen raw meat pet food samples were inoculated with *L. monocytogenes* (Li2 ATCC 19115, Lm68 BAA-839, EGDe) and *Salmonella enterica* DENT serovars (*S*. Dublin, *S.* Enteritidis, *S*. Newport, *S.* Typhimurium) cocktails (~8 log CFU/mL) and exposed to eBeam doses of 4, 7, 12.5, and 25 kGy. Following treatment, the efficacy of eBeam doses was measured using the decimal log (D_{10} values) reduction.

Results: D_{10} values were calculated for *L. monocytogenes* and the *Salmonella* DENT cocktail. Aerobic plate counts were also performed to assess total bioburden reduction. *Listeria monocytogenes* was generally more susceptible to eBeam treatment than *Salmonella* spp.,

except in frozen meat products where relatively greater resistance was observed. eBeam doses of 4–7 kGy were effective across most products. A dose of 4 kGy resulted in approximately 2–6 log reductions, while **7** kGy achieved approximately 4–6 log reductions of *L. monocytogenes* and the *Salmonella* DENT cocktail. At 7 kGy, approximately 4–7 log reductions of aerobic bioburden were achieved across most products.

Significance: Electron beam (eBeam) irradiation provides an effective non-chemical, non-thermal inactivation technology to eliminate *L. monocytogenes* and *Salmonella* spp. in frozen and freeze-dried raw pet food. Therefore as eBeam doses are approved for human food, raw pet foods can be made microbiologically safe.

T2-11: POST-PROCESSING CONTROL OF SALMONELLA ENTERICA IN RAW PET FOOD USING HIGH-PRESSURE PROCESSING AND NATURAL ANTIMICROBIALS

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Presentation type/Format: Technical

Category: Animal and Pet Food Safety

Abstract:

Introduction: Raw meat-based pet foods require effective microbial control strategies to manage *Salmonella* contamination during refrigerated storage. High pressure processing (HPP) is widely implemented to enhance microbiological safety in these products. Clean-label, ingredient-based antimicrobial systems may function as complementary hurdle to support HPP performance and improve post-processing microbial stability.

Purpose: To validate the effectiveness of a natural flavor-based antimicrobial system used in combination with HPP for controlling *Salmonella* spp. in raw pet food during 48 h of refrigerated storage.

Methods: Two treatments were evaluated: a negative control without antimicrobials (T1) and a formulation containing a natural flavor-based antimicrobial system (T2; 3.50% EW 100L + 0.50% EW S100). Treatments were inoculated with a four-strain antibiotic-resistant *Salmonella*

spp. cocktail (Newport, Heidelberg, St. Paul, Hadar). Initial populations were determined by plating inoculated samples in triplicate on tryptic soy agar supplemented with 100 µg/mL ampicillin. Following HPP, surviving *Salmonella* populations were enumerated immediately and after 24 and 48 h of storage at 4 ± 1°C. Statistical analysis was performed using one-way ANOVA (JMP Pro v15.1.0; p □ 0.05).

Results: Initial *Salmonella* populations ranged from 7.78–7.93 log CFU/g. Immediately post-HPP, populations were reduced to 1.00 log CFU/g in T1 and 1.98 log CFU/g in T2, corresponding to reductions >5 log CFU/g. During refrigerated storage, recovery of injured *Salmonella* cells was observed in the control. At 24 and 48 h post-HPP, *Salmonella* populations increased in T1 to 2.60 and 3.35 log CFU/g, while T2 maintained lower levels at 1.18 and 1.40 log CFU/g, respectively (p □ 0.05).

Significance: These findings demonstrate that a natural flavor-based antimicrobial system combined with HPP enhances post-processing *Salmonella* control during refrigerated storage.

T2-12: USE OF BACTERIOPHAGE AGAINST MULTI-DRUG-RESISTANT SALMONELLA IN DRY PET FOOD KIBBLES

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Presentation type/Format: Technical

Category: Animal and Pet Food Safety

Abstract:

Introduction: Pet foods are widely recognized as a significant source of *Salmonella* among pet owners. Close and frequent association between pets, pet foods, and pet owners increases this risk. Recent multistate outbreaks linked to dry pet foods have involved multidrug-resistant (MDR) *Salmonella*.

Purpose: To evaluate the efficacy of commercially available bacteriophage SalmoFresh^{TM} in reducing two MDR *Salmonella* serovars in vitro and in kibbles.

Methods: Standardized 5 log CFU/mL (in vitro) and 5 log CFU/g (in kibble) of *Salmonella* Infantis-4 and Typhimurium-92 inoculated matrices were treated with bacteriophage at multiplicities of infection (MOI) of 0, 100, 1000, and 10000. The in vitro samples were stored at 4°C and were analyzed at 0, 2, 6, 12, 24, and 48 h. Whereas, treated kibbles were stored at 25°C and analyzed at 0, 6, 12, 24, 48, and 72 h post-treatment. The samples were plated on TSA plates, followed by 24 h of incubation before counting. A total of 3 replications were

completed for each study. Mean reductions were compared across MOIs and time points using a two-way ANOVA (α = 0.01).

Results: Under in vitro conditions, phage treatment caused dose- and time-dependent reductions from 0 h and up to ~4-log CFU/mL reduction ($p < 0.001$) by 48 h for both serovars. While 100 MOI yielded <1-log reduction, both serovars declined to non-detectable levels by 48 h at higher MOIs. In kibble, both MOI and time had significant effects, where 10,000 MOI achieved ~2–3 log CFU/g reduction by 72 h. The 10,000 MOI significantly ($p < 0.001$) reduced *Salmonella* compared to other treatments. Declines in control kibbles suggest the dry matrix also contributed to reductions.

Significance: Bacteriophage produced clear dose- and time-dependent reductions of *Salmonella* in vitro and in dry pet food. These findings indicate that bacteriophage application is a promising biological intervention for reducing *Salmonella* contamination in pet foods and associated household exposure risks.

Award Application: Developing Scientist Award

T3-01: SYNERGISTIC ANTIMICROBIAL AND IMMUNOMODULATORY EFFECTS OF LACTIPLANTIBACILLUS PLANTARUM AND BERRY POMACE EXTRACTS AGAINST AVIAN PATHOGENIC E. COLI

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Presentation type/Format: Technical

Category: Plant-Based Alternative Products

Abstract:

Introduction: *Lactiplantibacillus plantarum* (LP) is a predominant probiotic with well-documented antimicrobial and immunomodulatory properties. Plant-derived byproducts such as berry pomace extracts (BPE) possess antimicrobial and prebiotic potential that supports beneficial microbiota. When combined, this synbiotic formulation can be leveraged to enhance efficacy against Avian Pathogenic *Escherichia coli* (APEC), a major poultry pathogen with zoonotic implication.

Purpose: This study evaluated the antimicrobial and immunomodulatory effects of synbiotics against APEC as alternatives for enhancing poultry health.

Methods: Inhibitory and bactericidal concentrations of BPE against APEC were determined. LP was grown in BPE supplemented media to obtain cell-free cultural supernatants ($CFCS^{BPE}$). APEC growth kinetics and changes in survival, stress-response, and virulence-associated gene expressions were evaluated following treatment with $CFCS^{BPE}$/BPE alone. Membrane

integrity was analyzed by confocal microscopy and quantitative permeability assays. Additionally, HD-11 cells were used to assess interaction with APEC in the presence or absence of LP and/or BPE. Statistical significance was analyzed using two-way ANOVA with Tukey's post-hoc test.

Results: BPE exhibited inhibitory activity at 1mg GAE/mL and promoted LP growth at 0.5mg GAE/mL, demonstrating its prebiotic characteristics. $CFCS^{BPE}$ reduced APEC counts by >3 log CFU/mL within 24h and achieved complete elimination by 48h (p<0.001). This was associated with significant downregulation of vital genes (*iss, fyuA, ompC, luxS, rpoS,* and *murD)* (p<0.05). Both treatments induced a time-dependent increase in membrane permeability. LP pre-treatment significantly enhanced macrophage bactericidal activity (p<0.05) without altering bacterial uptake efficiency.

Significance: These results highlight the synergistic antimicrobial mechanisms of LP and BPE and their capacity for immunomodulation as a sustainable intervention strategy.

Award Application: Developing Scientist Award

T3-02: FOODBORNE PATHOGEN OUTBREAKS AND RECALLS LINKED TO LEGUME FOODS AND SPROUTS, WORLDWIDE, 2012–2025

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Presentation type/Format: Technical

Category: Plant-Based Alternative Products

Abstract:

Introduction: With the global population predicted to reach 9.7 billion by 2050 and the market for resource-efficient plant-based foods set to reach USD 28.9 billion by 2024, it is essential to assess the risks of foodborne diseases associated with these alternatives in order to ensure that they are safe as consumption continues to grow.

Purpose: The study describes recent outbreaks and recalls of foodborne pathogens associated with legume-based foods and analyzes trends in the possible interventions.

Methods: We examined the outbreaks and recalls related to legumes and calculated the total number of outbreaks, recalls, diseases, hospitalizations and deaths by legume product and pathogen type. We searched the EFSA, ECDC, CFIA, CDC, OzFoodNet and FDA datasets for legume-related outbreaks and recalls between 2012 and 2025. We analyzed the characteristics of the outbreak, such as geography, seasonality, and food preparation settings using statistical tests (Fisher's exact test, chi square, Kruskal-Wallis) in the SAS (alpha value of 0.05).

Results: A total of 112 outbreaks were identified, resulting in 3,101 illnesses, most of which

were associated with beans (56.3%), sprouts (31.9%) and chickpeas (5.9%). Of the 91 outbreaks, 22 were multi-state (24.2%) and 90 were single-state (75.8%). While *Listeria monocytogenes* was associated with 6.6% of outbreaks, it was associated with 81.8% of deaths associated with legume-based foods. Between 2012 and 2025, the majority of recalled products were contaminated with *Listeria* (44.2%) or *Salmonella* (50.0%). Whereas peanuts were recalled more frequently (51.5%), despite their small contribution to outbreaks (4.4%). Although sprouts represented 9.0% of recalls, they were associated with 52.2% of hospitalizations and 36.4% of deaths.

Significance: Identifying persistent pathogens and food groups at increased risk of contamination is of the utmost importance for the development of strategies to reduce pathogens in plant-based food.

Award Application: Developing Scientist Award

T3-03: GENETICALLY CLOSE LISTERIA MONOCYTOGENES ISOLATES SHOW DIVERGENT GROWTH UNDER COLD-SMOKED SALMON PROCESSING STRESSES

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Presentation type/Format: Technical

Category: Seafood

Abstract:

Introduction: *Listeria monocytogenes* may proliferate in cold-smoked salmon, so challenge tests performed by food business operators should capture strain-to-strain variability under processing stresses.

Purpose: To test whether genomic proximity among smoked-salmon isolates predicts growth under salting, smoking, and refrigeration.

Methods: Nine isolates from cold-smoked salmon plus one reference strain (n=10; CC121, CC26, CC204, CC9) were grown in modified BHI under 3% NaCl, liquid smoke (0.7 mg phenols/100 g), 8°C, and cumulative 8°C+3% NaCl±smoke. Maximum growth rates (μmax, h^{-1}) were estimated from optical density kinetics and/or daily plate counts during 12 d at 8°C; strain effects were assessed by ANOVA with multiple comparisons. The 10 genomes were Illumina-sequenced (2×250 bp), assembled, and compared by cgMLST (Pasteur 1,748-locus scheme) to compute allelic distances.

Results: Under optimal conditions, μmax ranged from 0.638 to 1.151 h^{-1} and decreased

under 3% NaCl (0.590–0.820 h^{-1}) and liquid smoke (0.321–0.872 h^{-1}). One-way ANOVA with post-hoc multiple comparisons showed significant between-strain differences in μmax under the tested stress conditions (p<0.05). Three CC204 isolates (IG565, IG792, HPL114BA2) were extremely close by cgMLST (1–6 allelic differences; same complex type), yet showed contrasting phenotypes: under liquid smoke, μmax was 0.321 h^{-1} for IG565 vs 0.417–0.517 h^{-1} for IG792/HPL114BA2. At 8°C, overall μmax ranged from 0.076 to 0.101 h^{-1}, but IG565 showed no growth after 12 d (also under 8°C+3% NaCl and 8°C+3% NaCl+smoke), whereas all other isolates grew; under 8°C+3% NaCl+smoke, μmax ranged from 0.065 to 0.080 h^{-1} among growing strains.

Significance: Even within a single cgMLST complex type, phenotypes can diverge; therefore, strain selection for cold-smoked salmon challenge tests should not rely solely on CC/cgMLST but include phenotypic screening under relevant cumulative stresses.

T3-04: PREVALENCE, MULTIDRUG RESISTANCE AND MULTIPLE ANTIBIOTIC RESISTANCE INDEX OF VIBRIO CHOLERAE AND VIBRIO PARAHAEMOLYTICUS IN FERAL FISH FROM TWO MAJOR DAMS IN IBADAN, NIGERIA

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Presentation type/Format: Technical

Category: Seafood

Abstract:

Introduction: The rise of AMR has significantly complicated treatment options in humans and animals, posing a substantial threat to public health. The prevalence of multidrug-resistant *Vibrio cholerae* (Vc) and *Vibrio parahaemolyticus* (Vp) in feral fishes has emerged as a critical public health concern, potentially contributing to cholera transmission in Nigeria.

Purpose: This study assesses the prevalence and antibiotic resistance profiles of Vc and Vp in feral fish, focusing on the risks and implications for food safety and disease management in Nigeria.

Methods: 83 feral fishes (±250g) were collected from Eleyele and Asejire dams in Ibadan, Oyo State. Liver, intestine, and muscle tissues were enriched in buffered peptone water and incubated at 30℃ for 24 hours. The enriched samples were streaked on TCBS Agar, incubated at 30℃ for 24 hours, and subjected to biochemical tests for identification. Antibiotic sensitivity was assessed using the Kirby-Bauer test, with results interpreted according to CLSI (2024).

Results: Vc and Vp prevalence were 100% and 72.7% in Eleyele dam, and 100% and 50% in

Asejire dam, respectively. Both species exhibited 100% resistance to Ampiclox, Cefotaxime, Cefuroxime, Imipenem/Cilastatin, and Cefexime. Vc showed additional resistance to Amoxicillin Clavulanate (100%), Ceftriaxone Sulbactam (93%), and other antibiotics, while Vp displayed similar resistance patterns. Both isolates were sensitive to Ciprofloxacin, and *Vibrio cholerae* was also sensitive to Ofloxacin. The multiple antibiotic-resistant index (MARI) ranged from 0.44 to 1 for Vc and 0.63 to 0.94 for Vp, indicating a high level of multidrug resistance.

Significance: The detection of these MDR-vibrios in feral fishes underscores significant food safety concerns. The elevated AMR profiles highlight potential treatment failures in humans and aquatic animals, exacerbating the global AMR challenge.

T3-05: PESI-LIKE MEGAPLASMIDS: ASSESSING DISSEMINATION LIKELIHOOD AND DETERMINANTS OF HORIZONTAL TRANSFER USING GENOMIC LANGUAGE MODELS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Emerging *Salmonella enterica* serovar Infantis carries pESI-like megaplasmids encoding antimicrobial resistance and virulence determinants, and is linked to rising poultry-associated human infections; available evidence suggests these megaplasmids could disseminate to other clinically and economically relevant bacteria.

Purpose: Identify chromosomal factors associated with pESI-like carriage and predict dissemination risk to other virulent serovars and species.

Methods: We compiled high-quality assemblies from EnteroBase, public repositories and an internal collection, applied QC filters to asses completeness, contamination and fragmentation. Then we detected and filtered pESI-like elements using a custom database plus plasmid reconstructions. Pretrained genomic language models were fine-tuned on tokenized contig chunks from carrier and non-carrier genomes; chunk outputs were aggregated into genome-level embeddings and classified. SHAP was used to map feature

importance back to sequence regions. Finally an embedding-based probability metric ranking prioritized non-Infantis isolates for in vitro conjugation, competition and maintenance assays.

Results: The pipeline produced separable embeddings and recurrent genomic signatures that localized to prophage regions, metabolic loci and partition/stability systems plausibly involved in plasmid maintenance. SHAP-guided mapping enabled locus-level hypotheses explaining carrier status. The probability metric shortlisted a small set of high-priority non-Infantis isolates from the internal collection for experimental validation.

Significance: Interpretable language-model embeddings applied at scale offer a transferable, data-driven framework to prioritize high-risk plasmid-host associations and to guide targeted surveillance and mitigation efforts against AMR dissemination.

Award Application: Developing Scientist Award

T3-06: SURVIVAL OF EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI STRAINS DURING MUNICIPAL BIOSOLIDS TREATMENT AND POTENTIAL IMPLICATIONS FOR FOOD AND WATER SAFETY

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Recent research has demonstrated that naturalized wastewater *Escherichia coli* (NWEC) and extraintestinal pathogenic *E. coli* (ExPEC) are extremely resilient to sewage treatment, raising concerns about pathogen persistence in the food-water nexus.

Purpose: The goal of this research was to determine whether ExPEC pathotypes could survive municipal biosolids treatment processes.

Methods: A library of *E. coli* strains was isolated from municipal biosolid samples (anaerobic digested and thickened sludge). Purified isolates were confirmed to be *E. coli* by PCR (i.e., *uidA* gene) and by biochemical identification on a VITEK 2® Automated Bacterial Identification System. Virulence gene (VG) screening by PCR (*ibeA, fyuA, chuA,* and *iroN*) was done to identify potential ExPEC strains, including screening for ST131 strains (a notable pandemic strain).

Strains positive for virulence genes were subjected to whole-genome sequencing (WGS) followed by phylogenetic analysis and detailed VG characterization.

Results: 52 of 180 (28.9%) library isolates were positive for two or more ExPEC-associated virulence genes, with 9 strains (5.0%) containing all 4 virulence genes. Of these 52 strains, only four (7.7%) were ST131 positive, and 46 (88.5%) were positive for both *chuA* and *fyuA*. Repetitive extragenic palindromic PCR (rep-PCR) revealed that 19 of the 52 strains appeared clonally-related, leaving 33 potential ExPEC strains (18.3%) to be genome sequenced. Preliminary analysis suggests that some strains may represent clinically important isolates. A more thorough genomic analysis on phylogenetic-relatedness and VG composition will be presented at the conference.

Significance: ExPEC appear to survive biosolids treatment processes, raising questions about potential risks that human biosolids might pose as agricultural soil amendments for food production.

Award Application: Developing Scientist Award

T3-07: PSEUDOMONAS FLUORESCENS A506 INHIBITS ESCHERICHIA COLI O157:H7 ON ROMAINE LETTUCE BY INDUCING METAL STRESS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Contamination of romaine lettuce with *Escherichia coli* O157:H7 (EcO157) remains a persistent food-safety concern. The biocontrol strain *Pseudomonas fluorescens* A506 suppresses EcO157 strain MD41 on romaine, but the mode of action is unclear.

Purpose: To elucidate how A506 inhibits MD41 on lettuce.

Methods: MD41 was inoculated onto romaine leaves with or without prior colonization by A506 at 28 C. RNA was collected 24 h post-inoculation. MD41 siderophore gene expression was measured by RT-qPCR, and whole transcriptome profiles of both MD41 and A506 were generated by RNA-seq.

Results: RT-qPCR showed reduced expression of MD41 enterobactin genes in the presence of A506, arguing against iron competition as the inhibition mechanism. RNA-seq revealed strong and broad downregulation of MD41 motility, chemotaxis, and metabolic genes by

A506, while copper and other metal-stress genes were among the most highly induced under the influence of A506 versus MD41 alone, suggesting A506 causes metal toxicity in MD41. MD41 also exhibited signatures of A506-associated low oxygen (upregulation of *pflA, nifJ* and *frd*) and amino acid deficiency (upregulation of *pheP, aroP,* and *brnQ*) stresses, conditions that are known to cause endogenous copper toxicity in *E. coli* and may arise as A506 depletes oxygen and nutrients in leaf-surface water droplets where MD41 co-resides. Contrarily, RNA-seq detected no differential regulation of MD41 enterobactin genes in response to A506. Additionally, A506-induced low oxygen may promote MD41 fermentation and acidification, increasing iron bioavailability on leaves and further supporting our observations from population and RT-qPCR studies that iron depletion by A506 is unlikely to mediate A506 biocontrol of MD41 on lettuce.

Significance: These findings uncover a novel mechanism of bacterial inhibition by A506 and provide a new perspective on the interactions between food borne pathogens and leaf epiphytes.

Award Application: Developing Scientist Award

T3-08: DEVELOPMENT AND VALIDATION OF A MULTIPLEX PCR FOR FIVE COMMON SEROVARS OF SALMONELLA (MPCRSE+ST+SH+STHO+SINF)

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: A rapid and reliable laboratory method to identify *Salmonella* colonies and for distinguishing among the most common serovars could mitigate the health burden attributed to foodborne salmonellosis. We previously validated a multiplex PCR for *Salmonella* Typhimurium and *Salmonella* Enteritidis and now included newly designed PCR primer pairs for three additional serovars, namely, Heidelberg, Thompson and Infantis.

Methods: We targeted unique DNA fragments in the genomes of various *Salmonella* serovars and developed primer pairs for the PCR amplification of appropriately-sized targets for the genus *Salmonella,* each serovar and for an internal control to ensure optimal resolution and visualization of PCR products.

Results: The multiplex PCR detected the serovars Heidelberg (123 bp), Infantis (255 and 441bp), Typhimurium (523bp), Enteritidis (612 bp), and Thompson (808 bp) as well as two genus-specific targets namely, *invA* (211 bp) and *iroB* (309 bp) and a ribosomal DNA fragment (1,026 bp) serving as an internal control. Upon testing a panel of *Salmonella* organisms consisting of Heidelberg (n=113), Typhimurium (n=104), Infantis (n=106), Enteritidis (n=100), and Thompson (n=73), the PCR accurately assigned the serovar for 484 of 498 *Salmonella* organisms, accuracy of 97.6%. No *Salmonella* belonging to other serovars was wrongly classified (0/100). All non-*Salmonella* organisms tested negative in the assay (0/50; 100% specificity). Wrong serovar designations (n=12, or 2.4%) occurred when multiple

serovars were present in a sample or because of a misleading result from the submitting lab. Only one sample (serovar Thompson) was confirmed to be a true negative by whole genome sequencing.

Significance: As a reliable and rapid confirmatory tool for *Salmonella* identification and the designation of the five most common serovars causing illnesses, the validated multiplex PCR should reduce the delay encountered during food safety investigations involving *Salmonella*.

T3-09: HIGH-THROUGHPUT NEXT-DAY ISOLATION OF PURE SALMONELLA COLONIES FROM PRODUCE USING DIGITAL CULTURING AND PHENOTYPING

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: A critical bottleneck in food safety surveillance is the lack of efficient tools to rapidly obtain pure *Salmonella* isolates from complex food samples; conventional, manual isolation methods require at least four days to yield pure colonies necessary for definitive downstream molecular analysis (e.g., MALDI-TOF or WGS).

Purpose: This study aimed to demonstrate a novel, high-throughput digital culturing and phenotyping (DCP) method for the rapid isolation of pure, omics-ready *Salmonella* from fresh produce.

Methods: Fifteen 25g spinach samples were inoculated with 5 CFUs of *Salmonella* Newport; one sample served as a negative control. After 22 hours of selective pre-enrichment at 42°C in BPW with antibiotics, an aliquot of each sample was mixed with anti-*Salmonella* antibody conjugated magnetic beads to enrich the target and remove background microbiota. The resulting purified sample was then incubated for 12 hours in a 96-well plate containing XLT4 agar. Time-lapse images were automatically collected and analyzed in real-time to identify discrete, pure *Salmonella* colonies. The top-ranked colonies

were then automatically aspirated by a robot-controlled pipette and plated for quantitative validation.

Results: The image analysis algorithm correctly detected all 15 inoculated samples and the negative control, achieving 100% detection accuracy. A total of 88 discrete colonies were robotically picked from the 15 positive samples. Subsequent quantitative validation of recovered colonies confirmed that each pure isolate contained at least 10^5 *Salmonella* CFUs and maintained purity exceeding 90%, a concentration sufficient for direct downstream molecular analysis.

Significance: This automated "samples in, pure colonies out" method addresses the core time-to-isolation bottleneck in high-throughput *Salmonella* testing, making pure, omics-ready colonies available in under 36 hours (next-day) and accelerating critical food safety analyses by multiple days.

T3-10: RAPID CLASSIFICATION OF NATIVE AND VACCINE STRAINS OF SALMONELLA TYPHIMURIUM USING WHOLE-CELL MALDI-TOF MASS SPECTROMETRY

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Live-attenuated vaccines are a pre-harvest intervention to reduce *Salmonella* colonization in food animals. However, vaccine strains can persist through production and be detected in food products. Identifying these vaccine strains is crucial to mitigating any unintended effects on an establishment's *Salmonella* performance metrics, but current identification methods often rely on labor-intensive molecular techniques.

Purpose: This study evaluated whole-cell MALDI-TOF MS as a rapid and cost-effective alternative for distinguishing native *Salmonella* Typhimurium (*S.* Typhimurium) strains from a vaccine strain of *S.* Typhimurium (AviPro Megan Vac 1).

Methods: Native *S.* Typhimurium strains (n = 247) and isolates of Megan Vac 1 (n = 45) were cultured on sheep blood agar and prepared using direct transfer and extended direct transfer (formic acid overlay) methods. Spectra were acquired on the MALDI Biotyper sirius System. Multivariate analyses and machine learning classifiers were developed to distinguish and predict vaccine isolates.

Results: The spectral profiles of native and vaccine strains differed for direct and extended direct transfer methods (PERMANOVA, $p < 0.0001$). Multiple peaks showed significantly different intensities between the two groups (Wilcoxon rank-sum, $p < 0.05$). Random Forest

models based on peak intensities achieved 100% predictive accuracy when using spectra from both preparation methods. No single peak fully discriminated between native and vaccine strains, with models achieving a maximum accuracy of 94%.

Significance: MALDI-TOF MS, a technology commonly used in diagnostic laboratories, can effectively and quickly distinguish the Megan Vac 1 vaccine strain from native *S.* Typhimurium strains. Implementing this approach could result in cost savings, support the continued use of effective pre-harvest vaccination strategies, and improve the efficacy of regulatory performance assessments, ultimately enhancing food safety and public health.

T3-11: COMPARATIVE ANALYSIS OF SALMONELLA SEROTYPE DETERMINATION USING BETTERCALLSAL, SEQSERO2, AND SISTR

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Sequencing is increasingly being used to determine *Salmonella* serovar as it can be faster and more objective than traditional serology (TS). There are several bioinformatic tools that analyze sequence data to determine serovar. Understanding these tools and their accuracy is important to establish a robust workflow to identify *Salmonella* serotypes.

Purpose: The purpose of this work is to understand the degree of agreement between the three bioinformatic tools and TS.

Methods: Pure *Salmonella* isolates (258 samples comprising 45 different serotypes) and one intentionally mixed isolate underwent DNA extraction. The resulting DNA was used for Oxford Nanopore Technologies (ONT) sequencing with an ONT library prep kit. Samples were sequenced briefly and results were analyzed using three bioinformatic tools: SISTR, SeqSero2, and BetterCallSal (BCS). Results were compared between the different pipelines and TS.

Results: Marked differences were observed when comparing the agreement between the

analysis tools and the TS results. SISTR agreed with TS, SeqSero2, and BCS for 98.84%, 97.68%, and 95.75% of samples, respectively. SeqSero2 agreed with TS for 97.68% of samples and with BCS for 94.59% of samples. BCS agreed with TS for 94.59% of samples. The three bioinformatic pipelines agreed for 94.59% of samples, while the three pipelines agreed with TS for 93.43% of samples. When using the bioinformatic pipelines together and discarding results that fail to meet criteria (analysis errors), the combined pipelines agreed with TS in 99.23% of cases.

Significance: By optimizing the workflow to take advantage of the strengths of the different tools, sequencing for serology can be used to rapidly and accurately determine *Salmonella* serology with little to no errors in pure isolates across a variety of different serotypes and strains.

T3-12: DIGITAL PCR ASSAY FOR THE DETECTION AND QUANTIFICATION OF SALMONELLA LEVELS IN CHICKEN BREAST

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* is a leading cause of multistate foodborne outbreaks in the United States. Although USDA-FSIS monitors poultry using performance standards, routine testing still largely reports presence/absence and does not quantify pathogen load. Because contamination levels strongly drive risk, especially for high-load events and outbreak-associated serovars, rapid, accurate *Salmonella* quantification is critical for surveillance and outbreak prevention.

Purpose: To develop and validate a digital PCR (dPCR) workflow for the quantification of *Salmonella* contamination in chicken breast samples.

Methods: Pure-culture *Salmonella* strains belonging to serogroups Enteritidis (n=2), i,4[5],12, i:- (n=2), and Typhimurium (n=2) were cultured overnight twice, serially diluted, and cold-stressed at 4°C for 48 hours. Plate counts were used to calculate the inoculation volume. The 325g chicken breast was weighed into filter bags, inoculated at 0.1, 1, and 10 CFU/g, and

enriched with 1000 mL of modified buffered peptone water (mBPW) with supplements for 6 hours at 35±2°C. Direct cells from enrichments with no DNA extraction were used for dPCR assay targeting the *inv*A gene, and data were analyzed using QIAcuity Software.

Results: The dPCR assay showed high tolerance for DNA and PCR inhibitors. The assays accurately detected all cold-stressed *Salmonella* in inoculated chicken breast samples following a six-hour enrichment. Log_{10} dPCR concentrations (copies/µL) accurately estimated the inoculated levels, and inoculum CFU/g and dPCR-derived copies/µL were not significantly different ($p>0.05$).

Significance: This extraction-free, short-enrichment dPCR workflow enables rapid, accurate *Salmonella* quantification in chicken breast, overcoming the limitations of presence/absence testing. Quantitative results can support risk-based product disposition, earlier interventions, regulatory verification, and faster outbreak response.

Award Application: Developing Scientist Award

T4-01: BEHAVIOR OF SALMONELLA AND LISTERIA MONOCYTOGENES IN FROZEN BLUEBERRY AT TWO INOCULUM LEVELS

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Frozen berries have been implicated in foodborne illnesses. Blueberry is commonly found in frozen mixed-berry blends.

Purpose: This study evaluated the survival behavior of *Salmonella* and *Listeria monocytogenes* in frozen blueberry at two inoculum levels during frozen storage.

Methods: Ten-gram portions of intact blueberry were spot inoculated with inoculum cocktails of rifampicin-resistant *Salmonella* (5 strains) or *L. monocytogenes* (6 strains) at ~10^5 log CFU/g (high inoculum) or ~10^2 logCFU/g (low inoculum) and stored at -18°C. Bacteria were enumerated at 0,0.02,1,3,6,10,15,30,60 and 90d post-inoculation (N=10). At each timepoint, samples were mixed with 20mL of peptone water and stomached for 90s at low speed. Samples were serially diluted and plated on tryptic soy agar+rifampicin (80ppm)+0.1% sodium pyruvate. Summary statistics and linear regression models were used to model die-off, with significance defined as $p<0.05$.

Results: At a high inoculum, *Salmonella* reduced by 1.64 $\log_{10}$CFU/g, with no detectable

counts after 90d. Low-inoculum populations declined from 2.15±0.13 $\log_{10}$CFU/g at 0d to 0.53±0.48 $\log_{10}$CFU by 1d, followed by intermittent recovery and eventual non-detection by 90d. *L. monocytogenes* high inoculum level reports a reduction of 3.17 $\log_{10}$CFU/g, no detectable counts were observed after 90d. Low-inoculum populations decreased rapidly from 1.88±0.16 $\log_{10}$CFU/g at 0d to sporadic detection by 1d (1/10 positives) and were consistently below the limit of detection (LOD=0.30 $\log_{10}$CFU/g) from 10d onward. Based on linear models, significant differences were observed in die-off between high and low inoculum levels in both bacteria.

Significance: These findings show that pathogen survival trends in frozen blueberry depend on initial contamination levels. Incorporating multiple inoculum levels into laboratory-based frozen storage studies will improve interpretation of die-off kinetics and support more accurate risk assessments for frozen produce.

Award Application: Developing Scientist Award

T4-02: E. COLI O157:H7 EXPLOITS INCREASED NUTRIENT AND TEMPERATURE CONDITIONS TO GROW IN SIMULATED AGRICULTURAL RUNOFF

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Agricultural runoff in irrigation water can transport pathogens to contaminate produce commodities, causing human illness. Survival and growth of *E. coli* O157:H7 in runoff under pre-harvest agricultural conditions is not fully understood.

Purpose: To determine growth/survival patterns of *E. coli* O157:H7 at low levels in agricultural runoff under non-optimal temperatures representing the mean daily high and low water temperatures in Yuma, AZ.

Methods: Non-sterile soil runoff formulations were prepared with agricultural pond water and soils collected from a USDA-ARS farm in Maryland and were amended or not with heat-treated poultry pellets, a fertilizer used in fruit/vegetable production. *E. coli* O157:H7 REPEXH01 was inoculated into amended or unamended runoff (ca. 2 $\log_{10}$ CFU/mL), then incubated at low (13°C), high (22°C), and dynamic (12-hour cycles between 22°C/13°C) temperatures. *E. coli* O157:H7 levels were determined on MacConkey agar supplemented with sorbitol, cefixime-tellurite, and chloramphenicol (50 µg/mL) for up to 240h. Data were fit to the Baranyi model to measure growth parameters and compared using an ANOVA with

post-hoc Tukey HSD test. Each treatment was replicated in duplicate. Microbial communities across the treatment types will be analyzed after 16S sequencing.

Results: *E. coli* O157:H7 had a significantly ($P < 0.0001$) higher growth rate (m) and final population (*N*max) in amended soil runoff at 22°C (m=0.078±0.031, *N*max=4.58±0.037 $\log_{10}$ CFU/mL) compared to 22°C/13°C (m = 0.022±0.0019, *N*max=3.64±0.024 $\log_{10}$ CFU/mL). *E. coli* O157:H7 levels in unamended runoff fell below <1$\log_{10}$ CFU/mL at 22°C and 22°C/13°C after 96 and 144h, respectively; levels in amended runoff at 13°C were stable.

Significance: *E. coli* O157:H7 required higher or dynamic temperatures and additional nutrients from amendments to grow over 10 days, indicating agricultural runoff can support *E. coli* O157:H7 growth instead of survival under specific conditions.

T4-03: SEED-ASSOCIATED LISTERIA MONOCYTOGENES DRIVES MICROGREEN CONTAMINATION DURING GERMINATION AND GROWTH

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Microgreens are commonly consumed raw and present food safety concerns due to the absence of post-harvest kill steps. As with sprouts, seed-associated contamination can be critical for the introduction and persistence of *Listeria* in microgreen production systems.

Purpose: This study evaluated how seed contamination and cultivation matrices influence *Listeria monocytogenes* contamination of microgreens during germination and growth.

Methods: Daikon seeds were spray inoculated with a four-strain *Listeria monocytogenes* cocktail (3 log CFU/g) and dried overnight. Microgreens were grown for 10 days using biostrate pads (BP), vermiculite–perlite (VP), or soil with cattle compost (SC). Microgreens, substrates, and irrigation water were sampled on days 1, 3, 7, and 10 for *Listeria* enumeration using modified Oxford agar. The study followed a completely randomized

design with six trays per matrix. Data were analyzed using GraphPad Prism (P □ 0.05).

Results: Significant *Listeria* populations were recovered from microgreens, substrates, and irrigation water throughout production. On day 1, BP- and VP-grown microgreens supported higher *Listeria* populations (5.8–6.5 log CFU/g) than SC-grown microgreens (4.7 log CFU/g; P □ 0.05). Although pathogen populations declined over time, BP and VP microgreens maintained >4.3 log CFU/g by day 10, whereas Listeria levels in SC microgreens declined to approximately 1.2 log CFU/g (P □ 0.05). Recovery from substrates and irrigation water was also higher in BP and VP systems than in SC systems.

Significance: These findings demonstrate that seed-associated *Listeria monocytogenes* and cultivation matrix selection strongly influence contamination during microgreen production. Hence, seed sanitation and substrate choice represent critical control points for reducing food safety risk.

Award Application: Developing Scientist Award

T4-04: IN-FIELD VALIDATION OF POSTHARVEST WATER MANAGEMENT PRACTICES TO PREVENT CROSS-CONTAMINATION IN APPLE PACKINGHOUSES

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Postharvest water systems in apple packing, including flumes and dump tanks, move and wash fruit but can enable microbial cross-contamination, if not properly managed.

Purpose: This study evaluated whether minimum sanitizer concentrations in commercial flumes in Michigan and Virginia prevent microbial cross-contamination under operating conditions.

Methods: Field experiments were conducted in four commercial apple packinghouses in Michigan and Virginia. Apples were sampled before flumes (Produce-In) and after one or two flumes (Produce-$Out_{1,2}$). Microbial indicators including Aerobic Plate Count (APC), Enterobacteriaceae (EB), Total Coliforms (TC), generic *Escherichia coli* (EC) and water

parameters (turbidity, pH, temperature, sanitizer concentration) were monitored for up to 80 h. Microbial loads were quantified via serial dilution and Petrifilm plating. Linear models compared sampling points and packinghouses and assessed links between water quality and microbial loads.

Results: In Michigan, sanitizer levels varied: PAA 15–180 ppm, chlorine 7–50 ppm; Virginia chlorine 6–25 ppm. No significant APC differences were observed between Produce-Out$_1$ or $_2$ and Produce-In (-0.11 or -0.19 log CFU/apple). EB showed no significant changes (0.003 or -0.13 log CFU/apple). TC was similar for Produce-Out$_1$ vs. Produce-In (0.14 log CFU/apple) but increased at Produce-Out$_2$ (1.07 log CFU/apple; $p<0.001$). EC occurred in <2% of Michigan samples; Virginia had no TC or EC. Flume water averaged 1.37±1.14 log CFU/mL (APC), 0.26±0.70 (EB), and 0.21±0.64 (TC). No microbial reductions occurred, confirming washing is not a kill step.

Significance: Well-managed flume water did not increase microbial loads on apples during extended operation. Findings support risk-based water management using sanitizer concentration, turbidity, and indicator organisms (APC, EB, TC) rather than fixed water-change intervals.

Award Application: Developing Scientist Award

T4-05: CHARACTERIZING THE IMPACT OF FREEZE-DAMAGE WATERSOAK ON THE DYNAMICS OF L. MONOCYTOGENES AND E. COLI O157:H7 ON CHOPPED ROMAINE LETTUCE

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: While quality defects may be considered a sensory problem, prior research suggests that they may also enhance pathogen survival on produce. Understanding whether defects, such as freeze-damage watersoak, can impact the dynamics of pathogens on leafy greens can inform product management strategies.

Purpose: To characterize the dynamics of *L. monocytogenes* (LM) and *E. coli* O157:H7 (EC) on defect free romaine (DF) and watersoaked romaine (WS) at 40C, 12C and 21C.

Methods: Chopped romaine was stored at 4C (i.e., DF) or -20C for 30 mins followed by thawing at 4C (to generate WS damage). Samples were spot inoculated, separately, with two strains of LM and one strain of EC. Inoculated samples were stored at: (i) 4C for 18 d, (ii) 12C for 9 d, or (iii) 21C for 1 d and the concentration of LM and EC was determined throughout shelf life. The trial was replicated four times using romaine from separate production lots.

We calculated the net increase or decrease, respectively, by subtracting the concentration on d 0 from the maximum or minimum concentration observed during the trial.

Results: Across all four trials, we observed an increase in LM concentration, which was occasionally followed by die-off. The net increase of LM on WS was greater, compared to that on DF, across all temperatures; for example, the mean ± standard deviation of the $\log_{10}$ net increase of LM strains on DF and WS, respectively, was: (i) 0.7 ± 0.3 and 2.0 ± 0.8 at 4C and (ii) 1.7 ± 0.3 and 3.1 ± 0.3 at 21C. At 21C, EC showed a $\log_{10}$ net increase of 2.2 ± 0.2 and 3.7 ± 0.8 on DF and WS leaves. Comparatively, at 12C, EC sometimes showed either a net increase or decrease, or an increase followed by a decrease. On both DF and WS at 4C, EC typically showed a net decrease to levels below the plating detection limit of 2.3 $\log_{10}$CFU/leaf.

Significance: Defects, such as watersoak, can enhance pathogen growth on romaine, highlighting the need to consider such defects when assessing produce safety risks.

T4-06: EFFECTIVENESS OF UV-C LIGHT AND HYDROGEN PEROXIDE IN A PILOT SCALE BRUSH BED FOR CONTROLLING LISTERIA INNOCUA AND GENERIC ESCHERICHIA COLI ON APPLES

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Fresh apples are widely consumed as ready-to-eat products, making their microbiological safety a critical concern for the food industry. UV-C light and hydrogen peroxide (H_2O_2) are two promising non-thermal technologies that can be applied within commercial packinghouse brush bed systems for microbial control.

Purpose: This project evaluated the efficacy of H_2O_2 and UV-C light (254 nm) in the reduction of *Listeria innocua* and generic *E. coli* on apple surfaces during pilot-scale brush-bed spray bar interventions.

Methods: Apples were either inoculated (6 log CFU/apple) with a three-strain cocktail of *Listeria innocua* or generic *E. coli*. Two trials were conducted using two dosages of UV-C light (150 and 300 J/m^2) each including 10 apples per treatment combination: (1) UV-C treatment followed by H_2O_2, (2) H_2O_2 treatment followed by UV-C exposure and (3) UV-C and H_2O_2 simultaneously. A pilot scale brush-bed moving at 18.5 hz, equipped with a 6 nozzle spray bar

delivering 3% H_2O_2 (w/v) at a total flow rate of 30 mL/s (1.8 L/min) and equipped with two UV-C lights (254 nm) was utilized. Bacterial populations were enumerated by spread plating and reductions were compared to non-treated samples.

Results: All treatments were effective at reducing both bacterial populations on apples, relative to controls (5.5–6.0 log CFU/apple). *L. innocua* populations were reduced by approximately 3.4–3.6 log CFU/apple across treatments, while *E. coli* reductions ranged from approximately 2.4–3.1 log CFU/apple, with many treated samples reaching the limit of detection (2.3 log CFU/apple). Across treatments, the 300 J/m^2 dosages generally produced greater log reductions than 150 J/m^2, with simultaneous H_2O_2 and UVC application yielding the largest reductions overall.

Significance: These findings support the implementation of multiple, complementary interventions to reduce microbial risk in commercial apple handling and processing environments.

Award Application: Developing Scientist Award

T4-07: APPLICATION OF GASEOUS OZONE AND NON-THERMAL PLASMA AS ECO-FRIENDLY ALTERNATIVES TO CHLORINE FOR ALFALFA SEED AND SPROUT DECONTAMINATION

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Multiple foodborne disease outbreaks have been linked to alfalfa sprouts contaminated with Shiga toxin-producing *Escherichia coli* (STEC), with seeds identified as the primary source of contamination.

Purpose: To evaluate the efficacy of aqueous ozone and non-thermal plasma for reducing STEC on alfalfa seeds and sprouts at different contamination levels and to assess their effects on germination and sprout quality.

Methods: Alfalfa seeds or sprouts were inoculated with STEC O157:H7 EDL 933 at high (10^6 CFU/g) or low (10^3 CFU/g) levels and incubated for 30 min at ~22°C, then dried for 1–2 h in a laminar flow cabinet. Treatments included (i) non-thermal plasma (NTP; dry or mist) and (ii) continuous aqueous ozone (5 ppm) applied for 2, 10 or 15 min. Calcium hypochlorite ($Ca(OCl)_2$) served as a control at 50 ppm for sprouts and 20,000 ppm for seeds for 2 or 15 min. STEC populations were determined and germination percentage, and sprout length, weight, and color were evaluated. Data were analyzed in GraphPad software.

Results: On seeds, ozone (5 ppm, 15 min) reduced STEC by 1.1 and 1.6 log CFU/g, at high and low inoculum levels, respectively, while mist NTP achieved 1.7 and 1.8 log CFU/g, respectively. $Ca(OCl)_2$ (20,000 ppm, 15 min) showed comparable efficacy ($p > 0.05$) at high inoculum, but more reduction ($p < 0.05$) at low levels. On sprouts, ozone reduced STEC by 1.3 and 2.9 log CFU/g, whereas mist NTP achieved 0.9 and 1.7 log CFU/g reductions at the high and low inocula, respectively. Short (2-min) ozone or NTP treatments at low level were less effective than $Ca(OCl)_2$ (50 ppm) ($p < 0.05$), while no significant difference was found at the high inoculum levels. No significant adverse effects ($p > 0.05$) on germination or sprouts quality were detected.

Significance: Ozone and NTP significantly reduced STEC on alfalfa seeds and sprouts without affecting quality, supporting their potential as residue-free alternatives to chlorine-based sanitizers.

Award Application: Developing Scientist Award

T4-08: LONGITUDINAL SURVEY OF SALMONELLA PREVALENCE IN CALIFORNIA'S CENTRAL COAST

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: *Salmonella* contamination of fresh fruits and vegetables can cause serious illness and death. Since the California central coast region is a major production area, it is important to understand the reservoirs and possible routes of *Salmonella* contamination of fresh produce within this region.

Purpose: To assess the *Salmonella* population recovered in the California Longitudinal Study in relation to diversity, clinical relevance, and resistance profiles.

Methods: Environmental samples, including water (tailwater/surface), sediment, livestock and wildlife feces, soil and biological soil amendments (BSA), were collected and analyzed for *Salmonella* using modified FDA-BAM methods. All isolates were subjected to whole genome sequencing and analysis using standard FDA methods.

Results: Of the samples tested, 5.4% (177/3274) were *Salmonella* positive with 606 isolates representing 41 serovars; Muenchen (n=297), Thompson (n=26), Oranienburg (n=23) and Infantis (n=22) were common. Most isolates were recovered from tailwater/sediment (n=263) followed by other water sources (n=149), feces (n=92), and soil/BSA (n=102). Numerous wildlife isolates came from coyote feces (56/82). Several isolates were within 30 SNPs of clinical isolates, warranting further study. Some isolates contained antimicrobial, biocide and heavy metal resistance genes. Four Infantis isolates appear to contain the large pESI plasmid associated with multidrug resistance.

Significance: Defining the diversity and reservoirs of *Salmonella* circulating in agricultural landscapes advances our understanding of pathogen ecology and transmission routes. This information permits growers to perform enhanced risk assessments and implement evidence-based mitigation strategies along with aiding outbreak investigations.

T4-09: SYSTEMIC TRANSFER AND POSTHARVEST PERSISTENCE OF ESCHERICHIA COLI O157:H7 IN NFT HYDROPONIC BIB LETTUCE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: NFT hydroponic systems are increasingly used for leafy green production, yet limited data exist on whether a single contaminated plant can disseminate enteric pathogens through the system and contaminate harvestable product.

Purpose: Quantify transfer, persistence, and postharvest fate of *Escherichia coli* O157:H7 and generic *E. coli* (GEC) in NFT-grown Bib lettuce.

Methods: Four NFT benches (24 plants each) were grown under controlled conditions (23.9°C, 14-h photoperiod, pH 6.5, EC 1.3). Three benches had one inoculated plant per trough (6 plants; ~4.2 log CFU/plant) with O157 and GEC; one was a control. At 0, 3, 6, 12, and 24 days, four plants per bench were sampled (leaves and rockwool), along with hydroponic solution and trough surfaces. O157 was detected using CHROMagar O157 + rifampicin and NEOGEN Molecular Detection Assay 2. Three trials were conducted, including one with

postharvest storage. Detection probabilities were estimated by binomial models (K = 5). In the postharvest trial, 96 plants were stored at 5°C for 21 days and analyzed by MPN/direct plating, with clamshells assessed by swab enrichment.

Results: Hydroponic solution was 100% positive at days 3–6, declined by day 12, and was negative by day 24. Rockwool showed persistent contamination (9/9 plants at days 3–12; 8/9 at day 24), indicating a stable root-zone reservoir. Leaf contamination was intermittent, with 5 of 12 plants positive at day 3 and 2 of 12 at day 12, irrespective of strain (O157 or GEC). In the postharvest study, 3 of 96 plants were GEC-positive after 7–21 days of storage, all originating from plants nearest the inoculated source. Using day-3 harvest data and binomial modeling, the probability that at least one of five neighboring plants in a six-plant trough would become contaminated was 90%.

Significance: Under the inoculation conditions log 4.2 CFU/plant, adjacent-plant contamination occurred supporting the need for validated preventive controls in NFT systems.

T4-10: PATHOGENIC SHIGA TOXIN-PRODUCING ESCHERICHIA COLI DISTRIBUTION AND TRANSMISSION PATHWAYS IN CALIFORNIA'S CENTRAL COASTAL GROWING REGION: A LONGITUDINAL ENVIRONMENTAL SURVEY

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: In response to repeated STEC O157:H7 outbreaks linked to romaine lettuce

grown in central coastal California, a 5-year longitudinal study was conducted throughout this region from 2020–2025 to assess environmental factors contributing to the persistence and spread of STEC within this agricultural area.

Methods: Samples (animal feces (domesticated and wild), water, air (passive and active methods), soil, and sediment) were collected from public and private sites, enriched, and STEC were detected and recovered using a modified FDA BAM method. All STEC isolates underwent whole genome sequencing to assess serotype, virulence gene prevalence, genetic relationships and spatial distribution.

Results: A diverse subset of STEC serotypes were isolated from 6,134 samples tested, including STEC O157:H7 and other top six human illness-causing serotypes. Most study isolates were either O26:H11 (n = 89) or O157:H7 (n = 93) and were recovered from every matrix type except air, with multiple instances of genetically related strains recovered at different sampling points and across different matrices. Prevalence of serotypes O103:H2 (n=10), O111:H8 (n=4), and O121:H19 (n=1) was lower and associated with animal feces (cattle, sheep, dove, deer, coyote and feral pig). Serotype O145:H28 (n=1) was recovered from sediment. In addition to Shiga toxin encoding genes, all contained the key virulence marker, intimin, encoded by *eae*.

Significance: From a One Health framework, the recovery of highly pathogenic STEC from multiple matrices, including domesticated and wild animals, with genetically related strains across different sample types and locations suggests active transmission pathways between environmental reservoirs and informs potential intervention strategies to reduce food safety risks in this California leafy greens growing region.

T4-11: PERSISTENCE OF SALMONELLA SPP. AND LISTERIA MONOCYTOGENES IN HYDROPONIC LETTUCE PRODUCTION BASED ON SOILLESS SUBSTRATE TYPE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Soilless substrates used in lettuce hydroponic systems may differentially support the persistence of human pathogens.

Purpose: This study evaluated the impact of soilless substrate type with or without plants on the persistence of *Salmonella* and *Listeria monocytogenes* in an indoor hydroponic lettuce production system over 14 days.

Methods: On day 0, rockwool (RW) and low-density foam (LDF) substrates in sub-irrigated nutrient solution were inoculated with *Salmonella* or *Listeria* cocktail at an initial concentration of ~6 log colony forming units (CFU) per milliliter. Treatments included planted and non-planted substrates in a factorial completely randomized design with three biological replicates. Samples were collected on days 0, 3, 5, 7,10, and 14. Samples were processed, and bacteria were enumerated on selective media. Analysis of covariance (ANCOVA) was used to assess the effect of day, planting status, substrate type, bacteria type, and their interactions, with nutrient solution temperature, pH, and electrical conductivity as covariates.

Results: Bacteria concentration ($\log_{10}$ CFU/substrate) reduced significantly over time (p<0.001). Substrate type had a substantial effect on pathogens, with higher concentrations in RW compared to LDF (p<0.001). *Listeria* and *Salmonella* also show distinct persistence

patterns (p<0.001). Temperature (p<0.001) and pH (p=0.027) influenced bacterial persistence, while planting status did not (p=0.30). Significant interactions ($p < 0.001$) were observed between day and bacteria type; substrate type, bacteria type, and day; and substrate type and bacteria type, indicating differential persistence dynamics across substrates over time under controlled indoor conditions for hydroponic lettuce production.

Significance: These findings provide insights into optimizing substrate selection to enhance food safety in hydroponic lettuce production.

T4-12: FOODBORNE PATHOGEN TRANSFER AND PERSISTENCE IN INTEGRATED CROP-LIVESTOCK SYSTEMS: SHEEP GRAZING IN ORGANIC NUT ORCHARDS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Sheep grazing in nut orchards can enhance sustainability and soil quality, however, microbial populations dynamics and associated food safety risks remain poorly characterized, as ruminants are known carriers of foodborne pathogens.

Purpose: To evaluate food safety risks and foodborne disease pathogen introduction and persistence in nut orchard environments following sheep grazing events.

Methods: A repeated cross-sectional study was conducted across six organic nut orchards in California's Sacramento and San Joaquin Valleys, comparing grazed plots to non-grazed controls. Thirty composite soil samples per site were collected prior to grazing, 7 days post-

grazing, and every 30 days until harvest time (7 visits/site; n=1,080 total). Sheep fecal samples were collected during grazing, and wildlife fecal samples were collected opportunistically. Leaf and nut samples were collected at 120 days post-grazing and at harvest. All samples were enriched and confirmed via PCR for the presence of *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* (STEC). Generic *E. coli* (gEc) in soil was quantified using Petrifilm™ plates and linear mixed models were used to compare grazed and ungrazed plots.

Results: The pathogen prevalence in sheep fecal samples was 35% (n=22/60) with STEC detected in 26%, *S. enterica* in 8% and *E. coli* O157:H7 in 1.5% of fecal samples. Wildlife fecal samples had a 25% pathogen prevalence rate (n=13/52). Pathogen detection in soil and leaf/nut samples was rare at 1.4% (n=15/1,080) and 1% (n=2/198), respectively. Grazing significantly increased gEc concentrations in soil from Day 7 to Day 60 post grazing ($p < 0.001$), peaking at Day 7 post-grazing (13-fold increase), and dissipating by Day 90.

Significance: While pathogens were detected in animal feces, evidence of transfer or persistence to orchard soils was limited. These findings inform management strategies to optimize crop-livestock integration while prioritizing food safety.

T5-01: ANTIFUNGAL ACTIVITY OF LACTIPLANTIBACILLUS PLANTARUM PMO 08 AND ITS POTENTIAL APPLICATION IN YOGURT

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Antifungal lactic acid bacteria have emerged as bioprotective cultures to control acidotolerant and psychrotolerant spoilage fungi in yogurt, providing an alternative to chemical preservatives.

Purpose: This study aimed to investigate the antifungal activity of *Lactiplantibacillus plantarum* PMO 08 and its potential application as bioprotective cultures in yogurt.

Methods: The antifungal activity of *L. plantarum* PMO 08 was evaluated by an agar spot assay against *Cladosporium piniponderosae, C. colombiae, C. oxysporum* and *Fusarium annulatum*, previously isolated from a yogurt production process. The antifungal activity of CFS produced by *L. plantarum* PMO 08 was assessed after heat (60–121°C), pH (4–7) and enzymatic (catalase, trypsin, and proteinase K) treatments. The metabolic profiles of the CFS were assessed by untargeted GC-TOF/MS analysis, and compounds were annotated with mass spectral matching (based on a match □700) by in-house library. Furthermore, yogurt

fermented with *L. plantarum* PMO08 (PMO 08 yogurt) was separately inoculated with each of four fungal species at two inoculation levels (0.5 or 1.0 log CFU/mL in yogurt), and fungal growth was monitored at 4°C for 50 days. Yogurt fermented without *L. plantarum* PMO 08 served as a control.

Results: *L. plantarum* PMO 08 inhibited the growth of all tested fungi, with the strongest inhibition against *F. annulatum*. The antifungal activity of CFS was stable after heat and enzymatic treatments but was reduced after pH adjustment to 5–7, suggesting that organic acids contribute to the antifungal activity. A total of 89 metabolites were annotated including eight organic acids. In PMO 08 yogurt, fungal counts of three *Cladosporium* species remained under 1.0 log CFU/mL over 50 days. For *F. annulatum*, final counts were 1.7 ± 0.2 log CFU/mL lower than those of the control.

Significance: *L. plantarum* PMO 08 shows high potential as bioprotective cultures for yogurt through the production of antifungal metabolites.

T5-02: SPECIES-LEVEL CHARACTERIZATION OF THE SPOILAGE-ASSOCIATED MICROBIOTA IN RETAIL GROUND BEEF

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Case-ready ground beef packaged in overwrapped trays is commonly distributed to retail stores in modified atmosphere, yet the species-level composition of its spoilage microbiota remains poorly characterized.

Purpose: This study aimed to characterize the spoilage-associated microbiota in ground beef in overwrapped trays using both culture-based and sequencing methods.

Methods: Fifty-four packages of ground beef in overwrapped trays, with fat content ranging from 4% to 27%, were purchased from six retail stores (A–F) in Fort Collins, Colorado. Packages were stored in a retail display case at 3°C until four days past the labeled expiration date. Total aerobic bacteria were enumerated by conventional plate counting methods. The spoilage microbiota was characterized at the species level using full-length 16S rRNA gene amplicon sequencing (Nanopore technology). Differences in total bacterial load (by store and fat content) were assessed using ANOVA, while differences in bacterial community composition were analyzed using PERMANOVA; a *P*-value of < 0.05 was considered significant.

Results: The total aerobic bacteria ranged between 5.4 and 8.6 log CFU/g with a median value of 6.9 log CFU/g and did not vary ($P > 0.05$) among retail stores. However, the bacterial composition differed at the species level, with *Carnobacterium divergens* showing the highest relative abundance in store A (22.4%), *Pseudomonas fragi* in store B (26.1%), *Pseudolactococcus piscium* in store C (26.7%) and F (31.9%), *Leuconostoc gelidum* in store D (40.3%), and *Latilactobacillus sakei* in store E (30.6%). Ground beef fat content did not ($P > 0.05$) have an effect on bacterial counts or microbiota composition.

Significance: This study improves our understanding of the bacterial composition in ground beef at the species level, which will inform more effective strategies to improve the shelf life of beef products.

T5-03: GAINING INSIGHTS INTO EDIBLE MUSHROOMS' SPOILAGE KINETICS BASED ON MICROBIAL POPULATION DYNAMICS: ROLE OF INITIAL MICROBIOTA AND SURFACE STRUCTURAL DIFFERENCES IN WHITE, CREMINI, OYSTER, AND SHIITAKE MUSHROOMS

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Structural and biological heterogeneity create niches that shape microbial colonization and evolution during spoilage. The role of these structure-driven differences in determining microbial dynamics across mushroom types remains poorly understood.

Purpose: To determine how structural heterogeneity in white-WH, cremini-CR, oyster-OY, and shiitake-SH mushrooms influences microbial colonization and dynamics during storage.

Methods: Samples were stored at 4 and 12°C until unsuitable for consumption. Samples were homogenized, plated on tryptic soy agar, and incubated prior to extracting the DNA

from collected colonies. Mushrooms were sourced from 2 growing rooms (biological replicates) and plated in duplicate. Microbial communities were characterized using 16S rRNA high-throughput sequencing (Illumina HiSeq 2500). Microbial diversity and community composition were evaluated using Shannon and Simpson indices, and UniFrac-based PCoA, respectively. Relative abundance bar plots illustrated phylum and genus-level differences.

Results: Mushroom type was the primary driver of microbial community composition, with greater influence than storage temperature or biological replication. WH and CR clustered distinctly from OY and SH, further indicating structure-driven differences. Spoilage communities were dominated by Proteobacteria, Firmicutes, and Actinobacteria, with *Pseudomonas,* Enterobacteriaceae*, Serratia, Bacillus*, and *Glutamicibacter* prevalent across samples. Initial microbial loads and early taxonomic dominance strongly influenced microbial dynamics, with shifts in genus-level occurring between mushroom type and biological replicates. Storage temperature and mushroom-specific characteristics modulated these dynamics, generating divergent spoilage profiles.

Significance: Mushroom structural characteristics play significant role in microbial colonization and growth, driving distinct spoilage dynamics and enabling targeted strategies to improve shelf life.

Award Application: Developing Scientist Award

T5-04: ACIDIC TREATMENTS FOR INACTIVATION OF O157 AND NON-O157 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI BIOFILMS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: As high as 80% of bacterial infections in humans can be linked to microbial biofilms. Shiga toxin-producing *Escherichia coli* are important foodborne pathogens of public health concern associated with an array of liquid and solid food products and are capable of forming bacterial biofilm on various biotic and abiotic surfaces.

Purpose: Current study investigated the impact of malic and citric acids for the inactivation of wild-type and rifampicin-resistant Shiga toxin-producing *Escherichia coli*. Seven serogroups of Shiga toxin-producing *Escherichia coli* i.e., O157, O26, O45, O103, O111, O121, and O145 were studied.

Methods: The biofilm studies were randomized complete block designs for 0 (planktonic cells) to 14 days. Data were statistically analyzed using Tukey-adjusted mean separation at the type 1 error level of 5%. Planktonic and sessile cells of the wild-type and rifampicin-resistant pathogen were grown on stainless steel coupons (Finish 2B), half-submerged in 3% milk to maximize the biofilm formation in the air-liquid interface.

Results: Results show that both tested antimicrobials could lead to up to >99.9% reduction (*P*

<0.05) of the pathogen when tested against planktonic cells and early stages of biofilm. While the same treatment was less effective for the elimination of one- and two-week mature biofilms. The O157 and non-O157 serogroups exhibited similar (P □ 0.05) sensitivity to the antimicrobials

Significance: The treatments were equally effective ($P > 0.05$) against the wild-type and rifampicin-resistant phenotypes. Thus, results of future public health microbiology validation studies against wild-type pathogen could most probably be used interchangeably for prevention/treatment of rifampicin-resistant phenotypes as well.

Award Application: Developing Scientist Award

T5-05: ULTRAVIOLET INACTIVATION (UV-C) OF BACTERIAL AND VIRUS AEROSOLS IN AIR

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: In food processing facilities, airborne bacterial and viral aerosols can serve as vectors for microbial cross-contamination, settling on food contact surfaces, equipment, and products leading to spoilage and contamination of food.

Purpose: This study evaluates the effectiveness of UV-C treatment a non-chemical approach for inactivating bacterial and viral aerosols in air.

Methods: Model microorganisms included food-relevant bacteria (*Staphylococcus aureus*, *Pseudomonas fragi*, *Listeria monocytogenes*) and bacteriophages (Qβ and MS2) as viral surrogates. Aerosols were generated using a 4-jet Blaustein Atomizing Module atomizer. Bacterial aerosols were exposed to UV-C in a 2-lamp orientation, while bacteriophages used a 4-lamp orientation with low-pressure mercury lamps mounted outside of quartz sleeves. Aerosols passed through the sleeve, with UV dose controlled by airflow-modulated residence time. Microbes were collected via impinger in peptone water or CM buffer, enumerated by standard plating for bacteria and spot plate assay using *Escherichia coli* hosts for bacteriophages.

Results: Following aerosolization, the recovered concentration of the different microorganism were *Staphylococcus aureus* 4.05 ± 0.16 log CFU·mL^{-1}, *Pseudomonas fragi* 3.91 ± 0.04 log CFU·mL^{-1}, *Listeria monocytogenes* 5.90 ± 0.22 log CFU·mL^{-1}, MS2 7.27 ± 0.07 log PFU·mL^{-1} and Qβ 7.27 log PFU·mL^{-1}. UV-C treatment achieved complete inactivation of bacterial aerosols (no countable colonies in any samples). For bacteriophages, MS2 showed a maximum log reduction of 3.84 ± 0.04 log PFU·mL^{-1} (delivered fluence 77.05 mJ/cm^2), and Qβ 4.37 log PFU·mL^{-1} (45.9 mJ/cm^2). These outcomes demonstrate the system's robust capacity to eliminate airborne pathogens.

Significance: This study addresses critical needs in food safety by validating scalable UV-C air disinfection to reduce bacterial and viral aerosols in processing environments, preventing cross-contamination and minimize risks of foodborne outbreaks.

Award Application: Developing Scientist Award

T5-06: POSTBIOTICS IN AERATED WATER REDUCE SALMONELLA BIOFILMS IN DAIRY PROCESSING ENVIRONMENTS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: *Salmonella* is a major cause of foodborne illness and has been linked to dairy product contamination. Biofilms on food contact surfaces can act as reservoirs that increase food safety risk.

Purpose: This study evaluated postbiotics delivered in aerated nanobubble water to reduce *Salmonella* persistence in dairy processing environments.

Methods: Two *Salmonella* Enteritidis isolates (SE 12 and SE 21) were used to test treatments against planktonic populations, surface adhesion, and established biofilms on stainless steel and polystyrene. Aerated water was generated using nanobubble technology (30 mg/L dissolved oxygen). Postbiotics from *Lactococcus lactis* NRRL-B-663 and *Lactobacillus johnsonii* NRRL-B-2178 were applied at 40% v/v. Treatments were postbiotics in aerated water or tap water; 200 ppm chlorine was a control. Assays were run with and without 10% UHT milk; survivors were enumerated by serial dilution. Experiments were randomized, two independent trials were conducted with three replicates per treatment and analyzed in R ($p < 0.05$).

Results: Aerated water alone reduced *Salmonella* planktonic populations compared with the untreated control. With aerated water, postbiotics reduced *Salmonella* adhesion on stainless

steel and polystyrene surfaces by 0.5 to 1.0 log CFU. In established biofilms, the combination reduced *Salmonella* by 1.3 to 2.2 log CFU compared with aerated water alone and 200 ppm chlorine. Similar effects were observed for both isolates and in the presence of 10% UHT milk.

Significance: *Salmonella* biofilms increase cross-contamination risk in dairy processing environments. Postbiotics delivered in aerated nanobubble water increased antimicrobial and anti-biofilm activity and may support food safety-focused sanitation strategies.

Award Application: Developing Scientist Award

T6-01: ENHANCING MICROBIAL SAFETY OF LOW-MOISTURE FOODS: ACTIVE PACKAGING APPROACH TO MITIGATE LISTERIA AND SALMONELLA ON PECANS DURING STORAGE

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: Low-moisture foods (LMFs) such as nuts, though shelf stable, remain vulnerable to post-process contamination by *Listeria monocytogenes* and *Salmonella enterica* necessitating the need for post-processing interventions.

Purpose: This study aimed to enhance the microbial safety of LMFs through antimicrobial bionanocomposite (BNC) films integrated into active packaging.

Methods: Four nanocellulose-based BNC film formulations incorporating antimicrobials: 0.33% (w/v) nisin (BNC-N), 0.1% (v/v) oregano essential oil (BNC-O), a combination of nisin + oregano essential oil (BNC-ON), and a control film without antimicrobials (BNC-C) were evaluated for antimicrobial activity using an *in situ* challenge study on pecans. Pecan halves (a_w = 0.59) were inoculated (6.38 log CFU/g) with cocktails of *Listeria* or *Salmonella* and air-dried at 20°C for 48 h. Inoculated pecans (50 g) were packaged with individual BNC films and stored at 20°C and 55% RH with periodic sampling for microbial enumeration until it reached LOD (1.70 log CFU/g). Experiments were performed in triplicate and analyzed by ANOVA with Tukey's test ($p < 0.05$).

Results: Treatment and storage time significantly influenced pathogen survival ($p<0.05$), with rapid early and slower later reductions. *Listeria* declined to the LOD by day 60, whereas

Salmonella persisted through day 120, with control samples remaining at 4.20 log CFU/g. Among treatments, BNC-ON consistently resulted in the lowest (p<0.05) pathogen survival. By day 120, *Salmonella* populations in BNC-ON (3.57 log CFU/g) were significantly lower (p<0.05) than those in BNC-C, BNC-N, and BNC-O by ~ 0.63, 0.32, and 0.27 log CFU/g, respectively. Similarly, by day 60, *Listeria* populations in BNC-ON, BNC-O, and BNC-N were below detectable levels, whereas BNC-C samples remained at 1.86 log CFU/g.

Significance: Antimicrobial active packaging tested on pecans shows potential as a clean-label, sustainable approach for controlling post-process contamination in LMFs.

Award Application: Developing Scientist Award

T6-02: DIGITAL NORMALIZATION OF RAW MILK CONSUMPTION: A DATA-DRIVEN ANALYSIS OF FOOD SAFETY RISK DISCOURSE ON YOUTUBE

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Presentation type/Format: Technical

Category: Data Management and Analytics

Abstract:

Introduction: Consumption of raw milk presents a well-established food safety risk; however, online media increasingly portrays this practice as routine, beneficial, or low risk, potentially undermining public health guidance.

Purpose: To examine how digital exposure and social reinforcement within YouTube discourse contribute to the normalization and acceptance of raw milk consumption.

Methods: YouTube data on raw milk were analyzed (1186 videos; 78432 comments). Videos were collected using predefined YouTube Data API queries and limited to peer-generated content, excluding regulatory and public health channels. Exposure was measured by views and posting frequency, credibility by engagement and channel features. Comments were analyzed with NLP (topic modeling, stance/sentiment) to quantify normalization, resistance, and risk-minimization narratives. Structural models tested links among exposure, credibility, normalization, and risk-acceptance framing.

Results: Topic modeling identified dominant narratives emphasizing perceived health benefits, naturalness, and distrust of pasteurization. Comments expressing normalization or minimization of food safety risk made up 62% of classified comments, compared with 21% expressing explicit safety concerns and 17% neutral or ambiguous discourse. Videos in the highest exposure quartile showed a significantly greater proportion of normalization

language than lower-exposure videos ($p<0.05$). Engagement-based credibility cues were positively associated with normalization signals, and mediation analysis showed that social normalization partially mediated the relationship between exposure intensity and risk-acceptance framing.

Significance: These findings suggest that YouTube can normalize unsafe food practices through repeated exposure and peer reinforcement. Incorporating digital discourse analysis into food safety surveillance may enable earlier detection of emerging risk narratives and support more targeted risk communication.

T6-03: LETTUCE KNOW: ASSESSING WILLINGNESS TO PAY FOR IRRADIATED PRODUCE

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Irradiation is a tool used to destroy insects and microorganisms in food to promote food safety. It is not widely used on commercially available food in the United States in part because of limited consumer awareness and acceptance.

Purpose: The objective of this study was to compare factors that are important to consumers when purchasing produce, including irradiation for food safety.

Methods: We conducted a discrete choice experiment via survey at the 2025 Minnesota State Fair among adults aged >18 years. Participants were asked in six separate scenarios to choose between two bags of prepackaged lettuce with varying attributes: cost ($3.00, $4.50, or $6.00), shelf life (best before date 4, 7, or 10 days away), locally grown (or not locally grown), organically grown (or conventionally grown), and irradiated to destroy harmful bacteria (or thoroughly washed and ready to enjoy). A mixed logit regression model was fit to evaluate preferences and marginal willingness to pay was calculated from model estimates. Participants were asked additional questions regarding awareness of irradiation and willingness to purchase irradiated foods.

Results: Overall, 1,173 fair goers completed at least one discrete choice set. Increasing cost and irradiation were associated with lower odds of purchase and locally grown, organically

grown, and shelf life were associated with increased odds of purchase. The odds of purchase were 28% lower for irradiated compared to thoroughly washed products (OR=0.72; CI: 0.68, 0.75). On average, participants were willing to pay $0.60 less for irradiated compared to thoroughly washed lettuce. Despite this, 945 (81.0%) of participants reported being somewhat or very likely to purchase irradiated leafy greens.

Significance: Although the majority of participants reported a willingness to purchase irradiated leafy greens, they would require a significant discount to compensate for their overwhelming preference for thoroughly washed lettuce.

Award Application: Developing Scientist Award

T6-04: INTEGRATING CLIMATE INDICATORS INTO FOODBORNE DISEASE SURVEILLANCE USING ROUTINELY AVAILABLE DATA IN LOW-RESOURCE SETTINGS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Climate extremes can disrupt water, sanitation, and food systems, yet routine foodborne/enteric disease surveillance rarely integrate climate indicators in low-resource settings.

Purpose: Demonstrate a reproducible framework for linking gridded precipitation indicators to population-based diarrhea surveillance data and to quantify associations between precipitation extremes and pediatric diarrhea in Ethiopia.

Methods: Georeferenced Ethiopian Demographic and Health Survey clusters were linked to gridded precipitation surfaces (CHIRPS) to derive monthly precipitation anomalies and indicators of drought and wet extremes. Survey-weighted logistic regression models estimated associations with childhood diarrhea, adjusting for child, household, and geographic characteristics (age, sex, urbanicity, household income, etc.). Both same-month and one-month lagged climate exposures were assessed.

Results: Monthly precipitation indicators were linked to 20,734 of 48,782 child observations (42.5%). In weighted analyses (n=17,974), diarrhea prevalence was 21.6% in non-drought months versus 18.7% in drought months, and 19.3% in non-wet-extreme months versus 24.7% in wet-extreme months. In adjusted models (n=8,709), neither drought (OR 0.78; 95%CI:0.56–1.08) nor wet extremes (OR 0.94; 95%CI:0.76–1.17) were associated with increased odds of diarrhea. Child age (OR 0.98 per month; 95%CI:0.98–0.98) and household income (lowest vs highest quintile: OR 1.45; 95%CI:1.07–1.96) were strongly associated. Lagged analyses showed no significant associations for drought (OR 0.53, 95%CI:0.22–1.27) or wet extremes (OR 0.78, 95%CI:0.38–1.60).

Significance: Climate-linked, surveillance-ready indicators using widely available datasets can be adapted to other hazards and pathogen-specific outcomes to support climate-informed enteric disease monitoring in low-resource settings. Differences from historically observed wet-season patterns may reflect increasing rainfall variability in Ethiopia.

T6-05: BEYOND PREVALENCE: A RISK-BASED FRAMEWORK FOR SELECTING SALMONELLA SEROVAR TARGETS IN POULTRY

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Targets for *Salmonella* adulterant microbial criteria – such as those recently proposed by USDA-FSIS on poultry – should be based on risk integrating both contribution to foodborne illnesses and frequency in foods, rather than separately as currently done.

Purpose: To use a risk-based quantitative framework to propose highest risk (HR) *Salmonella* serovar targets in chicken and turkey.

Methods: We estimated serovar-specific proportions of contaminated poultry product lots (FSIS regulatory sampling, 2016–2021) and attributed outbreak illnesses (National Outbreak Reporting System, 2016–2021) using Bayesian hierarchical models, then computed a risk multiplier (RM) as the ratio of illnesses to frequency in product. Serovars were ranked by RM using the Surface Under the Cumulative Rank Curve (SUCRA), where RM>1 indicates above average risk and SUCRA=1 indicates full certainty of highest RM. Three serovars with the highest RM and SUCRA values were designated as HR and compared with the three FSIS-

proposed targets (FSIS-T).

Results: HR chicken serovars were the same as FSIS-T, *S.* I 4,[5],12:i:-(RM = 13.4; 95% CrI:9.64–19.14; SUCRA=1), Enteritidis (2.0;1.82–2.18;0.63), and Typhimurium (1.0;0.79–1.21;0.17). The trio accounted for 64.9%(854/1,315) of attributed illnesses while contaminating 2.7%(3,666/134,671) of sampled lots. HR turkey serovars were *S.* Enteritidis(32.2;16.8–69.8;1), Newport(3.5;1.6–7.5;0.83), and I 4,[5],12:i:-(2.0;1.3–3.2;0.67) while FSIS-T *S.* Typhimurium and Muenchen had RM=0 (no attributed illnesses), and *S.* Hadar had a RM=1.5(1.1–2.1;0.09). Contamination of sampled turkey lots was 0.84%(70/8,345) for HR serovars vs 3.1%(262) for FSIS-T, and contribution to attributed illnesses was 48.8%(166/339) for HR serovars but only 15.6%(53) for FSIS-T.

Significance: Proposed HR targets in chicken align with the FSIS-T, while HR serovars in turkey would result in >70% less lots being considered adulterated and greater public health gains.

T6-06: FOOD SOURCE ATTRIBUTION METHODS FOR CAMPYLOBACTERIOSIS: A SCOPING REVIEW

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: *Campylobacter* is a leading cause of bacterial foodborne illness globally and efforts to quantify the relative roles of various food, water, animal, and environmental sources often point in different directions, impeding prioritization of risk mitigation strategies.

Purpose: The Interagency Food Safety Analytics Collaboration (IFSAC) aims to summarize the available recent literature on methods and quantitative estimates of *Campylobacter* food source attribution.

Methods: This scoping review applied a search strategy across multiple databases to identify relevant peer-reviewed studies, primarily in English, published from 1/1/2010 through 4/1/2024. After de-duplication, citation abstracts were screened against inclusion (e.g., “includes foodborne exposures”) and exclusion criteria (e.g., “does not include *Campylobacter*”) by two independent reviewers; subsequent full text review involved more

stringent criteria. Meta-data such as the type, years, and geographic regions of studies were collected during both phases, and additional data on quantitative estimates was extracted.

Results: Literature search identified 3270 de-duplicated studies, abstract review excluded 2961 articles, and full text review excluded a further 309, resulting in 131 articles with quantitative *Campylobacter* source attribution estimates. The most common attribution methods identified were microbial subtype modeling (42 studies), case-control studies (some incorporating subtype modeling; 25), analyses of outbreak data (21), and expert elicitations (15). The most common regions reflected in the literature included Europe (60), North America (28), and Australia/New Zealand (17).

Significance: Information from this scoping review will help contextualize IFSAC *Campylobacter* attribution estimates, identify alternative estimates that might be used by IFSAC, and inform future analytical efforts to identify sources of non-outbreak-associated campylobacteriosis.

T6-07: ADJACENT LAND-USE GENERALLY NOT ASSOCIATED WITH NON-O157 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IN SURFACE WATER AND SEDIMENT FROM CALIFORNIA CENTRAL COAST AGRICULTURAL REGION

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Environmental persistence of Shiga toxin-producing *Escherichia coli* (STEC) in the California central coast agricultural region may pose a risk to produce safety.

Purpose: To evaluate the relationship between adjacent land use and non-O157 STEC presence in surface water and sediment in this region.

Methods: Samples of river water, agricultural tailwater, and associated sediment were collected from August 2020 to April 2025 from 51 sites along public roads and analyzed for non-O157 STEC using FDA BAM methods. For each site, linear distance to rangeland and riparian zones, along with adjacent land-use composition within a 460 m radius of each site was quantified for seven categories (reservoirs, surface water, cropland, developed land, riparian, wildland, rangeland).

Results: Cluster analysis grouped adjacent land-use into four clusters: (1) primarily cropland, with small amounts of riparian and developed land; (2) primarily cropland, with small amounts of developed land; (3) rangeland dominant, with a mixture of land-use categories except open water, and (4) riparian dominant, with a mixture of land-use categories except wild and rangeland. For all sites, prevalence for non-O157 STEC and STEC O157 was 3.5% (48/1368) and 0.5% (7/1368), respectively. There were 860 sediment and 508 water samples. Distance to the edge of rangeland or riparian zones was not significantly associated with non-O157 STEC occurrence when adjusted for repeated sampling of sites. Although sites with both rangeland and riparian zones within 900 m exhibited a higher prevalence of non-O157 STEC, none of the land-use composition clusters had a significantly different non-O157 STEC prevalence from each other.

Significance: The risk of non-O157 STEC contamination of surface water and sediment was not readily associated with adjacent land use composition but may be related to the joint proximity to rangeland and riparian-dominated sites for this region.

T6-08: RETROSPECTIVE NEAREST NEIGHBOR CASE-CONTROL STUDY TO ATTRIBUTE SPORADIC CAMPYLOBACTERIOSIS IN THE UNITED STATES TO SPECIFIC FOOD AND NON-FOOD SOURCES

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: While *Campylobacter* is the leading bacterial cause of diarrheal illness in the United States, determining the contribution of different sources to campylobacteriosis remains challenging and primarily relies on foodborne illness outbreak data. However, outbreak-associated cases account for <0.5% of campylobacteriosis infections.

Purpose: This study was developed by the Interagency Food Safety Analytics Collaboration to

determine if a novel analytic approach, nearest neighbors case-control (NNCC), using FoodNet's surveillance (17,633 cases; 2016–2018) and Population Survey (38,743 controls) data, could be used to retrospectively attribute sporadic campylobacteriosis to any of 56 food and non-food exposures.

Methods: Multiple imputation of missing exposure data was performed. For each imputed dataset, NNCC was implemented, and Bayesian regression used to quantify odds ratios and population attributable fractions.

Results: Consuming chicken, beef, eggs, fish, and raw/undercooked beef, as well as contact with live poultry and untreated surface water had the highest population risks. High individual risk was associated with consuming liver pate, and raw seafood, and contact with ill animals. Except for watermelon, produce consumption was not associated with increased population or individual risk. These results were generally consistent with past studies. Unlike outbreak data, sporadic case data may untangle individual and population risk when analyzed with appropriate controls.

Significance: This study demonstrates the value of using exposure data collected during routine active surveillance in a NNCC-framework to attribute sporadic illness to food and non-food sources. Estimates of individual and population risk generated using these methods could be used to guide public health and regulatory resources.

T6-09: A NOVEL PATHOGEN COMMODITY PAIR: INVESTIGATION OF A MULTISTATE OUTBREAK OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O121:H19 INFECTIONS LINKED TO ORGANIC WHOLE AND BABY CARROTS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: In 2024, FDA, CDC, and state/local partners investigated an STEC O121:H19 outbreak linked to organic whole and baby carrots, resulting in 48 illnesses, 20 hospitalizations, one case of HUS, and one death.

Purpose: To review investigational efforts to identify the source of illnesses and potential contributing factors that led to contamination.

Methods: A case was defined as a laboratory-confirmed infection of STEC O121:H19 yielding an isolate related by WGS within 0–9 alleles with isolation dates from 9/18/2024–11/13/2024. States and CDC interviewed ill people with standard questionnaires. FDA conducted traceback analysis, a Farm Inspection at Farm A, and a Preventive Controls Inspection at Manufacturer B. Environmental and product sampling was conducted following guidance from the FDA BAM.

Results: Of the 37 people interviewed, 33 (89%) reported eating carrots prior to illness. Of those who remembered further details, 14/17 reported consuming organic carrots. FDA and state partners conducted traceback investigations for organic carrots associated with illnesses. All cases traced consumed bagged whole or baby carrots sourced from one distributor supplied by either Farm A or Manufacturer B; where comingling occurred. FDA conducted a Farm Inspection at Farm A, noting cleaning and sanitation issues; a non-outbreak strain of *E. coli* was also detected. A Preventive Controls inspection conducted at Manufacturer B noted several issues including an unidentified hazard requiring a preventive control. The distributor recalled potentially contaminated organic whole and baby carrots, and several downstream recalls were issued.

Significance: This is the first reported outbreak of STEC O121:H19 infections associated with carrots and underscores how comingling of product can complicate traceback investigations. The investigation also demonstrates how coordination between state and local partners can significantly accelerate identifying a specific brand or product type.

T6-10: RECENT SURVEILLANCE AND BURDEN TRENDS RELATED TO SALMONELLA AND POULTRY IN CANADA

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Salmonellosis is a leading cause of foodborne illnesses, hospitalizations and deaths in Canada. A key transmission route for domestically acquired salmonellosis is through poultry consumption.

Purpose: To use integrated surveillance data and burden of illness research to describe the surveillance and burden trends related to *Salmonella* and poultry in Canada to inform government and industry interventions.

Methods: Surveillance data from FoodNet Canada and the National Enteric Surveillance Program were analysed to determine trends of domestically acquired salmonellosis and *Salmonella* Enteritidis (SE) specifically. Burden of illness multipliers were applied to estimate the associated illnesses, hospitalizations and economic costs of domestically acquired, poultry related salmonellosis in Canada.

Results: *Salmonella* incidence has been consistently high from 2010–2018 with SE being the predominate serovar. Decreases were seen from 2019–2021 for all *Salmonella* and SE as a result of collective food safety efforts from industry and government and in part the COVID-19 pandemic. As of 2022, rates of both *Salmonella* and SE have been trending up to pre-pandemic levels, in both domestic and international travel related cases, with poultry being a key driver of the domestic illness. FoodNet Canada retail surveillance data shows that SE remains a key serovar throughout the chicken processing continuum. The estimated costs related to domestically acquired poultry associated salmonellosis is $7.35 million CAD/year.

Significance: Surveillance and burden trends indicate that domestically acquired, poultry associated salmonellosis remains a food safety and public health concern. Continued diligence and increasing collaborative efforts to mitigate the risks of illness and decrease the associated burden and economic costs are needed.

T6-11: INCLUDING SEAFOOD EXPOSURES IMPROVES DIAGNOSTIC PERFORMANCE OF VIBRIOSIS CULTURE-INDEPENDENT DIAGNOSTIC TESTS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Diagnostic capability for vibriosis has been expanded by introduction of culture-independent diagnostic tests (CIDTs); however, false positive results have been reported.

Purpose: To better understand the validity of vibriosis CIDTs, this analysis aimed to identify correlates of false positivity and explore the impact of relevant exposure criteria on positive predictive value (PPV).

Methods: Epidemiological data were analyzed for non-*V. cholerae* CIDT-positive vibriosis cases reported to CDC with a gastrointestinal sample source and a valid culture result. PPV was calculated overall and by reported seafood consumption. To explore associations with false positivity, bivariate logistic regression was conducted with demographic, clinical, and exposure variables (significance $P<0.05$).

Results: From 2017–2024, 5,324 CIDT-positive cases were reported meeting these criteria,

1,845 of which were culture-positive (PPV=35%). Restricting analyses to only patients who ate any seafood, molluscan bivalves, or raw bivalves increased PPV to 49%, 65% and 72%, respectively. In bivariate comparisons, a false-positive CIDT result was more likely if patients were female (OR=1.7) or located in Gulf Coast or noncoastal states (ORs=1.3, 1.7), and less likely in patients aged 30–59 (OR=0.53) or located in Pacific states (OR=0.35). Patients who had underlying health conditions, were hospitalized, or died were more likely to be false positive (ORs=1.7, 1.6, 5.2). Odds of false positivity were significantly lower if patients consumed any seafood, any bivalves, or raw bivalves (ORs=0.12, 0.11, 0.09).

Significance: Inclusion of relevant seafood exposures improved the predictive value of vibriosis CIDT results to a level comparable with CIDTs for other pathogens, and patient consumption of seafood was associated with approximately 90% lower odds of false positivity. Including consumption of seafood in CIDT diagnostic criteria could improve accuracy and specificity of CIDTs for vibriosis.

T6-12: FOODBORNE CRYPTOSPORIDIOSIS – A LITERATURE REVIEW ON SUSPECTED FOOD VEHICLES AND INFLUENCING FACTORS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Increasing numbers of cryptosporidiosis cases in Germany in recent years and worldwide reports on foodborne cryptosporidiosis outbreaks trigger the demand to enhance knowledge on food exposure pathways and drivers.

Purpose: In order to inform food safety risk managers, reports on foodborne cryptosporidiosis outbreaks were analysed for information on causative foods and influencing factors.

Methods: A Web of Science systematic literature search was performed in December 2024. Two search strategies were combined: 'TI=(*Cryptosporidium*) AND AB=(food)' (208 results) and 'TI=(Cryptosporidi*) AND (TI=(case*) OR TI=(outbreak*))' (429 results). The research was complemented by observations systematically collected during early warning and crisis prevention activities to include recent outbreaks.

Results: We analyzed 56 foodborne cryptosporidiosis outbreaks that have been documented from 1983 to 2025. Overall, the outbreaks resulted in 3,303 cases, with an average of 59

cases per outbreak. The outbreaks were primarily caused by the species *Cryptosporidium parvum*. Epidemiological investigations could link most of the outbreaks to a food vehicle, although *Cryptosporidium* spp. could rarely be isolated from the suspected source. Causative food vehicles were lettuce, salads, vegetables, fruit juices, raw milk and curd cheese. Various influencing factors such as primary contamination or recontamination in combination with hygiene deficiencies and/or processing errors have been identified for foods of animal origin, whereas factors for plant foods were irrigation or washing with contaminated water and insufficient cleaning of the harvest.

Significance: Providing food safety managers with information on where to put control measures and inform food control services on foods to be tested for *Cryptosporidium* spp. can possibly help to avoid future outbreaks and lower the cryptosporidiosis disease burden.

T7-01: VALIDATION OF THE NEOGEN® MOLECULAR DETECTION ASSAY 2 FOR THE DETECTION OF SALMONELLA, E. COLI O157, SHIGA TOXIN-PRODUCING ESCHERICHIA COLI FROM RAW BEEF AND RAW PORK SAMPLING CLOTHS, AND RAW CHICKEN PARTS AND RAW TURKEY CARCASS SAMPLING MITTS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: The Neogen Molecular Detection Assays 2 is used with the Molecular Detection System to detect microbial organisms. The assay uses loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification.

Purpose: A matrix extension to the AOAC Official Methods of Analysis℠ (OMA) of the Molecular Detection Assay 2 (MDA2)- *Salmonella* and *E. coli* O157 (including H7) and *Performance Tested Methods*℠ (PTM) of the MDA2- STEC Gene Screen (*stx* and *eae*) to include raw beef and raw pork sampling cloths, and raw chicken parts and raw turkey carcass sampling mitts.

Methods: The validation matrix extension study consisted of 30 samples at 3 levels (5 uninoculated control, 20 at fractional positive, and 5 high) in a method comparison study of

the MDA2- *Salmonella*, *E. coli* O157 (including H7), and STEC (*stx* and *eae*) against the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG) reference methods for raw beef and raw pork sampling cloths (375 g test portions), raw chicken parts sampling mitts (4 lb test portions), and raw turkey carcasses sampling mitts (one turkey carcass per test portion).

Results: Uninoculated samples were negative, the high samples were all positive, and fractions were achieved for all matrices. The matrix study showed no significant difference in performance between the Molecular Detection Assay 2 and the MLG reference methods according to the AOAC Probability of Detection (POD) statistical model.

Significance: The MDA2 is an accurate method for detecting *Salmonella*, *E. coli* O157, and Shiga toxin-producing *E. coli* across all matrices evaluated. The real-time results enable analysts and food manufacturing personnel to respond promptly to presumptive positive pathogen detection, allowing them to identify potential adulteration and implement corrective action in a timely manner.

T7-02: COMPARISON OF CAMPYLOBACTER DIVERSITY FROM USDA-FSIS COLLECTED POULTRY PRODUCTS USING TWO ENRICHMENT METHODS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: In 2021 FSIS began using a new *Campylobacter* enrichment medium to analyze poultry samples, so it is important to evaluate differences in genetic diversity among *Campylobacter* populations isolated via the former and current enrichment method.

Purpose: Identifying changes in diversity that coincide with a method change will provide evidence that method change is a possible confounding factor for trend analyses.

Methods: Sequences of *Campylobacter* (isolated from 2018–2025) obtained from FSIS collected poultry samples were analyzed to determine *Campylobacter* species distribution, assess the frequency of Sequence Types (STs), and rank of ST. The Shannon Diversity Index (SDI) was computed on STs to evaluate diversity of populations from the two enrichment methods. Additionally, the top 10 STs for each enrichment method were compared using Spearman's rank correlation.

Results: We evaluated similar time periods, 2018–2021 and 2022–2025 for the former and

current enrichment methods, respectively. This included 12747 sequences from the former enrichment method and 12016 from the current enrichment method. The distribution of *Campylobacter* species was not significantly different between enrichment methods. SDI values were 4.21 and 4.11 for the former and current methods, respectively. Bootstrap resampling (n=1000) was used to generate confidence intervals and demonstrated no significant difference between SDI values. Rankings of STs were highly correlated between enrichment methods (Spearman's Rank Correlation: 0.93, *p*-value: 0.0025).

Significance: While FSIS has already demonstrated equivalence in isolate recovery between the former and current enrichment methods, this is the first time isolate diversity has been compared for the two enrichment methods. The analysis demonstrates that changing the *Campylobacter* enrichment media should not be considered a confounding factor in future trend analyses that span time periods that utilize both methods.

T7-03: A TARGETED AMPLICON SEQUENCING ASSAY FOR RAPID AND ACCURATE GENOTYPING OF CRONOBACTER STRAINS FROM FOOD AND ENVIRONMENTAL SAMPLES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Cronobacter* are found in dry environments and may cause severe or fatal infections in premature or immunocompromised infants particularly *C. sakazakii.*

Purpose: Develop a rapid next-generation sequencing application for accurate taxonomic identification and genotyping of *Cronobacter* in powdered infant formula (PIF).

Methods: A *Cronobacter* Targeted Amplicon Sequencing (TAS) panel with 192 targets was evaluated. First, limit of detection of *C. sakazakii* BAA894 and *C. malonaticus* E615 was assessed using 0.005 to 50 ng DNA with 0, 100, 500, 1000ng of background DNA. Second, 100g PIF samples were enriched according to BAM Ch. 29 and enumerated. PIF enrichment cultures were inoculated with four levels of BAA894 with or without 5.9 logs of *E. coli* EDL933 as competitor. Uninoculated PIF cultures and PIF with EDL933 served as control.

Four DNA extraction methods were evaluated. Finally, BAA894 was detected with TAS after enrichment in two types of inoculated 100g PIF and DNA extraction with two different methods.

Results: The TAS detected 0.025-0.5 ng target DNA depending on background level, but 0.3-5 ng target was optimal. In PIF enrichment cultures, *Cronobacter* could be subtyped at species and strain levels at concentrations of 524 cells/µL, and at species level at concentrations of 73 cells/µL, even with 5.9 logs/µL of microbial competitor. While 1-2 cells pre-enrichment to 100g PIF could be detected with TAS after enrichment, four cells were optimal for accurate species and strain level identification. DNA prepared with the four extraction methods generated target sequences using the TAS protocol.

Significance: The TAS panel successfully identified *Cronobacter* species and discriminated at strain level. Background bacteria didn't affect identification accuracy. Combined with standard enrichment, single-cell detection and genotyping were achieved, prior to isolation, highlighting its potential to be incorporated in conventional microbiological testing workflows.

T7-04: EVALUATION AND VALIDATION OF METHODS FOR THE DETECTION OF CRONOBACTER IN ENVIRONMENTAL SAMPLES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Cronobacter* is a pathogen that may cause life-threatening infections in neonates. To reduce risk of *Cronobacter* contamination in powdered infant formula, it is critical to monitor environment of production facilities.

Purpose: To evaluate and validate *Cronobacter* detection methods for environmental monitoring, employing enrichment schemes and chromogenic agars from ISO22964:2017 and FDA's internal method.

Methods: Stainless steel surfaces were inoculated with *Cronobacter* and 1 of 8 microbial competitors at a 1:1 ratio in 8 trials. After 18 h drying, cells were scraped from surfaces and added to enrichment broth. Growth of *Cronobacter* and its competitors was monitored by enumeration at multiple time points. Comparisons were made between primary enrichment in BPW for 19±1 h vs. 24±2 h, followed by secondary selective enrichment in R&F vs. CSB broth. Selective agars DFI, R&F, CCI, and ESIA were also evaluated for *Cronobacter* recovery. An optimized protocol was then validated against FDA internal method at fractional *Cronobacter* level with 10-fold competitor.

Results: *Cronobacter* levels at 18 h and 24 h in BPW primary enrichment did not differ significantly (n=8; mean diff. 0.03 log CFU/mL; $p = 0.69$). During secondary enrichment, *Cronobacter* outgrew competitors by average of 0.61 log CFU/mL in CSB, while competitors outgrew *Cronobacter* by 1.54 log CFU/mL in R&F. CSB recovered *Cronobacter* significantly better than R&F (n=8; mean diff. 2.15 log CFU/mL; $p = 0.0014$). The findings supported adopting 19±1 h for BPW primary enrichment and CSB for secondary enrichment. In a single-laboratory validation, FDA internal method detected 7/20 fractional test portions and revised method detected 8/20 (dPOD = 0.05), indicating no significant difference. All four chromogenic agars showed comparable performance in detection of positive test portions.

Significance: The new protocol can serve as environmental testing method in the FDA BAM and harmonizes with the ISO standard.

T7-05: DEVELOPMENT OF AN ENZYME PRETREATMENT THAT ENHANCES ENVIRONMENTAL SAMPLING WHEN BIOFILMS ARE PRESENT

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Biofilms on surfaces pose a threat to food industry facilities and can confound environmental sampling by preventing efficient bacterial collection. This may compromise testing effectiveness.

Purpose: This work developed an enzyme cocktail (EC) used before sample collection that releases viable bacteria from biofilms to improve sampling effectiveness.

Methods: Six proteases, 5 polysaccharidases and a DNase were screened as 2 enzyme cocktails (2EC) for antibiofilm activity (ABA) on biofilms of 7 food relevant bacteria in a 96-well plate format. ABA was determined using a Crystal Violet assay. The top 6 2ECs were also tested for cell release and toxicity. Fifteen 3 enzyme cocktails (3EC) were then compared using 2 biofilm models. The four top 3ECs were tested on a larger 52-strain bacterial panel (9 species), and also with biofilms grown on coupons made of 4 materials. A lead EC was selected and its product specifications assessed. Independent final efficacy testing (Q-laboratories) used an advanced multi-species biofilm model with 2”x 2” stainless steel (ss) coupons and *Listeria monocytogenes* collection and detection as the endpoint.

Results: Comparing the mean % Control values across the 52-strain panel, 3EC2 showed superior efficacy over the other 3ECs (Kruskal-Wallis/post-hoc Dunn's test 3EC2 over 3EC1 (*P*-0.0098), 3EC3 (*P*-0.0478), and 3EC4 (*P*-0.0028). 3EC2 had high ABA with just a 5 min treatment time (SD from Ctr, *t*-test *P*-<0.0001, mean % Ctr 29%, N= 7 exp) similar to 60 min treatment (SD from Ctr, *t*-test *P*-<0.0001, mean % Ctr 20%, N= 7 exp) and had 4^{o}C storage stability of >1 year. Testing by the advanced biofilm model demonstrated 3EC2 enabled substantial improvement in swab-based collection of *L. monocytogenes* from 48, 96, 144h biofilms over untreated controls.

Significance: This novel pretreatment, BioXpose, will enable more accurate and sensitive environmental testing in food industry facilities, reducing foodborne illnesses and recalls.

T7-06: SILENCING THE GRAIN: EMPLOYING TARGETED CAPTURE TO UNCOVER TRACE ARTHROPOD CONTAMINATION IN CEREAL BACKGROUNDS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Metagenomic Next-Generation Sequencing has transformed the identification of foodborne pathogens; however, its use in characterizing multicellular eukaryotic contaminants, such as arthropods, requires specialized workflows to attain species-level resolution in intricate food matrices.

Purpose: This research assesses a metagenomic workflow using hybridization-capture to increase detection sensitivity and taxonomic resolution of arthropod DNA added to food.

Methods: We designed a custom bait panel targeting ~2,000 arthropod species. In Experiment 1, genomic DNA (gDNA) from 30 insect species were mixed at equal (20 ng) and unequal (2–32 ng) masses with wheat flour gDNA. In Experiment 2, gDNA from five insect species were spiked into cornmeal gDNA at three loading levels (2.5–10 ng per species).

Libraries were enzymatically fragmented (35°C, 15 min) and hybridized for 18–20 hours at 65°C. Shotgun and enriched libraries were sequenced on an Illumina MiSeq platform. We used a hierarchical 30-bp mitochondrial K-mer pipeline to assign taxonomic groups.

Results: In wheat flour mixtures, targeted capture found all 30 target species with no false negatives, increasing on-target reads to 98.4% while depleting wheat DNA background by 1,400- to 2,000-fold. In cornmeal experiments, bait capture shifted datasets from corn-dominated to insect-dominated (87.9–99.6%) target reads, achieving up to 18.8x enrichment. At the lowest spike level, all five target species, including closely related species, *Tribolium castaneum* and *T. confusum*, were found and identified. Species-level profiles remained highly correlated (r = 0.9) between shotgun and bait capture libraries, preserving community structure.

Significance: This targeted metagenomic approach provides a scalable tool for identifying trace arthropod DNA, allowing the documentation of overall community structure and distinguishing between field and storage pests in real-life food samples, supporting food safety enforcement.

T7-07: QUANTIFYING SALMONELLA TYPHIMURIUM IN MIXED CULTURES USING HYPERSPECTRAL MICROSCOPY AND DEEP LEARNING WITH SYNTHETIC DATA

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Image-based pathogen detection enables rapid screening, but accurate detection and quantification in mixed cultures are limited by microbial background, motivating the use of artificial intelligence (AI).

Purpose: This study aims to develop and validate an AI-enabled hyperspectral microscopy workflow for detection and quantification of *Salmonella* Typhimurium in mixed cultures using synthetic training data to improve scalability.

Methods: Five bacterial species (*S.* Typhimurium, *E. coli* O157:H7*, Listeria monocytogenes, Staphylococcus aureus,* and *Pseudomonas fluorescens*) were freshly cultured for 16 h in tryptic soy broth with yeast extract at 37°C. Bacterial suspensions (2 µL) were placed on glass slides, air-dried, rehydrated with 1 µL of deionized water, and imaged using a darkfield hyperspectral microscope (100×, *n*=500). Monoculture hyperspectral data were normalized, reduced by principal component analysis, and converted into composite images to train deep learning object detection models augmented with synthetic mixed culture data. For the independent test dataset, non-target species were fixed at 10^5 CFU/mL and mixed with *S.*

Typhimurium ranging from 10^3–10^6 CFU/mL. Models were evaluated on this experimental mixed culture dataset using accuracy, and image-based enumeration was compared with selective plate counts to establish quantification performance.

Results: Detection accuracy for *S.* Typhimurium in mixed cultures improved from ~71% with monoculture-only training to 79% with synthetic mixed culture augmentation on the independent test dataset (*n*=100). Image-based enumeration showed a proportional relationship between detected instance counts and inoculum concentration (10^3–10^6 CFU/mL), consistent with selective plate counts.

Significance: This study demonstrates a scalable AI approach for pathogen detection and quantification that reduces reliance on extensive combinations of training data, supporting image-based applications in food safety.

Award Application: Developing Scientist Award

T7-08: OPTIMIZING PATHOGEN SURVEILLANCE: PERFORMANCE EVALUATION OF A TARGETED SALMONELLA SPP. QUANTIFICATION METHOD IN POULTRY PROCESSING

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* is one of the leading causes of foodborne illness worldwide, with the poultry industry as a major reservoir. Reliable, reproducible techniques are essential for industrial risk estimation and process monitoring. The SureCount™ *Salmonella* Multiplex PCR Kit (Thermo Fisher Scientific) integrates targeted identification and quantification of *S.* Enteritidis, *S.* Typhimurium, and *Salmonella* spp., with a short 7-hour enrichment, making it suitable for high-throughput surveillance.

Purpose: This study verified the kit performance for *Salmonella* quantification across poultry processing matrices, specifically carcass rinses and surface samples.

Methods: Laboratory and field trials were conducted. In laboratory trials, sixteen chicken portions were inoculated with 10, 1, and 0.1 CFU/mL of *S*. Infantis and quantified at 0 and 20 h post-inoculation. Additionally, chicken exudate previously inoculated with *S*. Infantis was used to contaminate six sterile stainless-steel surfaces (three replicates), which were sampled using sterile sponges. Inoculum concentrations were verified by plate count. In the field trial, processing plant samples (n = 759; 506 carcass rinses, 253 surfaces) were analyzed by comparing SureCount™ with a modified BAM-FDA protocol.

Results: In laboratory trials, the proportion of quantifiable SureCount™ results did not differ between 0 h and 20 h (Fisher's exact test, $p > 0.05$). In surface models (10–1,000 CFU), SureCount™ showed strong linearity ($R^2 = 0.9638$). After mathematical correction, the estimated/real ratio was 1.014 ± 0.06. In field samples, an association was found between SureCount™ and modified BAM results (χ^2, $p = 0.0001$), supporting its use in field surveillance workflows (PPV = 81%; NPV = 88%).

Significance: Results demonstrate robust performance of SureCount™ for quantifying *Salmonella* in poultry processing matrices under controlled and field conditions, supporting its applicability for industrial food safety monitoring.

Award Application: Developing Scientist Award

T7-09: INVESTIGATING USE OF MALDI-TOF MS AND FT-IR SPECTROSCOPY IN TYPING BACILLUS CEREUS SENSU LATO

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Bacillus cereus sensu lato* is a group of spore-forming and toxin-producing bacteria. Standard methods (such as ISO 7932:2004) cannot differentiate between the species of the group. Implementing Matrix-associated laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) and the complementary technique Fourier-transform infrared (FT-IR) spectroscopy are both promising to discriminate between the *B. cereus s.l.* species and strains.

Purpose: This study aims to evaluate the use of MALDI-TOF and FT-IR for identification within *B. cereus s.l.* and as tools for routine laboratories.

Methods: A MALDI-TOF and FT-IR database were constructed for 120 *B. cereus s.l.* strains isolated from different food types and cultured on tryptone soy agar (TSA). MALDI-TOF (m/z 200-20,000, Bruker Microflex® LT-SH) and FT-IR (4000–400 cm-1; IR Biotyper) spectra were

recorded from three technical and three biological replicate cultures. Data analysis was conducted in Bruker software, R and the open-source Quasar spectral mining tool. 30 *B. cereus s.l.* strains were cultured, prepared and analyzed under the same conditions as for database preparation. These strains were used to evaluate the typing performance of the MALDI-TOF and FT-IR databases. Each strain was additionally characterized using WGS to identify its species and lineage.

Results: Both MALDI-TOF and FT-IR showed a relatively high discriminative power to discriminate between *B. cereus s.l.* species and genetic lineages. Strains able to produce cereulide toxin were clearly separated from non-cereulide producers, although this was species dependent. The results with the independent test set (30 strains) demonstrated that both techniques could be implemented in a routine laboratory workflow. The discriminative power of FT-IR seemed to be slightly higher to MALDI-TOF.

Significance: Both MALDI-TOF and FT-IR spectroscopy can be useful in outbreak investigation and surveillance of *B. cereus s.l.* isolates.

Award Application: Developing Scientist Award

T7-10: IMPEDANCE SPECTROSCOPY-BASED BIOSENSOR FOR RAPID AND QUANTITATIVE DETECTION OF SALMONELLA IN TURKEY PRODUCTS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* remains a leading cause of foodborne illness in the United States. Despite control efforts, infection rates remain unchanged, highlighting the need for rapid and reliable detection technologies. Conventional methods require a long time to deliver results, limiting timely intervention during poultry processing.

Purpose: This study presents the development of a sensitive impedance-based biosensor combining positive dielectrophoretic (pDEP) manipulation with antibody-functionalized interdigitated electrodes (IDEs) for rapid, quantitative detection of multiple *Salmonella*

serotypes (Typhimurium, Enteritidis, Heidelberg, and Infantis) in poultry rinsate.

Methods: The biosensor integrates microfluidic channels with IDEs, using pDEP to concentrate and trap *Salmonella* cells. Three functional regions focus cells along the channel centerline, trap them via high electric field gradients, and detect them using immobilized polyvalent anti-*Salmonella* antibodies (serogroups C1, B, and D). Antigen-antibody binding produces measurable impedance changes indicating *Salmonella* presence. Spiked turkey rinsate samples from process-line endpoints and pre-chill stations were used to evaluate sensor performance under realistic processing conditions.

Results: The biosensor detected *Salmonella* from 1 to 3,000 CFU/ml, with a detection limit of 1 CFU/ml achieved within 70 minutes, including sample delivery, binding, and washing times. Impedance responses followed a logarithmic relationship with concentration, enabling quantitative analysis. Specificity testing showed negligible cross-reactivity with *L. monocytogenes* and *E. coli* O157:H7. The sensor also differentiated live *Salmonella* from peracetic acid-inactivated *Salmonella* at high concentrations.

Significance: This work demonstrates sensitive, accurate *Salmonella* detection in poultry processing, supporting early contamination identification, timely intervention, and improved food safety monitoring.

Award Application: Developing Scientist Award

T7-11: FT-IR SPECTROSCOPY IN BACILLUS CEREUS OUTBREAK RESEARCH AS A COST-EFFECTIVE SCREENING TOOL

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Isolate characterization and comparison are central aspects in foodborne outbreak investigation and is nowadays regularly performed using whole genome sequencing (WGS). Despite its high accuracy, WGS is relatively expensive, time-consuming, and requires specific expertise for data analysis.

Purpose: This study evaluates the use of Fourier-transform infrared (FT-IR) spectroscopy as rapid and cost-effective isolate typing alternative.

Methods: Presumptive *B. cereus* was isolated from a food sample (ISO 7932) and five fecal patient samples collected after a foodborne outbreak event. Overall, 51 isolates were screened using FT-IR. The isolates were also characterized by whole-genome sequencing (WGS; Illumina NovaSeq X platform, S4 PE150 XP) to evaluate the performance of FT-IR. Quantification of cereulide in all samples was performed using liquid chromatography

coupled to tandem mass spectroscopy (LC-MS/MS).

Results: 14 different strains were identified by core-genome multi-locus sequence typing (cgMLST) from WGS. Two strains were detected in the food and in four out of five patient samples, while cereulide toxin was detected in all samples. A representative set of 13 isolates was obtained (dereplication) by comparing the FT-IR spectra through three different clustering methods: hierarchical cluster analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA). The overall congruency of FT-IR with cgMLST in this dataset was 96.4% (Adjusted Wallace Index). This used dereplication strategy reduced the costs with 67% compared to WGS of all 51 isolates.

Significance: FT-IR spectroscopy can be a useful technique in the investigation of foodborne outbreaks, especially as a first-line screening tool to reduce the number of isolates for subsequent confirmation using WGS.

Award Application: Developing Scientist Award

T7-12: COMPARATIVE ANALYSIS OF WET AND DRY INOCULA ON THE SURVIVAL OF SALMONELLA TENNESSEE ON FRESH-CUT ‘GALA’ APPLES AND GREEN BELL PEPPERS POST FREEZE-DRY PROCESSING

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Freeze-drying (FD) is a non-thermal drying method used to create low-moisture foods by reducing water activity to a level that prevents microbial growth. Freeze-dried, fresh-cut produce may present a unique combination of food safety concerns due to multiple possible routes of contamination (e.g., wet- or dry-based) on fresh commodities, lack of kill step, and the ability of foodborne pathogens like *Salmonella* to persist in these foods over time. Microbial challenge studies are paramount to determining safety risks associated with foods, and conservatively consider probable, worst-case scenarios.

Purpose: Determine the effect of FD, time in storage, wet and dry contamination, and commodity on *Salmonella* survival on freeze-dried produce to inform challenge study design.

Methods: Fresh-cut, ‘Gala’ apple and green bell pepper slices (*n*=156) were inoculated with *Salmonella* Tennessee using a wet or dry (sand-based) inoculum at ca. 8 log CFU/g, freeze-

dried, and stored at 23°C for 28 d. *S*. Tennessee populations were enumerated on tryptic soy agar at 0, 3, 7, 14, 21, and 28 d in storage. The data were fit to a linear regression model with a three-way interaction between time (days), inoculum type, and commodity with log CFU/g reductions from inoculation as the outcome variable in RStudio V.4.5.2.

Results: There were no significant (p>0.05) changes in *S*. Tennessee population on dry inoculated bell pepper only during FD and throughout storage. Wet inoculated populations reduced by ca. 0.52–1.46 log CFU/g higher than dry inoculated populations while accounting for commodity and time in storage (p<0.0001). Populations on apple reduced by ca. 0.23–0.92 log CFU/g higher than on bell pepper at 14–28 d in storage while accounting for inoculation method (p<0.05).

Significance: Challenge study design should consider the synergistic and antagonistic effects of time in storage, inoculation method, and commodity for studies on *Salmonella* survival on freeze-dried produce.

Award Application: Developing Scientist Award

T8-01: ENVIRONMENTAL DRIVERS OF SOIL MICROBIOME AND SHIGA TOXIN-PRODUCING ESCHERICHIA COLI PRESENCE IN A CALIFORNIA CENTRAL COAST FRESH PRODUCE FIELD

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Environmental properties, weather events, and adjacent land use may influence the soil microbiome, potentially affecting the survival of Shiga toxin-producing *Escherichia coli* (STEC) in agricultural fields.

Purpose: To identify factors affecting soil bacterial composition and STEC presence in a produce field in the California central coast as part of the California Longitudinal Study.

Methods: Metagenomic sequencing was performed on 103 soil samples selected from 578 collected over 5 years along transects through a fresh produce field. Soil bacterial community profiles were compared, and correlations with environmental factors and STEC presence were analyzed. Statistical analyses were extended to include soil samples from four additional fields in the California central coast region (overall n=1,084) to examine relationships between STEC occurrence and environmental variables.

Results: Seasonality significantly influenced soil bacterial communities ($P<0.001$), which also exhibited correlations with average 7-day air temperature ($P<0.001$) and relative humidity ($P=0.004$). Soil from fallow and production areas in the field harbored different bacterial profiles ($P<0.001$), while adjacent habitat (riparian vs. cattle rangeland) had no significant effect ($P=0.115$). The STEC prevalence rate was 1.7% (10/578), with no association between STEC presence and soil microbiome composition ($P=0.083$). Across all five produce fields, STEC prevalence was 1.2%, and detection probability decreased with increasing $\log_{10}$ distance from riparian ($P=0.01$) or rangeland ($P=0.001$) habitat.

Significance: These results provide insight into factors affecting soil bacterial communities and their relationship with STEC presence in agricultural fields in the California central coast region that can be considered during risk assessments.

T8-02: CANTALOUPE FRUIT COLONIZATION VIA THE BLOSSOM ROUTE EXHIBITS A DOSE-RESPONSE PATTERN AT VERY LOW LEVELS OF INITIAL CHALLENGE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Recent recurring outbreaks of *Salmonella* have been associated with the consumption of cantaloupes.

Purpose: To investigate the ability of *Salmonella* to colonize and internalize cantaloupe fruit when applied to blossoms at varying low concentration contamination levels.

Methods: Cantaloupe plants (*Cucumis melo* ‘reticulatus’; cultivar ‘Primo’) were grown from

commercial seed and maintained in the NC State University BSL-3P phytotron greenhouse. *Salmonella* (a cocktail of outbreak serovars Javiana, Newport, Panama, Poona and Typhimurium) contamination was introduced to blossoms at logarithmic dilution inoculum concentrations, from ca. 4.6 $\log_{10}$ CFU down to <1 CFU/blossom. Surface and internal samples of mature fruits were processed and enriched for *Salmonella* in accordance with FDA BAM methods. Data were analyzed for prevalence of contamination [surface and inside (blossom versus stem side)].

Results: Of the cantaloupe fruit harvested from *Salmonella*-inoculated flowers at ca. 4.6 $\log_{10}$ CFU (n=57), 2.6 $\log_{10}$ CFU (n=28), and 1.6 $\log_{10}$ CFU (n=54), 87.7% (50/57), 71.4% (20/28) and 29.6% (16/54) were contaminated, and of those positive, 68.0% (34/50), 50.0% (10/20) and 31.3% (5/16) had evidence of internalization into the fruit pericarp, respectively. For those melons that *Salmonella* internalized (n=49), the majority (63.2%; 31/49) harbored *Salmonella* within the entire pericarp. *Salmonella* contamination was not observed when blossoms were inoculated with either ca. 0.6 $\log_{10}$ (n=49) or <1 CFU (n=34).

Significance: Findings from this controlled growing environment revealed a dose-response pattern, with cantaloupe contamination possible when blossoms were exposed to *Salmonella* at inocula concentrations as low as ca. 35 CFU during pre-harvest.

T8-03: SOILLESS SUBSTRATE TYPE IMPACTS TULANE VIRUS PERSISTENCE AND LOCALIZATION DURING RECIRCULATING DEEP-WATER CULTURE HYDROPONIC CULTIVATION OF LEAF LETTUCE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Food safety risks associated with hydroponic cultivation of leaf lettuce are not well characterized.

Purpose: The impact of soilless-substrate type on the persistence and localization of a human norovirus surrogate, Tulane virus (TV), in recirculating deep water culture (DWC) systems during the cultivation of leaf lettuce was investigated.

Methods: Nutrient solution (NS) in DWC systems containing 25-day old butterhead lettuce (cv. Rex) seedlings were inoculated with approximately 3 to 4 log PFU/mL of TV. Tulane virus concentration was determined in NS, soilless substrates (low-density foam [LDF], peat, and rockwool), roots, and leaves via virus plaque assay on post-inoculation day 0 (lettuce seedling), 2, 4, 7, 14, and 21 (mature lettuce head).

Results: For cultivation in LDF (n=2), the mean value of TV on day 0 was 4.18, 2.86, and 1.13 log in NS, LDF, and roots, respectively. The recovered TV on day 0 was 3.63 log (NS), 1.34 log (peat), and 1.55 log (roots) during cultivation using peat (n=1). Meanwhile, 3.02, 2.46, and 1.03 log TV were detected in NS, rockwool, and roots, respectively, on day 0 in the presence

of rockwool (n=1). By day 21, TV declined to undetectable levels regardless of sample type, during cultivation with peat. Similar TV decline was observed in root and NS, but <1 log reduction was detected in rockwool by day 21. For production using LDF, TV declined to <1 log regardless of sample type but persisted until day 21. Tulane virus was not detected in edible lettuce leaves at any time during the study.

Significance: The results from this study may be used to support substrate selection as a risk management practice during hydroponic lettuce cultivation. Although TV declined over the 21-day period, if not managed properly, this short-term persistence of enteric viruses indicates potential cross-contamination risks during pre- and post-harvest handling.

Award Application: Developing Scientist Award

T8-04: OZONE NANOBUBBLE TREATMENT OF PREHARVEST AGRICULTURAL WATER AS AN ALTERNATIVE MITIGATION STRATEGY UNDER THE FDA PRODUCE SAFETY RULE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Preharvest agricultural water has been repeatedly implicated as a potential source of produce contamination, and with few approved irrigation water treatments, there is a need to develop and validate alternative water treatments.

Purpose: This study evaluated ozone nanobubbles (ONB) as a non-chemical water treatment for mitigating microbial risk in preharvest irrigation water.

Methods: Lab trials of ONB treatments were performed using a standardized agricultural test water medium (99 mL) inoculated with a 7-strain cocktail of either Shiga toxin-producing *Escherichia coli* (STEC) or *Salmonella* (7 log CFU/mL) and mixed with an ONB solution (99 mL; 8

ppm ozone) for a 5-minute contact time. Experiments were conducted in triplicate at 12°C or 32°C and pH 6.5 or 8.4, and reductions were assessed by plating in quadruplicate onto selective media (n=96). In field trials, surface water was treated with ONB, and water samples were collected at various times (0, 5, 10, 30, 60 min) from the untreated water source and the first and last sprinkler heads. Samples were collected in triplicate at each location and sample time and tested for Total Coliforms (TC) and *E. coli* (EC) (n=45).

Results: ONB water treatment achieved a mean log reduction of 3.74 (3.20–4.11) for STEC and *Salmonella*, across all temperature and pH conditions, in lab trials with a 5-minute contact time. In the field, ozone treatment of surface waters resulted in nondetectable TC levels at the first sprinkler head within 5 min of continuous treatment and a maximum log reduction of 2.83 at the last sprinkler after 60 min. Similarly, EC levels were nondetectable at the first sprinkler after 5 min and after 60 min at the last sprinkler.

Significance: Ozone nanobubble treatment reduced foodborne pathogens in lab tests with test agricultural water and improved water quality in field tests, demonstrating its potential as an effective non-chemical preharvest water treatment mitigation.

T8-05: INFLUENCE OF MUSTARD GREEN COVER CROP CULTIVARS ON SALMONELLA ENTERICA SURVIVAL IN AGRICULTURAL SOIL

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: *Brassicaceae* cover crops, such as mustard greens (*Brassica juncea*), are widely used to improve soil health and as biofumigants for suppressing soilborne pathogens in agricultural soil, but their effect on foodborne pathogens has not been fully examined.

Purpose: To evaluate cultivar-mediated effects of eight mustard green varieties on *Salmonella enterica* population dynamics in agricultural soil stored within a biocabinet.

Methods: Eight mustard green cultivars were grown in a certified organic field. At flowering, whole plants were harvested, chopped into ~3 cm pieces, and incorporated into soil inoculated with rifampicin-resistant *Salmonella enterica* serovar Newport strain J1892 to an initial concentration of ~8 log CFU/g. Soil-biomass mixtures were placed in pots for each cultivar and gently tamped to retain compounds in the soil, and untreated soil served as the control. At each sampling time point (days 0, 1, 4, and 7), 10 g soil samples were collected

from each pot (n = 12), diluted in 90 mL BPW, and rinsate was serially diluted and plated onto XLT-4 agar for enumeration. Statistical analyses were performed using R.

Results: *Salmonella* populations increased significantly by day 1 across all treatments, including the untreated soil ($p < 0.05$). Over the 7-day sampling period, the untreated soil showed the highest reduction in *Salmonella* concentration (1.55 log CFU/g), compared with soils treated with mustard green (0.72 log CFU/g). The rate of *Salmonella* reduction in untreated soil was higher (0.57 log CFU/g/day), while mustard green-treated soils showed lower or comparable attenuation rates (0.18–0.40 log CFU/g/day).

Significance: Biofumigation with mustard green, as implemented in this study, may not provide reliable suppression of *Salmonella* in agricultural soils over the short term.

T8-06: EVALUATION OF UV-C LIGHT, PECAN SHELL EXTRACT, AND THERMAL TREATMENTS FOR PATHOGEN CONTROL IN HYDROPONIC SYSTEMS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Effective sanitation strategies are essential in hydroponic and controlled environment agriculture to limit foodborne pathogens that may attach to surfaces, form biofilms, and persist in nutrient-rich water and growing media.

Purpose: This study evaluated the efficacy of UV-C light, pecan shell extract (PSE), and thermal treatments in reducing pathogens on production surfaces, seeds, and growing substrates commonly used in hydroponic systems.

Methods: A *Listeria monocytogenes* cocktail was spot inoculated onto PVC, vinyl, and HDPE coupons and exposed to UV-C light for one minute (0.28 mW-s/cm^2). Lettuce seeds inoculated with *L. monocytogenes* were treated with PSE (1:10 w/v) via sodium alginate coating or by priming for six hours at 4°C or room temperature. Hydropriming served as a control. Additional trials using *E. coli* and *Pseudomonas* spp. evaluated PSE at 1:10, 1:20, and 1:30 w/v, and germination rates were examined. Thermal sanitation was tested using inoculated rockwool cubes (~7.9 log CFU/g), exposed to boiling water or steam (70, 80, and 90°C) for 10–600 seconds.

Results: High *Listeria* attachment (»5.61 log CFU/cm^2) was observed after seven days in coupons held in fertilizer solutions, while UV-C treatment effectively reduced pathogen levels after day seven »2.97 log CFU/cm^2 across all materials. PSE priming and coating achieved 3–4 log CFU/g reduction of *L. monocytogenes* in lettuce seeds. For *E. coli*, significant reductions were observed at 1:20 and 1:30 w/v, while *Pseudomonas*, showed the greatest reduction with 1:20 priming. PSE priming also significantly (P<0.05) increased germination (88.3%). Boiling water and 90°C steam reduced *L. monocytogenes* by 6 log CFU/g across all exposure times, while 70–80°C steam required exposures longer than 60 sec to achieve significant reductions.

Significance: These findings demonstrate that non-chemical interventions can effectively reduce microbial risks in hydroponic production systems.

Award Application: Developing Scientist Award

T8-07: SURVIVAL OF E. COLI TVS353 IN SOIL MESOCOSMS AT VARIOUS MOISTURE LEVELS IN A CONTROLLED DIURNAL TEMPERATURE ENVIRONMENT

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Light exposure is a critical factor influencing *E. coli* survival in agricultural soils under field conditions. This study evaluated the combined effects of soil moisture and light exposure on the survival of *E. coli* in agricultural soil under controlled under diurnal environment. Although effects of environmental variables on *E. coli* survival in soil have been evaluated in several longitudinal field studies, limited information is available on how specific factors and their interactions influence persistence under controlled conditions.

Purpose: To model the survival of *E. coli* in agricultural soil at varying moisture levels and light exposure using controlled environment designed to simulate diurnal pre-harvest field conditions.

Methods: Soil mesocosms were inoculated with *E. coli* TVS353, maintained at three moisture levels (100%, 75%, 50% field capacity), two light conditions (diurnal, complete dark), in triplicate under 14h day temperature of 25°C, 10h night at 15°C. Soil samples were collected twice from each pot and enumerated for *E. coli* on days 0, 1, 3, 7, 14, 28, 35, 56, and every 7 days thereafter, and survival dynamics were analyzed using linear mixed-effect analysis and biphasic microbial survival model.

Results: A significant decline in *E. coli* levels was observed over the sampling days *(p*<0.0001*)*. *E. coli* survived up to 140 days under higher soil moisture levels at 100% and 75%FC, dark conditions, whereas survival was 63 to 70 days at 50%FC incubated in the diurnal environment. Biphasic survival model fitted the data well and had satisfactory goodness of fit with R^2 and RMSE values ranging from 0.85 to 0.99 & 0.16 to 1.04, respectively. Biphasic modeling revealed higher initial inactivation rate constants under low moisture and light exposure conditions.

Significance: This study represents one of the first attempts to quantitatively assess the effect of light exposure on *E. coli* survival in agricultural soils across varying moisture levels.

Award Application: Developing Scientist Award

T8-08: EUGENOL INACTIVATION OF MULTIDRUG-RESISTANT SALMONELLA INFANTIS AND SALMONELLA SCHWARZENGRUND FOR MULTISEROTYPE BACTERIN DEVELOPMENT

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Multidrug-resistant (MDR) *Salmonella* Infantis and *Salmonella* Schwarzengrund contribute significantly to foodborne salmonellosis. Inactivated bacterins are selectively used to control emerging *Salmonella* serovars; however, conventional chemical inactivation methods, such as formalin, may alter antigenic structures and compromise immune responses.

Purpose: This study evaluated eugenol (EG), a plant-derived antimicrobial, as an alternative inactivation strategy for developing whole-cell multiserotype bacterins targeting MDR *Salmonella*.

Methods: *S.* Infantis and *S.* Schwarzengrund cultures, grown separately in Luria–Bertani broth (LB), were combined (1:1) and treated with EG at concentrations (0.01% to 1%) for 24 h at 37°C to determine sub-inhibitory (0.1%), minimum inhibitory (0.25%), and minimum

bactericidal (0.5%) concentrations. Then, combined cultures of serotypes (1:1) were grown with or without EG at the same concentrations to mid-log phase and subjected to heat treatment (60°C for 30 min). Bacterial viability and motility were assessed after 24h and 8h, respectively. Adhesion and invasion abilities were evaluated using Caco-2 cells, and expression of virulence genes (*hilA, hilD, invF, motA,* and *fliC*) was quantified using RT-qPCR. Experiments included appropriate controls and biological replicates (n=at least 6/treatment) and were analyzed using one-way ANOVA with Tukey's post hoc test.

Results: EG at 0.1%, when combined with heat treatment, resulted in complete loss of bacterial viability, motility, adhesion, and invasion, along with significant downregulation of all tested genes ($P<0.05$). EG alone also significantly reduced virulence traits and gene expression at 0.25%.

Significance: These results demonstrate that EG-based inactivation effectively suppresses key virulence characteristics of MDR *Salmonella*, supporting its potential as a safer alternative to chemical inactivation methods for bacterin development pending immunogenicity evaluation.

Award Application: Developing Scientist Award

T8-09: ENVIRONMENTAL DIVERSITY OF SALMONELLA NEWPORT IN THE U.S. ATLANTIC REGION

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Environmental surveys along the Virginia Eastern Shore (VES) have identified *Salmonella* as a persistent inhabitant of surface waters, with some investigating how the diversity is impacted by affected waterways tied to agricultural and animal industries.

Purpose: This study aims to assess the prevalence and ecology of *Salmonella* Newport, specifically PFGE Pattern JJPX01.0061, through environmental sampling among states with similar land use in the Atlantic region.

Methods: Environmental samples (water/sediment/scat) were collected over the summer months of three years (2014–NC, SC, GA; 2015–OH, PA, NJ; 2016–DE, MD), and over a full year

on the VES. In total, more than 1,600 samples were tested for *Salmonella* using FDA methods for complex environmental samples. All recovered isolates were sequenced for identification of *S*. Newport and subsequent phylogenetic analysis.

Results: Overall, 590 samples (35%) tested positive for *Salmonella*, yielding over 1,420 isolates (234 being Newport). South Atlantic samples had a higher prevalence than the Mid-Atlantic, and greater prevalence was seen in water samples. Newport isolates comprised at least five sequence types, with ST118 representing the majority. Phylogenetic analysis of ST118 suggests that while Newport-61 is an ST118, not all ST118 isolates are Newport-61s. This survey also supports the conclusion that Newport-61 is likely to be attributed to the Delmarva region of the U.S.

Significance: Differences among regions such as NC and GA, where tomato-associated *S*. Newport outbreaks have not been reported, and VA, where they are more frequent, may inform environmental stewardship and support development of practices to prevent pre-harvest contamination of fresh produce.

Award Application: Developing Scientist Award

T8-10: EFFECT OF ENVIRONMENTAL CONDITIONS AND SOIL AMENDMENTS ON E. COLI AND TOTAL COLIFORMS PERSISTENCE AND TRANSFER IN BELL PEPPER FIELDS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Raw manure improves soil health but poses pathogen risks; transfer dynamics from soil amendments to soil and fruit remain poorly defined.

Methods: This study evaluated microbial risk during bell pepper production under cow manure (CM), poultry litter (PL), or chemical fertilizer (NB), with/without tillage, in a 36-plot on-farm study across 2023–2024. Soils were sampled at weeks 1, 3, 6, and 12; peppers at weeks 13 and 14 for *E. coli* and total coliforms enumeration. Bell peppers (n=80) were inoculated with Nalidixic acid-resistant *E. coli* on tops or sides for a 5-day die-off study. Weather was recorded; GAMMs with 11 variables modeled microbial dynamics; linear mixed models assessed soil nutrient changes pre vs. post amendment; linear regressions assessed yield responses to amendments.

Results: CM plots had higher soil *E. coli* in weeks 1–3 (1.86-3.00 log CFU/g) than PL (<1.00-1.41) and NB (<1.00-1.11), declining across treatments by week 12 to 1.01-1.36. Total coliforms in 2023 began at 4.10-5.63 log CFU/g with 1.22-1.88 log CFU/g reductions by week 6; in 2024, manure plots resurged by week 12 (4.33-5.02), while NB remained lower and steadier (2.37-3.41). At harvest (weeks 13-14), *E. coli* on peppers was detected only in 2023 tilled NB (0.32 log CFU/pepper; others □0.27) and was non-detectable across treatments in

2024 (□0.357). Die-off on fruit was faster on tops than sides (0.39-0.57 vs 0.20-0.51 log CFU/cm^2/day); time since inoculation dominated, with environmental effects varying by year and site of inoculation. Despite soil nutrient level differences, yields were comparable, and treatment×method interactions were not significant.

Significance: These results support practical risk management for BSAAO use in produce by setting application-to-harvest intervals of >3 months and good agricultural practices (mulching, drip irrigation, and trellising). Environmental factors significantly influence microbial die-off on soil and produce surfaces.

Award Application: Developing Scientist Award

T8-11: SYSTEM MODEL FOR SWEET ONION PRODUCTION FIELDS IN SOUTHEASTERN UNITED STATES

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Preharvest contamination can arise from multiple interacting pathways, including wildlife intrusion, irrigation water. Sweet onion production is concentrated in the Vidalia region of GA, generating approximately $120–200 million annually from ~9,400 acres, accounts for about 40% of the U.S. sweet onion market. However, outbreaks linked to onions highlight the need to understand the pathway of foodborne pathogens in preharvest produce environments.

Purpose: To develop a systems-based model to predict *E. coli* contamination dynamics in sweet onion production fields.

Methods: A system model was developed in MATLAB to simulate contamination in a 1-acre sweet onion field (~58,080 plants). The model integrated contamination inputs from wildlife intrusion, irrigation water. Probability distributions for fecal deposition rates, and *E. coli* concentrations were taken from published studies. Rainfall impacts on irrigation pond contamination were modeled using a polynomial regression. The model simulated 1,000 iterations across 58,080 subplots over 365 days of the year. Multiple scenarios were evaluated, including baseline conditions, irrigation-only, local defecation-only, rainfall during the curing period.

Results: Under baseline conditions, soil contamination was driven by cumulative wildlife fecal deposition and irrigation water. Irrigation was identified as the dominant source of preharvest contamination, as irrigation-only scenarios produced contamination levels comparable to baseline conditions. Rainfall occurring one day before harvest increased predicted contamination by 1.17-fold relative to baseline. Sensitivity analyses demonstrated consistent log-scale reductions as baseline inputs were reduced.

Significance: System modeling framework provides comprehensive understanding, quantitative tool for pre-harvest contamination dynamics, which is useful for designing mitigation strategies in preharvest produce systems.

Award Application: Developing Scientist Award

T8-12: SURVIVAL OF PATHOGEN SURROGATE ESCHERICHIA COLI ON ROMAINE LETTUCE FOLLOWING APPLICATION OF CONTAMINATED WATER IN ARIZONA

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: According to the FDA FSMA Produce Safety Rule Subpart E, growers must determine whether scientifically valid mitigation measures are necessary, including increasing the interval between the final water application and harvest, when conducting Agricultural Water Assessments (AGWAs).

Purpose: To quantify the survival of green fluorescent protein-tagged *E. coli* TVS353 on romaine lettuce following a contaminated water application.

Methods: Two independent field trials were conducted in Yuma (Trial 1) and Maricopa (Trial 2), Arizona. Romaine lettuce plots were inoculated with high (~4.40 log CFU/mL) and low (~2.69 log CFU/mL) concentrations of *E. coli*, respectively, using a backpack sprayer. Whole-head and composite samples (N=150) were collected from 1- to 336-h and analyzed using a Most Probable Number (MPN) technique. Whole-head samples were weighed into individual

bags following a three-by-three dilution scheme of 250-, 25-, and 2.5-g, while composite samples were weighed at 375-, 37.5-, and 3.75-g. Samples were enriched with 1000mL of tryptic soy broth containing rifampicin, incubated (24h, 37 ± 2°C), and streaked onto tryptic soy agar with rifampicin. Plates were considered positive when colonies fluoresced under UV light. *E. coli* concentrations were estimated using an FDA BAM based MPN calculator.

Results: In Trial 1, head concentrations decreased by 0.52 ± 0.57 log MPN/100g by 336h. Composite samples showed no *E. coli* reductions (4.46 ± 0.00 log MPN/100g) by the final timepoint (192h), with only a transient decrease of 0.34 ± 0.46 log MPN/100g at 48h. In Trial 2, concentrations fell below the limit of detection by 70h for heads (<1.0 MPN/g) and by 190h for composites (<0.68 MPN/g), corresponding to >2.00- and >3.00-log MPN/100g reduction, respectively.

Significance: Die-off patterns varied between locations, highlighting that contamination load and site-specific conditions must be considered when applying findings to meet the Produce Safety Rule.

Award Application: Developing Scientist Award

T9-01: A FLUID-MILK SPOILAGE MODEL INTERFACE INCORPORATING REAL-TIME TEMPERATURE DATA FOR PRECISE SHELF-LIFE PREDICTIONS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: The short shelf life of fluid milk makes it a significant contributor to food waste, underscoring the importance of accurate shelf-life prediction, for which simulation models can serve as a powerful tool.

Purpose: The purpose of this work was to develop a modeling tool in combination with a corresponding digital infrastructure that enables fluid milk processors to make precise shelf-life predictions by utilizing real-time temperature data.

Methods: We established a digital infrastructure to monitor the temperature of fluid milk throughout the processing facility until delivery to a retail store. We used data collected through this infrastructure as input for a spoilage simulation model (accessible through a user interface https://mqip.shinyapps.io/PPI_Digital_Interface/), and compared the resulting output with that obtained using the model's default input parameters. Furthermore, we evaluated estimation adjustments for scenarios in which real-time data is used to document supply chain disruptions (e.g., a cooling failure, resulting in a temperature increase from 4°C to 10°C during delivery to the retail store).

Results: Using temperature data recorded through the digital infrastructure led to an adjustment of the median predicted proportion of spoiled containers on shelf-life day 28 from 10% when using default data to 20%, thus avoiding a potential overestimation of shelf life. Furthermore, application of our model enabled the adjustment of shelf-life predictions in the described disruption scenario. Here, the predicted median spoiled proportion on shelf-life day 28 was adjusted from 10% when the failure was not detected to 20% when the real-time temperature was documented.

Significance: Our user interface modeling tool will contribute to the adoption of prediction models in industry and aid in tackling future temperature control challenges.

Award Application: Developing Scientist Award

T9-02: A DYNAMIC PREDICTIVE MODEL FOR BACILLUS CEREUS GROWTH FROM SPORES IN TRYPTIC SOY BROTH UNDER TEMPERATURE-ABUSE CONDITIONS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: *Bacillus cereus* food poisoning often occurs due to temperature abuse of cooked foods, resulting in ~ 63,400 illnesses annually in the U.S.

Purpose: This study aims to develop a dynamic model to predict the potential growth behavior of *B. cereus* from spores in tryptic soy broth (TSB) under fluctuating temperature profiles.

Methods: A four-strain spore cocktail was heated at 80°C for 10 min, inoculated at 2~3 log CFU/mL to TSB, and incubated at isothermal temperatures from 10 to 54.4°C or at temperatures following two 12-h cycle sinusoidal profiles (10 to 30°C for 30 h and 25 to 45°C for 23 h). All tests were conducted in duplicate. The Baranyi model was used to fit the growth

curves at isothermal temperatures. The temperature dependence of the maximum specific growth rate (μ_{max}) and lag phase duration was described using the modified Ratkowsky model and hyperbolic function. The Baranyi differential equations were solved numerically using a fourth-order Runge–Kutta method to build a dynamic model. Acceptable prediction zone (APZ) analysis was used to evaluate the model performance.

Results: No growth was observed at 10°C or 54.4°C. Both primary and secondary models showed good agreement with the observed data, with satisfactory fit statistics (R^2 = 0.953–0.998 and RMSE = 0.073–0.904). The theoretical minimum and maximum growth temperatures were 8.94°C and 54.11°C, respectively. Most μ_{max} values reported in previous studies for plant-based cereal and non-cereal foods and meat and dairy products followed a similar temperature-dependent pattern and fell within the range predicted by the fitted model. The APZ results showed that 16 out of 17 prediction errors fell within -1.0–0.5 log CFU/mL.

Significance: This dynamic model well predicts *B. cereus* growth and could support temperature abuse risk assessment for the food industry and regulatory agencies. It will be included in the USDA Pathogen Modeling Program once published.

Award Application: Developing Scientist Award

T9-03: A QUANTITATIVE RISK ASSESSMENT OF SHIGA TOXIN-PRODUCING E. COLI (STEC) IN ORGANIC FRESH PRODUCE ASSOCIATED WITH COMPOST OF ANIMAL ORIGIN

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Organic farming often relies on compost of animal origin to improve soil

fertility. However, if composting is poorly processed or managed, it may introduce pathogens into soil and contaminate fresh produce. Quantitative risk assessment (QRA) can estimate preharvest risk and evaluate food safety practices in organic farming, yet compost pathway remains largely unaddressed in QRA.

Purpose: To estimate the risk of Shiga toxin-producing *E. coli* (STEC) contamination in fresh produce from composted manure using quantitative risk assessment approach.

Methods: A stochastic QRA was used to predict the risk of STEC contamination in organic fresh produce during preharvest. The output was estimated as the probability of contamination with 1 CFU per head romaine lettuce. The model incorporated animal manure, composting, storage, application to soil, and produce contamination. Runoff from compost storage pathway was also included to model contamination in produce from the storage to nearby water or adjacent fields. Baseline assumed improperly managed static cattle manure compost with an unfinished composting duration of 15–30 days. The model was built in R using 'quantrra' package with 10000 iterations, and global sensitivity analysis was used to identify the most influential inputs.

Results: The baseline model estimated a mean probability of 0.0284 (2.84%) that a harvested lettuce would contain 1 CFU of STEC. The maximum count estimated per produce was 22.87 CFU. Sensitivity analysis indicated the initial concentration of manure was the most important factors, followed by composting method and storage time. Compared with baseline, the finished dairy windrow compost with 30–45 days of storage reduced the contamination risk to 0.000073 (0.0073%).

Significance: This QRA model provides a better understanding of important factors contributing to food safety risks associated with compost and supports decision-making on best soil-amendment practices in organic farming.

Award Application: Developing Scientist Award

T9-04: CHARACTERIZING SAMPLING PLANS FOR IRRIGATION WATER WITH REGARDS TO THEIR STATISTICAL POWER AND IMPACT ON LEAFY GREENS RECALL RISK DUE TO SHIGA TOXIN-PRODUCING E. COLI

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: As irrigation water can be a vehicle for foodborne pathogen contamination of leafy greens, characterizing the power and risks associated with microbiological sampling plans for irrigation water can inform food safety practices.

Purpose: To compare sampling plans (n = 7) for irrigation water by their (i) power of rejecting water at various generic *E. coli* (gEC) levels and (ii) impact on the risk of a leafy greens recall due to contamination with Shiga toxin-producing *E. coli* (STEC).

Methods: We simulated sampling of water at various levels of gEC, while assuming that the gEC level was (i) homogeneously or (ii) normally distributed with a standard deviation of 0.99 $\log_{10}$ CFU/mL. We calculated the percent of samplings that resulted in rejection for each plan

at each gEC level. To estimate recall risk (RR), we simulated a worst-case scenario of irrigation for romaine with water that complied with each sampling plan, assuming a ratio of 4:1 gEC:STEC cells in water. The resulting estimate of STEC level on romaine at harvest was used as an input for a previously developed leafy greens risk model to calculate RR.

Results: The baseline sampling plan requires testing of three 100mL samples for gEC, of which 2 must test below 0 MPN/100mL while 1 can have up to 10 MPN/100mL; this plan had an (i) 80% power of rejecting water at a homogenous gEC level of 12.0 CFU/L and (ii) associated RR of 1 in 3,877 lots. If sample volume was increased 10-fold, we observed an (i) 80% power to reject water at a homogenous gEC level of 1.2 CFU/L and (ii) a RR of 1 in 4,293 lots. If we assumed a normal, rather than a homogenous, distribution of gEC, the gEC level that is detected at a power of 80% was approx. 1.7 – 2.7-fold lower for three plans, 1.7-fold higher for one plan and comparable for the remaining three plans.

Significance: Our findings provide initial insight into the efficacy of water sampling plans and highlight the need for data on gEC distributions in irrigation water.

T9-05: DEVELOPING A DATA-DRIVEN RISK MODEL TO PRIORITIZE PRODUCE ACTIVITIES: METHODOLOGICAL APPROACH AND CASE-STUDY EXAMPLES

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: A food safety program for produce encompasses a wide range of activities and initiatives. The prioritization of such activities requires utilizing both public health and other decision criteria.

Purpose: The purpose is to develop a data-driven and adaptive prioritization model to evaluate cross-functional activities and inform a risk-based operational plan.

Methods: A multi-criteria decision analysis approach was adapted from the FDA Risk-Ranking Model for Food Tracing and other tools. Public health (PH) and “other decision” (OD) criteria were developed, each with detailed scoring definitions on a scale of 1-3-9 corresponding to low-medium-high. The model was operationalized based on available data and subject matter expertise to evaluate activities in areas such as outbreak response, regulations and guidance, compliance, research, outreach and stakeholder engagement. A priority score was

determined for each activity from the PH and OD scores with normalization and criteria-group weighting.

Results: An iterative development process resulted in: i) PH criteria that evaluate the impacts on outbreaks and cost of illness, science-based control measures, industry-wide implementation, and emergency response; and ii) OD criteria that consider statutory requirements, stakeholder collaboration, affordability, and programmatic needs. The model generated priority scores ranging from a normalized score of <30 to 80 (out of 100) with ~50 unique scores. Sensitivity analyses evaluated the influence of individual criteria and criteria-group weighting schemes on the priority score distribution. Examples will be presented to illustrate the model's capacity to differentiate higher vs. lower ranked activities.

Significance: This model advances our methodologies for consistent, structured, and data-driven approaches to risk prioritization, in alignment with the Human Foods Program's mission to identify, assess, and prioritize public health risks.

T9-06: MODELING THE IMPACT OF MECHANICAL DAMAGE ON RISK OF A PRODUCT POSITIVE TEST FOR ESCHERICHIA COLI O157:H7 IN CHOPPED ROMAINE LETTUCE

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Postharvest quality defects (e.g., mechanical damage) in fresh produce are routinely assessed at receiving at retail. Although previous studies have shown that such defects can increase pathogen presence, they are rarely incorporated into quantitative microbial risk assessments (QMRAs), potentially leading to underestimation of food safety risk.

Purpose: To develop a QMRA that quantifies how mechanical damage (MD) impacts the risk of a product positive test for *Escherichia coli* O157:H7 (EC) on chopped romaine lettuce at retail.

Methods: A QMRA was developed to model EC concentration on chopped romaine from product arrival at the processor through retail storage. The model simulated 100,000

batches, each including 2,833 packages (225 g/package). Both baseline and MD scenarios assumed non-clustered contamination of EC across a batch; MD scenario additionally modeled the effect of MD. Detection of a product positive test at retail was evaluated by sampling of 5 packages per batch with a 25-g analytical unit per package.

Results: Under baseline and MD scenarios, 879 batches (0.879%) included at least one contaminated package with 1 CFU/package. The mean proportion of contaminated packages per batch was 0.65% (median of 0.56%; 5^{th}–95^{th} percentiles: 0.04–1.55%). Among contaminated packages, the mean concentration was 5.44 $\log_{10}$ CFU/package under baseline scenario (median of 2.79; with 5^{th}–95^{th} percentiles: 1.40–4.78%) and the mean concentration was 5.66 $\log_{10}$ CFU/package under MD scenario (median of 2.81; 5^{th}–95^{th} percentiles: 1.41–4.81%). Upon sampling at retail, 67 batches (0.067%) were classified as medium-high risk for a product positive test (1 in 1,000), 422 (0.422%) as medium-low risk (1 in 10,000 – 1 in 1,000), and 390 (0.39%) as low risk (<1 in 10,000).

Significance: By incorporating quality defects into QMRA, this work will ultimately provide retailers with a decision-support tool to set targets for acceptable MD.

Award Application: Developing Scientist Award

T9-07: DEVELOPING AN INTEGRATED FRAMEWORK FOR PRE-HARVEST SALMONELLA RISK REDUCTION AND MITIGATION ASSESSMENT IN U.S. BEEF SYSTEMS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Evaluating pre-harvest interventions (PHI) to mitigate *Salmonella* in beef requires translating changes in prevalence and load into public health outcomes.

Purpose: To design a quantitative framework to evaluate PHI in cattle according to their efficacy in reducing beef-attributed salmonellosis

Methods: We performed a narrative review to characterize pre-harvest *Salmonella* in beef cattle (i.e., prevalence, load, and serovar virulence) and compiled an inventory of PHIs and their efficacy. We analyzed publicly available *Salmonella* genomes and epidemiological data to classify higher virulence (HV) serovars. From these analyses, we identified variables for two model components: 1) pre-harvest component measuring how mitigations reduce *Salmonella* in cattle, and 2) existing beef processing model estimating human salmonellosis risk under different microbial criteria (MC).

Results: We identified 10,191 citations, yielding 323 studies for full text review, which were

synthesized into six PHI categories with efficacy estimates: vaccines (n=5), direct-fed microbials (15), supplements/medications (23), environmental modifications (3), phages (6), and other management practices (3). Genomic analysis of 95,452 *Salmonella* isolates identified *S.* Enteritidis, Newport, Braenderup, Typhimurium, 4,[5],12:i:-, Thompson, Uganda, Heidelberg, Dublin, and Infantis as HV serovars. Based on this, key modeling components are: heterogeneity in *Salmonella* load (e.g., lymph node, supershedders), prevalence (e.g., region, season), and virulence (e.g., prevalence, dose-response). PHIs will be probabilistically ranked by beef-attributed salmonellosis reduction. PHIs with 25% salmonellosis reduction (Healthy People 2030 target) will be considered impactful mitigation strategies.

Significance: A framework is proposed to quantitatively select PHIs for *Salmonella* control in beef by their post-harvest impact on human health risk.

T9-08: NOROVIRUS INFECTION RISKS ASSOCIATED WITH CONSUMPTION OF CONTAMINATED TOMATOES – AN APPLICATION OF A NOVEL QMRA-SEIR MODEL

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: The magnitude of norovirus infection risk to a population from consuming norovirus-contaminated produce is unknown.

Purpose: To estimate the individual- and population-level norovirus infection risks associated with a contaminated-tomato exposure event.

Methods: We developed a quantitative microbial risk assessment (QMRA)-linked SEIR model to estimate individual risk and population cases from consumption of norovirus-contaminated tomatoes. Norovirus GII.6 strains isolated from farm tomatoes and quantified

by digital RT-PCR were incorporated into a QMRA model to estimate individual infection risks (10,000 iterations, mc2d package) varying consumption rates and norovirus infectivity assumptions (1 to 1:1000). These risks were integrated into a SEIR model (N=100,000; 50% of population exposed to tomatoes) influencing the force of infection, to estimate the likelihood and size of outbreaks.

Results: Median norovirus infection risks from one-time exposure to contaminated tomatoes were highest among adults (21 to <60 years), 1.5×10^{-3} per day, 95th percentile range (3.3×10^{-5}, 2.0×10^{-1}) and lowest among children (<6 years), 1.9×10^{-4} per day, 95th percentile range (1.8×10^{-6}, 3.3×10^{-2}). Using the SEIR model, a large seeding event (50% population exposure; 1:100 infectivity ratio) resulted in 19 cases (95% UI: 1 to 37) resulting from primary exposure and 56 cases (95% UI: 1 to 256) from primary exposure and secondary person-to-person transmission. Seeding events representative of sporadic, low levels of contamination resulted in no outbreaks (population exposure < 5%). The likelihood and magnitude of an outbreak was most influenced by assumptions of norovirus viability (QMRA model) and percent of population exposed (SEIR model).

Significance: This study highlights the utility of integrated risk assessments to evaluate norovirus risk for individuals and populations and can inform agricultural and public health mitigation strategies.

T9-09: RISK OF MICROBIAL HAZARD EXPOSURE DURING NUTRIENT FILM TECHNIQUE (NFT) HYDROPONIC LEAFY GREEN PRODUCTION: A MODEL STUDY OF LISTERIA MONOCYTOGENES PERSISTENCE IN ROOT-PLUGS AND NUTRIENT SOLUTIONS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Foodborne illness outbreaks have been linked to leafy greens grown in hydroponics-controlled environment agriculture (CEA) systems. Data gaps exist regarding how foodborne pathogens may contaminate hydroponic (HP) systems. Nutrient Film Technique (NFT) hydroponic systems recirculate the nutrient solution (NS) between a reservoir and the plants' roots, potentially facilitating pathogen dissemination throughout the system.

Purpose: Simulate a *Listeria monocytogenes* (Lm) contamination event in a hydroponic lettuce production and evaluate if Lm persists over time.

Methods: In a 48-plant NFT system, 24 germinating plugs were inoculated with Lm (3×10^7 CFU/ml) and placed alternately with non-inoculated plugs. Bacterial persistence, and transfer were monitored weekly in random samples from plugs, water/NS (reservoir), and channel surface (swabs) for 28 days, using selective-enrichment and direct-plating colony-count techniques. After the 28-day crop cycle, the same NFT system was used to grow new lettuce to evaluate possible Lm transfer between cycles.

Results: All root-plugs were Lm positive on days 7, 14, 20, and 28 as detected by enrichment culture. Lm was detected in NS samples by direct-plating at 30 CFU/mL on day 20 but was undetectable by day 28 (on harvest). NS samples were Lm positive on days 7, 14, and 20 by enrichment but negative on day 28. At harvest, average Lm levels were 1.5×10^2 CFU/mL/g in non-inoculated root-plugs and 2.5×10^3 CFU/mL/g in inoculated plugs. No Lm was detected in any samples on the second cycle.

Significance: Results show that both non-inoculated and inoculated root-plugs remained Lm-positive until harvest, suggesting transfer of pathogens through the system. There was a gradual reduction of Lm load observed in water/nutrient solution. Nonetheless, transfer through water/NS to subsequent crops was not detected. This data can support efforts by growers and regulators to assess and verify the microbial safety of NFT-HP systems.

T9-10: STAGE MATTERS: COMPARING CAMPYLOBACTER CONTAMINATION HOTSPOTS ACROSS TWO BROILER PROCESSING FACILITIES VERSUS PREDICTIVE MODELS

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: *Campylobacter* remains a leading cause of foodborne illness globally, and broiler processing is a critical point for contamination control.

Purpose: The aim of the study was to compare *Campylobacter* prevalence patterns across two commercial broiler processing facilities and benchmark these findings against predictive estimates from a recent meta-analysis.

Methods: A total of 4,726 samples were collected from Facility A between January and November 2023, and 3,397 samples from Facility B between January and December 2023. Samples represented multiple processing stages, product types, and operational conditions. Statistical analyses were performed using SAS V9.4 with $\alpha = 0.05$. Prevalence was determined using frequency procedures, and associations were assessed using Chi-square and Fisher's exact tests. Odds ratios (OR), relative risk (RR), and risk differences (RD) were calculated to compare contamination patterns between facilities and across key factors.

Results: Overall, *Campylobacter* prevalence was significantly higher in Facility B (19%)

compared to Facility A (13%; $p < 0.0001$), with Facility B exhibiting a 49% higher risk (RR = 1.49; 95% CI: 1.35–1.65) and 61% higher odds (OR = 1.61; 95% CI: 1.42–1.81) of contamination. Variation across processing stages and sample types was substantial, with early stages such as rehang and mechanically separated meat (MDM) showing the highest contamination levels. Interactions between stage and product type amplified risk at these points, indicating that contamination control requires attention beyond primary chilling.

Significance: Observed contamination patterns exceeded predictive estimates from the literature, highlighting critical control points beyond primary chilling and underscoring the need for targeted interventions to reduce *Campylobacter* risk in broiler processing.

T9-11: PREDICTIVE MODELING OF CHLORINE EFFICACY IN CONTROLLING LISTERIA DURING APPLE DUMP TANK PROCESSING

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Apple dump tank systems are critical preventive control points where *Listeria monocytogenes* cross-contamination can occur when sanitizer efficacy is compromised. Apple packers are required to scientifically validate the efficacy of sanitizer-treated dump tanks, but facility-specific variability makes validation across all commercial operating conditions impractical.

Purpose: To develop and validate predictive models to describe chlorine efficacy in controlling *Listeria* cross-contamination during apple dump tank processing.

Methods: *Listeria innocua* was used as a surrogate for *L. monocytogenes* in a pilot-scale chlorinated simulated dump tank water system. A three-factor, three-level Box-Behnken design was used to evaluate the interactive effects of free chlorine (FC, 25–100 ppm),

inoculation level (IL, 4–6 log CFU/mL), and chemical oxygen demand (COD; 250–4000 ppm) on *Listeria* reduction and cross-contamination mitigation. Response surface methodology (RSM) models were developed in R and subsequently validated using commercial dump tank water collected from four apple packing facilities.

Results: The RSM models demonstrated high predictive accuracy (R^2 = 0.91–0.98) and strong agreement between predicted and observed values during validation using commercial water. FC was positively correlated with *Listeria* reduction and negatively correlated with the cross-contamination, whereas higher COD and IL significantly reduced chlorine efficacy. Results highlight the need for continuous monitoring of FC concentration and organic load during dump tank operations and the application of hurdle intervention strategies during postharvest apple handling.

Significance: The models provide apple packers with a practical, science-based tool to optimize chlorine disinfection and enhance microbial safety during postharvest apple handling. The modeling framework may also apply to other produce systems and foodborne pathogens.

Award Application: Developing Scientist Award

T9-12: EFFECTS OF HEAT, COLD, AND ACID STRESS ON SALMONELLA TYPHIMURIUM BEHAVIORS ON CHICKEN WINGS DURING LOW TEMPERATURE STORAGE

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Presentation type/Format: Technical

Category: Modeling and Risk Assessment

Abstract:

Introduction: Understanding the behavior of stressed pathogens is critical, as environmental stresses encountered during food processing can significantly influence their survival, growth, and virulence, with direct implications for food safety and risk assessment.

Purpose: The objective of this study was to investigate the survival and growth of unstressed and stressed *Salmonella* Typhimurium (ST) on chicken wings during low-temperature storage and to develop mathematical models for pathogen risk prediction.

Methods: Heat, cold, and acid-stressed ST (HSST, CSST, and ASST) were induced by simulating poultry processing conditions. Chicken wings inoculated with unstressed ST (USST) or stressed ST were stored at temperatures of 4, 10, and 20°C.

Results: The results demonstrated that stress effects on ST behavior were temperature- and stressor-dependent. Compared to USST, heat stress enhanced ST survival at both 4 and 10°C, whereas cold and acid stress accelerated ST reduction at 4°C but provided relative protection at 10°C. Exposing to 20°C, USST, HSST, CSST, and ASST displayed full growth curves. However, the growth kinetic parameters: lag phase, specific growth rate, or maximum population were affected by stress, particularly for acid-stressed cells. The Weibull model was developed to predict ST survival at 4 and 10°C, whereas the Huang model was developed to predict ST growth at 20°C. Model validation demonstrated that these models were suitable for describing the survival or growth of USST and stressed ST, with 70–90% of residual errors within ± 0.5 log CFU/mL rinsate.

Significance: These results indicated that environmental stresses could influence ST behavior in chicken wings, highlighting the importance of incorporating stress factors into model development and risk assessment.

T10-01: ENERGY-EFFICIENT DIELECTRIC BARRIER DISCHARGE PLASMA-ACTIVATED WATER FOR SALMONELLA INACTIVATION IN AQUEOUS SYSTEMS AND ON FRESH PRODUCE

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Plasma-activated water (PAW) is a promising non-thermal food safety intervention, but adoption is limited by high energy demand, variable antimicrobial efficacy, and reduced performance on complex produce surfaces. Two studies were conducted to (i) identify engineering factors governing PAW efficacy and energy efficiency and (ii) evaluate optimized PAW against *Salmonella* on fresh produce with different surface topographies.

Purpose: Study 1 examined gas–liquid mass transfer during PAW generation using a dielectric barrier discharge (DBD) reactor operated under a high-energy regime (126 W, 2 L/min airflow) and a low-energy, flow-optimized regime (38.7 W, 1 L/min airflow). Microbial inactivation against *Salmonella* and *Listeria monocytogenes* was evaluated in water and on sand carriers.

Methods: Physicochemical properties and energy consumption were assessed. Study 2 applied optimized PAW to tomatoes, cocktail cucumbers, and cantaloupes representing smooth and warty surfaces. Produce was spot-inoculated with a *Salmonella* cocktail (~10.7 log CFU/mL), treated for 1 or 2 min, and enumerated on TSAYE. Quality attributes were

monitored during 7-day storage at 4°C.

Results: The high-energy regime produced minimal pathogen reduction (0.43±0.20 log CFU/mL), likely due to loss of volatile oxidants. In contrast, the optimized regime retained higher dissolved ozone (0.53±0.01 mg/L), acidified PAW (pH 2.98±0.05), achieved >7 log CFU/mL *Listeria* inactivation, and reduced *Salmonella* by 6.58±0.30 log CFU/mL while lowering energy use by 69%. On produce, 1-min treatments yielded uniform reductions (~1.65 log CFU/mL), while 2-min treatments showed surface-dependent effects, with tomatoes achieving the highest reduction (3.62±0.09 log CFU/mL). No quality loss was observed.

Significance: Gas–liquid mass transfer, rather than electrical power, governs PAW efficacy, and flow-optimized, low-energy DBD-PAW effectively reduces *Salmonella* on fresh produce without compromising quality.

Award Application: Developing Scientist Award

T10-02: RAPID ON-SITE DETECTION OF SALMONELLA IN TURKEY PRODUCTS USING A PORTABLE FIBER-OPTIC SERS PLATFORM

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* remains a leading foodborne pathogen in the United States, causing approximately 1.35 million infections, 26,500 hospitalizations, and 420 deaths annually, motivating the need for a portable sensing system that replaces laboratory-based methods that are time-consuming, costly, and resource-intensive.

Purpose: The objective of this study is to develop and validate a portable fiber-optic Surface-

Enhanced Raman Spectroscopy (SERS) sensing system for on-site rapid detection of *Salmonella* in turkey products.

Methods: A compact SERS sensing platform was developed, integrating a 532-nm laser, fiber-optic SERS sensor, Raman spectrometer, BME280 environmental sensors, GPS module, custom 3D-printed mechanical components, and a 7-inch touchscreen LCD within a portable aluminum enclosure. The system is operated through a secure web-based interface employing HTTPS/TLS encryption, role-based authentication, and CSRF protection. Turkey rinsate samples were spiked with *Salmonella* Typhimurium at concentrations of 10, 100, and 1000 CFU/mL. Raman spectra were acquired using an automated workflow, averaged to reduce noise, and processed using baseline correction to extract characteristic pathogen-related spectral features.

Results: The system produced distinct and reproducible Raman signatures characteristic of *Salmonella,* exhibiting a clear concentration-dependent response across 10–1000 CFU/mL. Reliable detection was achieved at concentrations as low as 10 CFU/mL. Automated PDF reporting linked detection outcomes with environmental conditions, geolocation, sample identifiers, and timestamps to ensure traceability and reproducibility.

Significance: This portable fiber-optic SERS platform provides rapid detection of *Salmonella* in field environments. Its automated PDF result generation offers significant potential for streamlined on-site food safety screening in poultry processing applications.

Award Application: Developing Scientist Award

T10-03: WHERE, WHAT, AND HOW WE DEVELOP DIGITAL TOOLS FOR FOODBORNE PATHOGEN MODELING: A SCOPING REVIEW

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Digital tools are increasingly developed to model foodborne pathogens and inform food safety decisions, yet a comprehensive characterization of modeling goals and approaches, and code availability is lacking.

Purpose: This scoping review systematically characterized digital tools for foodborne pathogen modeling to evaluate the intended goals, methodological trends, and code availability.

Methods: We searched six databases (PubMed, Web of Science, FSTA, AGRICOLA, CAB Abstracts, Medline) for peer reviewed studies on digital tools that model foodborne

pathogens in food/agriculture matrices. We included original research published between 2012–2024 describing available digital tools. Records were imported into Covidence for automatic de-duplication and screening. Two reviewers independently screened titles, abstracts, and full texts. We extracted data on pathogen genus and species, first author geographic region, supply chain stage, modeling approach (mechanistic, data-driven, hybrid), and code availability from included studies.

Results: Of 7,181 records, 62 studies met the inclusion criteria. Code unavailability was the most frequent exclusion reason. *Salmonella* was the most frequently modeled pathogen (31%). Methodologically, risk assessments were most common (27%), followed by microbial kinetics (23%) and machine learning (19%). Mechanistic models accounted for (65%), while data-driven (27%) and hybrid (8%) remained limited, indicating that methodological evolution toward combining mechanistic and data-driven models is still in its early stages. Processing received the greatest attention (48%), while distribution was understudied (3%). Code was readily available in 81% of studies, with 19% available only upon request.

Significance: Our study reveals methodological trends and transparency gaps in digital tools for foodborne pathogen modeling and provides evidence to guide future research and advance reporting standards for transparency and reproducibility.

Award Application: Developing Scientist Award

T10-04: VALIDATION OF ENTEROCOCCUS FAECIUM AS A SURROGATE FOR SALMONELLA ENTERICA, ESCHERICHIA COLI O157:H7, AND LISTERIA MONOCYTOGENES DURING STEAM TREATMENT OF IN-SHELL PECANS

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Despite their low water activity (0.60–0.65), pecans have been recalled for possible foodborne pathogen contamination. Although steam conditioning may be used post-harvest to facilitate shelling, validated time–temperature parameters for achieving a 5-log reduction and a suitable surrogate for process validation are lacking.

Purpose: This study evaluated the efficacy of saturated steam in reducing *S. enterica, L. monocytogenes* and *E. coli* O157:H7 on in-shell pecans and suitability of *Enterococcus faecium* as a potential surrogate.

Methods: In-shell pecans inoculated with nalidixic acid-resistant strains (7.61 ± 0.48 log CFU/g) were steam treated at 70, 80 or 90 ± 1°C for 0–210 s (20 s come-up time). Microbial survivors were enumerated on agar media (nalidixic acid, 50 µg/mL). A log-linear model was fitted to determine bacterial thermal resistance (decimal reduction time, D-values). The

impact of a 5-log reduction treatment on kernel moisture content, water activity, and color was evaluated. Data were analyzed using one-way ANOVA with Tukey's HSD test.

Results: A 5-log reduction of *L. monocytogenes* was achieved within 150, 60, and 15 s at 70, 80 and 90°C, respectively, whereas *S. enterica* required 120, 90 and 25 s and *E. coli* O157:H7 required 210, 90, and 30 s at the respective temperatures. *E. faecium* only yielded 3.82 ± 0.25 log CFU/g reduction after 210 s at 70°C but achieved >5-log reduction within 90 s at 80°C and 30 s at 90°C. *Listeria* had lower D-values (40.08 ± 4.43, 11.52 ± 0.58, and 2.55 ± 0.20 s at 70, 80, and 90°C), whereas *E. faecium* had higher D-values (63.89 ± 11.07, 16.98 ± 1.48, and 5.63 ± 0.70 s) indicating most heat-resistant ($p < 0.05$). Steam treatment at 5D had minimal impact on kernel color while increasing moisture (5.56–7.82%) and water activity (0.57–0.63).

Significance: Steam treatment can be an effective post-harvest kill step for in-shell pecans and *Enterococcus faecium* served as a suitable surrogate during process validation.

Award Application: Developing Scientist Award

T10-05: A PLATFORM FOR HIGH-MASS-TRANSFER OZONE NANOBUBBLE DOSING WITH MINIMAL OFF-GASSING: PERFORMANCE ACROSS BENCH, PILOT, AND INDUSTRIAL FOOD PROCESSING APPLICATIONS

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Ozone is an effective antimicrobial; however, its adoption in food processing applications has been limited by the inability of systems to deliver sufficient ozone into solution without excessive off-gassing.

Purpose: This work evaluated a pure ozone nanobubble delivery platform specifically developed to maximize antimicrobial efficacy while minimizing atmospheric ozone release.

Methods: Over a multi-year development effort, the platform was evaluated across bench-, pilot-, and industrial-scale applications. Bench studies assessed reductions in pathogen levels in water and on relevant food-contact surfaces. Pilot-scale trials evaluated reductions of microbial indicators under continuous-flow conditions. Industrial trials assessed aerobic plate counts (APC), pathogen prevalence, and food-contact surface hygiene across protein and produce processing environments. In each trial, microbial outcomes were quantified, and dissolved and ambient ozone were monitored.

Results: Bench-scale testing demonstrated 3–4 log reductions of *Escherichia coli*, *Salmonella*,

and *Listeria* in water. Pilot-scale evaluations showed 2-log reductions of total coliforms and generic *E. coli* under flowing conditions. Industrial deployments across pork, poultry, produce, and human-grade pet food processing achieved 2–3 log APC reductions, consistent and significant reductions in *Salmonella* prevalence, and maintenance of pre-operational cleanliness levels on food-contact surfaces throughout production operations. Across two dozen trials, ambient ozone levels remained under OSHA safety limits (<0.1 ppm).

Significance: Pure ozone nanobubble delivery can overcome historical limitations, enabling antimicrobial performance across diverse applications. The platform supports ozone use in environments previously considered infeasible due to off-gassing and organic load constraints, positioning ozone nanobubbles as a viable non-chemical intervention for industrial food safety and sanitation systems.

T10-06: AI-DRIVEN ROBOTIC VISUAL INSPECTION AND INLINE SMART SENSING FOR REAL-TIME FOOD SAFETY CONTROL IN AUTOMATED PROCESSING LINES

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Increasing automation in food manufacturing has improved efficiency but has complicated real-time food safety inspection across complex processing lines.

Purpose: This study evaluated an AI-integrated collaborative robotic inspection system combined with inline smart sensors for hazard detection and food safety control during food processing.

Methods: A pilot-scale processing line was equipped with collaborative robots using AI-based visual inspection and inline smart sensors to monitor physical hazards, microbiological surrogate indicators (ATP-based hygiene proxies), and process hygiene parameters. The study included 120 production batches (average batch size: 500 kg) across three food matrices: ready-to-eat meat, fresh-cut vegetables, and dairy-based products. AI vision models were trained on 18,500 annotated images using cross-validation to detect foreign bodies, surface contamination patterns, and packaging integrity defects. Inline sensors

recorded temperature, moisture, and ATP-related hygiene proxies at 1-second intervals. Performance was compared with conventional manual inspection and periodic sampling. Data were analyzed using paired *t*-tests and logistic regression (α = 0.05).

Results: The robotic inspection system achieved a physical hazard detection sensitivity of 96.2%, compared with 78.4% for manual inspection ($p < 0.01$). Detection of early surface contamination indicators increased by 42%, allowing corrective actions before loss of process control. Inline sensors identified 18 process deviations per 1,000 batches versus 7 with traditional monitoring ($p < 0.05$). Undetected critical control point failures decreased from 3.1% to 0.9%, while false-positive rates remained below 4.5%.

Significance: AI-driven robotic inspection combined with inline sensing enables continuous, process-based food safety monitoring and reduces reliance on reactive end-product sampling, strengthening preventive control strategies in automated food processing.

T10-07: COLD PLASMA DISPROPORTIONATELY AFFECTS MICROBIAL DIVERSITY AND COMMUNITY COMPOSITION IN RAW CHICKEN

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Antimicrobial interventions can have a selective effect on certain microbial communities, affecting spoilage patterns and shelf life. Cold plasma induced modifications of the microbiota of meats have not been evaluated.

Purpose: Evaluate how cold plasma and plasma-activated H_2O_2 affect the microbial diversity and bacterial communities on raw chicken.

Methods: Chicken breast, drumstick, and skin sections (2×2×0.5 cm) were subjected to six treatments (control, H_2O_2, plasma 5 or 10 min, and plasma-activated H_2O_2 for 5 or 10 min (230V)) with three replicates (n=270). Samples were rinsed in Buffer Peptone Water, plated on Plate Count Agar, and incubated at 37°C for 48 h. Colonies were collected for DNA extraction and amplified for the full-length 16S rRNA gene using the 27F and 1492R primers. The PCR products were measured and sequenced on PacBio Sequel II. Similar reads (□97%) were grouped into OTUs using USEARCH. Each OTU was assigned a bacterial name in QIIME2 using the SILVA database. Alpha and beta diversity were analyzed using PCoA. ANOVA was used to compare groups, and LEfSe to identify which bacteria were more abundant within

treatments.

Results: *Pseudomonas lundensis* dominated untreated chicken samples (~0.99) and decreased after cold plasma treatment for 10 min (~0.01). Suppression of *Pseudomonas* (dominant taxon) led to treatment-dependent prominence of other bacteria [*Pantoea agglomerans* in breast (0.64) and skin (0.55)*, Bacillus* in 10-min plasma exposure (0.60), *Staphylococcus* in 10-min plasma-activated H_2O_2 (0.78)]. Phylogenetic tree analysis indicated that Gram-negative groups (*Pseudomonas, Pantoea*) contained multiple closely related lineages. Gram-positive groups showed either tight clustering (*Carnobacterium*) or clear separation into distinct lineages (*Bacillus).*

Significance: Cold plasma reduces dominant spoilage bacteria on raw chicken, and its effectiveness varies across chicken parts depending on the bacterial community present.

Award Application: Developing Scientist Award

T10-08: DEFINING ELECTROCHEMICAL PROCESS–STRUCTURE RELATIONSHIPS FOR SCALABLE MANUFACTURING OF MECHANO-BACTERICIDAL STAINLESS STEEL

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Electrochemically etched stainless steel 316L has been reported to inactivate foodborne pathogens, potentially through mechano-bactericidal (MB) disruption of cells arising from nanotexturing on 316L. However, the relationship between etching parameters and MB nanostructure formation remains poorly defined, impeding structural optimization and scale-up due to the narrow nano-texturing window within a vast multidimensional parameter space (e.g., voltage, electrolyte composition, etc.).

Purpose: This study tests whether polarization curves (current density vs applied voltage) can identify nanotexturing voltages, thereby accelerating optimization and scale-up of MB stainless steel.

Methods: We systematically examined the relationships between polarization curves and resultant nanostructures of 316L coupons etched in nitric acid (HNO_3) and perchloric acid

($HClO_4$) across a range of electrolyte concentrations.

Results: Atomic force microscopy (AFM) revealed that although etchant types (HNO_3, $HClO_4$), concentration, and etching area (0.38cm^2, 4.0cm^2) shifted the absolute nanotexturing voltages, nanotexture formation consistently occurred closely within the first peak of the corresponding polarization curves. Morphologies depended on etchant: HNO_3 promoted randomly-distributed, high-aspect-ratio nanostructures, with R_{rms} up to 7.0±0.8nm at 1.4V, whereas $HClO_4$ yielded periodic, parallel, jugged ridges at 3.4V (R_{rms}=4.3±1.7nm). At higher potentials associated with passivation and electropolishing, both systems transitioned to smooth surfaces with sparse features. Hydrodynamic factors, including etchant volume and stirring rate, had no significant influence on nanotexturing voltages (p>0.05), supporting reproducible nanostructure formation across setups.

Significance: This work demonstrates that polarization curves can guide etching condition selection to produce nanotextured, MB 316L, enabling faster morphological optimization and more efficient process scale-up.

T10-09: EVALUATION OF THE INACTIVATION CAPACITY OF LACTIC ACID AGAINST E. COLI O157:H7 ON WHOLE UNPEELED YELLOW ONIONS

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Fresh-cut onions have been linked to large multi-state outbreaks since 2020, with contamination sources still unclear. Soil, water, animal intrusion, and land-use history are implicated. Pathogens can persist through curing, transport, and storage and enter fresh-cut facilities—where no kill step exists—making validated sanitation and postharvest decontamination critical to outbreak prevention.

Purpose: The effectiveness of lactic acid (LA) as a postharvest sanitation treatment for reducing *Escherichia coli* O157:H7 (ECH) on the surface of whole, unpeeled yellow onions (WYO) was evaluated.

Methods: Five independent trials were conducted in a pilot-scale facility. In each trial, 24 bags (50 lb each) of WYO were dip inoculated with an ECH cocktail (□5 log CFU/onion) and held for 2 h. Three treatments were evaluated: 5.5% LA, 7.0% LA, and a natural die-off control. Bacterial recovery was assessed at 2, 24, and 192 h of storage at 5°C using five

replicates of eight onions per treatment. Surface recovery used moistened Micro-Tally® Mitt samplers, followed by centrifugation, plating, enrichment, and confirmation by selective and differential media, probe-based PCR, and MPN. Data were analyzed by ANOVA with mean separation at $\alpha = 0.05$.

Results: Lactic acid significantly reduced ECH recovery at all time points ($p < 0.05$). The control showed natural die-off, with recoveries >4 log CFU/sample at 2 h, 2.6 log CFU/sample at 24 h, and 0.9 log CFU/sample at 192 h. In contrast, both LA treatments showed no detectable ECH at 2 h and recoveries <0.4 log CFU/sample at 24 and 192 h, with LA-7.0% providing the greatest and most consistent reductions across all trials.

Significance: These results show that lactic acid is an effective postharvest intervention for whole onions, significantly reducing *E. coli* O157:H7 prior to fresh-cut processing. Validated LA treatments at this stage provide a critical preventive control in an industry with few effective alternatives.

T10-10: INACTIVATION OF SALMONELLA ON FROZEN RASPBERRIES, STRAWBERRIES, AND BLUEBERRIES USING A GAS PHASE HYDROXYL RADICAL PROCESS

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Frozen soft fruit have been implicated in foodborne illness outbreaks linked to contamination by bacterial pathogens. A challenge in decontaminating frozen soft fruit is the need to maintain the product in a frozen state and have no negative effects on quality.

Purpose: To optimize a gas phase hydroxyl-radical process for inactivating *Salmonella* on frozen soft fruit without negatively affecting product quality.

Methods: A cocktail of *Salmonella* (Enteritidis, Typhimurium, Montevideo, Newport, Dublin) was spray inoculated onto 50 g of frozen berries (raspberries, strawberries, or blueberries) that were returned to the freezer for 24h prior to testing. The operating parameters of the hydroxyl radical process were UV-C dose (177–355 mJ/cm^2) and 3% v/v hydrogen peroxide flow rate (20–40mL/min). The samples were returned to -20°C for 24h prior to determining *Salmonella* survivors and quality metrics (color).

Results: The average *Salmonella* loading on frozen berries was 4.98±0.16 log CFU/g. It was

found that the log reduction of *Salmonella* was independent of hydrogen peroxide flow rate and UV-C dose within the range tested. There was a significant difference ($p<0.05$) in *Salmonella* counts of hydroxyl radical-treated samples compared to non-treated controls. On blueberries, the maximal log count reduction (LCR) of *Salmonella* was 2.53±0.45. This was similar to strawberries that recorded a LCR of 2.22±0.14. Raspberries supported a greater LCR of 3.75±0.10. The berries remained frozen throughout treatment with no negative effect on color, except for raspberries which became lighter in color during post-treatment storage. This was reduced by post-treatment spray of catalase.

Significance: A gas phase hydroxyl radical process can inactivate *Salmonella* introduced onto frozen soft fruit without negatively affecting quality. The treatment provides a preventative control to enhance the food safety of frozen fruit.

Award Application: Developing Scientist Award

T10-11: INACTIVATION OF FOODBORNE MICROORGANISMS IN WHOLE MILK USING A DOUBLE HELIX UV-C SYSTEM

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Whole milk (WM) is an optically challenging matrix for UV-C processing due to its high fat and protein content, resulting in extreme absorption, scattering, and limited light penetration under continuous-flow conditions.

Purpose: This study evaluated the microbial inactivation kinetics of co-inoculated vegetative pathogens and a viral surrogate in WM using a custom-designed double-helix Dean-flow UV-C system.

Methods: WM (4.5% fat, 3.5% protein) was processed using a continuous-flow UV-C system made of fluorinated ethylene propylene tubing (4 mm internal diameter) double-helically wound around four centrally placed amalgam lamps (254 nm). Serpentine bends (3.1 cm radius) enhanced the radial mixing. The system was operated at a flow rate of 46 $L \cdot h^{-1}$ (Re = 1167, Dean number = 296, residence time = 20.97 seconds). WM was co-inoculated with MS2 bacteriophage*, E. coli* O157:H7, and *Salmonella* Muenchen, and microbial survivors were enumerated after successive UV-C passes using selective chromogenic media. Reduction equivalent fluence (REF) was quantified using MS2 inactivation kinetics.

Results: At 254 nm, WM exhibited absorption and reduced scattering coefficients of 30.72 ± 0.01 cm^{-1} and 53.57 cm^{-1}, respectively, with a refractive index of 1.397. MS2 exhibited log

reductions of 0.64 ± 0.14, 1.11 ± 0.06, and 1.84 ± 0.03 after the first, second, and third passes, corresponding to delivered fluence values of 13.34, 23.27, and 38.67 mJ·cm^{-2}, respectively, with linear inactivation kinetics ($R^2 > 0.99$). After two passes, co-inoculated *E. coli* O157:H7 and *Salmonella* Muenchen achieved 6.06-log and 5.51-log reductions, respectively, corresponding to a reduction equivalent fluence (REF) of approximately 24.85 mJ·cm^{-2}.

Significance: This next-generation UV-C system overcomes severe optical limitations of WM and achieves regulatory-relevant microbial inactivation under continuous-flow conditions, supporting its application as a non-thermal dairy processing technology.

Award Application: Developing Scientist Award

T10-12: EVALUATING THE EFFECTS OF WALL MATERIALS ON THE VIABILITY AND LONG-TERM STORAGE STABILITY OF BACILLUS COAGULANS ENCAPSULATED BY ULTRASONIC SPRAY FREEZE DRYING

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: *Bacillus coagulans* is a widely used probiotic with potential health benefits; however, its viability and stability can be reduced during ultrasonic spray freeze drying (USFD).

Purpose: The current study aims to investigate the effects of wall materials on the viability and long-term storage stability of *Bacillus coagulans* after USFD.

Methods: *Bacillus coagulans* (ATCC 8038) was cultured in 2 L tryptic soy broth (37°C, 150 rpm, 24 h), harvested by centrifugation (4500 g, 10 min, 4°C), and resuspended in 50 mL phosphate-buffered saline (PBS). Wall material consisted of 5% (w/v) maltodextrin and 5% (w/v) skim milk was mixed with cell suspension (1: 10, v/v) and atomized using an ultrasonic nozzle (50 kHz and 45% amplitude, air pressure 8 psi), and fed into liquid nitrogen (feed rate 10 mL/min), followed by freeze drying (□87°C, 40 mT, 48 h). A control was performed by mixing bacterial cells in PBS without wall material, and all experiments were conducted in triplicate. The lyophilized sample was stored at 22°C, 4°C, and -20°C over time to determine stability. The probiotic powders (100 ± 10 mg) were rehydrated with 9.9 mL PBS,

serially diluted, plated on tryptic soy agar, and incubated at 37°C for 48 h. Viable cell concentration (CFU/g) was measured on days 0, 7, and 14. Statistical analysis was performed using ANOVA and post hoc Tukey's HSD test ($\alpha = 0.05$).

Results: The viability of encapsulated *B. coagulans* was significantly improved with a 1.44 ± 0.10 log CFU/g reduction in viable cells, compared to control (2.56 ± 0.43 log CFU/g, $p < 0.05$). USFD-encapsulated *B. coagulans* exhibited significantly higher stability at -20°C with a 0.50 ± 0.07 log CFU/g cell reduction after 14 days, compared to 4°C, 22°C (1.12 ± 0.09 and 1.87 ± 0.37 log CFU/g, respectively, $p < 0.05$).

Significance: Encapsulation of *B. coagulans* with maltodextrin and skim milk enhanced the viability and long-term stability after USFD, showing promise as wall materials for probiotic protection.

Award Application: Developing Scientist Award

T11-01: PROCESS VALIDATION: CAN FERMENTATION TO REDUCE PH AND HPP ENHANCE PATHOGEN INACTIVATION DURING AIR DRYING OF DROËWORS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: 5 log reduction in *E. coli* during the manufacture of Droëwors presents challenges due to its unique drying process. The use of pH reduction and HPP in conjunction with air drying have not been assessed with respect to achieving a 5 log reduction in *E. coli*.

Purpose: Assess the effect of pH reduction by fermentation and HPP to enhance *E. coli* O157:H7 inactivation during air drying of Droëwors

Methods: Whole muscle beef trimmings inoculated (~8 log CFU/g) with a 5 strain cocktail of *E. coli* O157:H7 were dipped in Perasafe FC-100 (388ppm) and then minced coarsely (#3) and mixed with dry spice seasoning (0.6% dextrose, 1.8% salt, 123.8ppm nitrite) stuffed into 20mm sheep casing and dried at 26°C and 80% RH for 24h and then at 20°C and 30% RH for up to 10 days to manufacture Droëwors. 3 batches without or with starter culture (USP-100) to reduce pH were produced and subjected to HPP (600 MPa, 180s) when the Droëwors reached a_w of 0.85 and 0.8. Samples were plated on SMAC and log reduction values from *E. coli* counts were analyzed using GLM with post-hoc pairwise comparisons (emmeans, Tukey HSD, R 4.4.0).

Results: Droëwors reached a shelf-stable a_w of <0.85 within 5–7 days of drying, and a pathogen reduction of 3.29±0.05-3.47±0.03 log CFU/g. Inclusion of a starter culture reduced the pH from 5.42±0.02–4.73±0.03 during the same time, but failed to result in any significant increase in pathogen reduction compared to Droëwors produced without starter culture (pH 5.47±0.02). HPP of Droëwors with a a_w of 0.85 and 0.80 resulted in a further pathogen inactivation of 2.45±0.03 log CFU/g, resulting in a □5 log CFU/g pathogen inactivation.

Significance: HPP at 600 MPa for 180s can be successfully used in conjunction with drying to achieve a □5 log *E. coli* O157:H7 inactivation and is most effective when HPP is applied prior to the Droëwors reaching a aw of □0.80. Processors can use HPP without any significant modification to their drying process to produce a safe product.

T11-02: VALIDATION OF A NEW METHOD FOR ENUMERATION OF CAMPYLOBACTER SPP. IN POULTRY: OUTCOMES FROM A NATIONAL RING TRIAL

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Campylobacteriosis is a notifiable foodborne illness in New Zealand, predominantly attributed to poultry. Through sustained, multi-year regulatory and industry interventions, New Zealand Food Safety (NZFS), with the poultry industry, achieved a 20% reduction in campylobacteriosis notifications (from 88 to 70/100,000 population). NZFS continues efforts for further reductions by 2030. However, as industry hygiene practices improve, more poultry carcasses show *Campylobacter* levels below the detection threshold (2.3 $\log_{10}$ CFU per carcass rinsate) of the current National Microbiological Database (NMD) method. To address this, a more sensitive Relative Centrifugal Force (RCF) method was developed by adding centrifugation to the NMD protocol.

Methods: The new method was initially validated by the reference laboratory, followed by an interlaboratory study involving six accredited laboratories and the reference laboratory. Each of the seven laboratories analyzed 12 samples, for a total of 84 samples, with high or low inoculum (~10^4 and ~10^2 CFU/70 mL) of the reference strain *Campylobacter jejuni* NZRM 3498/ NCTC 11392 in a chicken rinsate matrix, using RCF and NMD methods.

Results: Although RCF yielded slightly lower mean counts (3.91 $\log_{10}$) than NMD (4.00 $\log_{10}$),

it demonstrated improved reproducibility, with a lower coefficient of variation (RCF: 4.9%, NMD: 6.0%). At the lower inoculum level, 41 out of 42 samples returned detectable results with the RCF method compared to only 30 using the NMD method, showing RCF lowered detection limit (1.47 $\log_{10}$ CFU per carcass rinsate).

Significance: Further validation using poultry-derived *Campylobacter* strains is underway, with the method showing potential to support more sensitive enumeration in poultry monitoring programs where pathogen prevalence and concentrations are declining.

T11-03: COMPARATIVE EVALUATION OF ILLUMINA, OXFORD NANOPORE, AND PACBIO SEQUENCING PLATFORMS FOR METAGENOMIC RECOVERY AND PHYLOGENETIC ANALYSIS OF STEC O157:H7 FROM BACTERIAL ENRICHMENTS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Accurate metagenomic analysis and pathogen genome recovery from bacterial enrichments, especially Shiga toxin-producing *Escherichia coli* (STEC), is critical for foodborne pathogen surveillance and outbreak investigations. Sequencing platform selection impacts genomic data quality and utility for public health use.

Purpose: This study compared three sequencing methods, Illumina (A), Oxford Nanopore Technology (B), and Pacific Bioscience (C), for their ability to accurately perform metagenomic analysis and recover STEC O157:H7 genomes from bacterial enrichments.

Methods: Romaine lettuce plants were sequentially harvested using knives contaminated with STEC O157:H7 at two inoculum levels (5.4 or 2.4 $\log_{10}$ CFU/ml) or PBS controls. In total, 91 lettuce heads were analyzed for O157:H7 presence using modified FDA-BAM methods. Five enrichment samples (3 positive and 2 negative by qPCR for STEC) were sequenced on a

NextSeq 2000, a GridION and a Sequel IIe. Bioinformatics analysis was performed using Centriflaken. It assembles the reads using flye assembler, classifies the contigs with Kraken2, and performs in silico analysis for MLST, AMR, serotyping, and virulotyping.

Results: Method B generated the longest reads and achieved the most complete genome assemblies with better synteny than C, successfully closing genomes for the spiked O157:H7 strain. C assemblies demonstrated superior nucleotide accuracy and correctly placed assemblies within phylogenetic clusters during SNP analysis. Both B and C platforms successfully identified the correct serotype (O157:H7), sequence type (ST-11), and virulence gene profiles. A assemblies were highly fragmented and failed to accurately match the spiked strain in phylogenetic analysis.

Significance: While B excelled at genome completion, C provided superior accuracy for SNP-based phylogenetic analysis. Both long-read platforms significantly outperformed A for metagenomic recovery of STEC O157:H7 genomes from enrichment cultures.

T11-04: PERACETIC ACID INDUCES VBNC SALMONELLA TYPHIMURIUM IN PRODUCE WASH WATER

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Sublethal sanitizer exposures can cause bacteria to enter a viable but non-culturable (VBNC) state, potentially limiting assessments of sanitizer efficacy. The impacts of peracetic acid (PAA) on VBNC in foodborne pathogens and in the context of microbial interactions in wash water systems remain unclear.

Purpose: To evaluate VBNC in *Salmonella* Typhimurium following PAA treatment in the presence and absence of antimicrobial-resistant (AMR) plant-associated commensals.

Methods: *S.* Typhimurium was exposed to 10, 20, and 30 ppm PAA in water for 1 min. After treatment, 0.1% sodium thiosulfate was used to neutralize residual sanitizer, and cells were enumerated via plate count. In parallel, total viable cells were quantified using propidium monoazide (PMA)-qPCR targeting *invA*. To evaluate wash water-relevant microbial interaction effects on *S.* Typhimurium response to PAA, AMR strains of *Serratia ureilytica* and *Pseudomonas fulva* that were previously isolated from leafy greens were co-inoculated with the pathogen 1:1 prior to 20 ppm treatment.

Results: In the model system, *S.* Typhimurium starting at 8.6 log CFU/ml was reduced to 4.5 log, 1.5 log, and to a level below detection limit (< 1 log CFU/ml) following 10, 20, and 30 ppm exposure, respectively. In contrast, PMA-qPCR detected persistent populations around 7.3 (2.8-VBNC), 7.1 (5.6-VBNC), and 6.4 (6.4-VBNC) log cells/ml, respectively. Co-inoculation with *S. ureilytica* protected *S.* Typhimurium, yielding 4 log and 1 log higher concentrations when using plate count and PMA-qPCR methods. *P. fulva* also conferred protective effects.

Significance: Sanitizers are routinely used in fresh produce wash water to reduce pathogen loads and prevent cross-contamination. Our pilot study indicates that PAA sanitizer can induce a VBNC state in *S.* Typhimurium that can be enhanced by background microbiota. These findings highlight limitations of using culture-based methods alone for evaluating sanitizer performance.

T11-05: SEASONAL CHARACTERIZATION OF THE BUFFALO MILK MICROBIOME IN SOUTH AUSTRALIA

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Buffalo milk contains a diverse microbial community with potential relevance to probiotic development, yet its microbial ecology remains largely uncharacterized in Australia.

Purpose: This study aimed to define the microbial composition and seasonal dynamics of raw buffalo milk from South Australia, with emphasis on the persistence of probiotic-associated taxa.

Methods: Raw milk samples were collected monthly over one year. In total, 36 samples were analyzed by 16S rRNA gene sequencing to characterize community structure and diversity, and one pooled seasonal sample per season was analyzed using shotgun metagenomics to assess functional potential. Alpha and beta diversity analyses were performed to determine seasonal variation.

Results: Both sequencing approaches reveal a complex, seasonally dynamic microbiome. Diversity was lowest in summer and highest in autumn ($p < 0.05$). Across 15 detected phyla, temporal shifts were observed in Actinobacteria, Proteobacteria, and Firmicutes, with clear seasonal clustering ($p < 0.001$). These shifts may influence raw milk quality and the stability of probiotic-associated taxa. Genus-level shifts were observed across seasons, with cooler months enriched in *Staphylococcus*, *Alloiococcus*, and *Brachyspira*, and warmer months enriched in *Aeromonas* and *Pseudomonas*. Shotgun sequencing confirmed

Pseudomonas dominance in autumn and identified *Enterobacter* as prevalent in summer. Importantly, lactic acid bacteria were consistently detected year-round, indicating that these probiotic-associated genera form a stable component of the raw milk microbiome despite broader seasonal fluctuations.

Significance: This first year-round characterization of Australian buffalo milk provides foundational insights into its microbial ecology. These findings support evidence-based strain selection, functional assessment, and the development of safe fermented buffalo-milk products for the Australian dairy industry.

T11-06: SALMONELLA SEROVAR INFANTIS REPJFX01 GEOGRAPHIC SOURCE ATTRIBUTION THROUGH CORE SNP AND PHYLOGEOGRAPHIC ANALYSES

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: REPJFX01 is a multidrug-resistant *Salmonella* Infantis REP (reoccurring, emerging, and persisting) strain associated with sporadic illnesses and outbreaks in the U.S. linked to chicken consumption and international travel.

Purpose: Identify single nucleotide polymorphisms (SNPs) for geographical source attribution and assess the global emergence and spread of REPJFX01.

Methods: Assemblies of (a) clinical U.S. REPJFX01 isolates with international travel data collected by FoodNet (n=390), (b) non-U.S. and non-clinical REPJFX01 isolates (n=274), and (c) previously analyzed REP and non-REP *S.* Infantis isolates (n=228) were obtained from NCBI

Pathogen Detection. kSNP4 was used to create a phylogenetic tree based on core SNPs of all genomes (n=892) and to identify core SNPs for geographic source attribution. Additionally, 232 isolates were selected for phylogeographic analysis using BEAST X.

Results: Overall, 28/30 REPJFX01 isolates associated with international travel destinations clustered with environmental isolates from the travel destination. We identified one core SNP that supports assignment of clinical isolates to the South America/Asia clade with 99% sensitivity and 96% specificity. We also identified 25 other core SNPs that were associated with isolates from either a continent or four specific countries. The phylogeographic analysis indicated that the common ancestor of REPJFX01 existed in Peru ca. 2009 (Prob.=1) while the ancestor of the U.S. isolates existed ca. 2010 (Prob.=0.92).

Significance: These results demonstrate that SNPs can help determine whether an infection with REPJFX01 was acquired domestically or internationally prior to an interview. Identifying potential events leading to spread of REPJFX01 can support more targeted public health guidance.

T11-07: BIO-MAPPING AND SANITATION ASSESSMENT OF FOODBORNE PATHOGENS IN A SMALL-SCALE POULTRY PROCESSING PLANT

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Identifying contamination points for foodborne pathogens like *Salmonella* and *Campylobacter* during poultry processing remains a food safety priority.

Purpose: This longitudinal study evaluated foodborne pathogens on carcasses and processing water rinses across processing stages and equipment.

Methods: A small processing plant was visited twice monthly for 11 months (each visit represents a different flock). Samples collected include carcass and water rinses (N=220) from five key processing stages: live receiving, rehang, ceca, bird wash and post-chill, with eight equipment swabs at pre/post-sanitation (N=352). Samples were analyzed for *Salmonella* and *Campylobacter* using standard culture methods and quantified with the BAX® system. Confirmed isolates were identified and data were analyzed using Pearson's Chi-square test in JMP.

Results: Overall, *Salmonella* and *Campylobacter* prevalence was 34% (196/572) and 43% (244/572), respectively. In carcass rinse, *Salmonella* was significantly higher ($P<0.05$) at receiving, rehang, and bird wash, with a 23% reduction from receiving to post-chill. *Campylobacter* was similar ($P>0.05$) across processing stages with 9% reduction. In general, both pathogens were low in water rinse samples. *Salmonella* load ranged from 0 to 4.4 $\log_{10}$

CFU/mL, while *Campylobacter* was 1.0-5.0 log CFU/mL in the carcass rinse. Impressively, sanitation reduced prevalence on equipment by 36% (*Salmonella*) and 56% (*Campylobacter*), with low levels of both pathogens on the evisceration equipment. Pathogen load reduced from an average of 0.14 log CFU/mL (*Salmonella*) and 1.93 log CFU/mL (*Campylobacter*) to below detection limit post sanitation. A total of six *Salmonella* serogroups were detected and C_2-C_3 (78%) was the most abundant, while *C. jejuni* dominated the samples (48%), with some mixed *coli/jejuni* observed.

Significance: Identifying key contamination points during processing can help further reduce pathogen contamination and enhance food safety equally.

Award Application: Developing Scientist Award

T11-08: MECHANISMS OF SALMONELLA SURVIVAL AND CROSS-CONTAMINATION DYNAMICS DURING VARIOUS STAGES OF POULTRY PROCESSING

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Immersion scalding/chilling are vital processing steps but may permit bacteria cross-contamination when water quality or sanitizer performance are compromised.

Purpose: This study quantified *Salmonella* transfer dynamics for simulated poultry scalding and chilling, evaluating the impact of peracetic acid (PAA), water volume, pH, temperature, and organic load on controlling cross-contamination.

Methods: Fresh chicken thighs/legs (100±20 g; 20 pieces/run) were used in scalding (45–60°C; pH 4, 7, 10) and chilling (4°C; 2–5 L) systems. A five-serovar *Salmonella enterica* cocktail (10^7–10^9 CFU/mL) was applied to chicken pieces or directly to tank water. Cross-contamination was evaluated with PAA (0–75 ppm), under continuous/batch dosing. Water chemistry (PAA, pH, total dissolved solids, conductivity) was tracked and bacterial levels in water/on chicken were quantified by plate counts (BGS agar; 10^2 CFU/mL detection limit). Experiments were performed in triplicate.

Results: In PAA absence, *Salmonella* rapidly disseminated, reaching 9.0×10^6 CFU in 5 L of

chiller water and 3.2×10^5 CFU per uninoculated piece within 30 min. When tank water contained 1.7×10^9 CFU/mL *Salmonella*, uninoculated pieces accumulated 2×10^6 CFU within 10 min. Continuous PAA showed a concentration response: 20 ppm allowed steady water contamination (up to 2.9×10^6 CFU in 5 L), 30 ppm reduced but didn't eliminate transfer, 50 ppm PAA drove water contamination below detection with sporadic surface recoveries (7×10^3 CFU), and 75 ppm PAA eliminated detectable cross-contamination. During scalding, acidic conditions (pH 4) showed greatest reductions, with 1Log lower survival than pH 7, 10 at 52–56°C, even with high organic load.

Significance: Immersion systems can rapidly amplify *Salmonella* contamination without proper controls. Maintaining 75 ppm PAA in chillers and acidic scalding conditions are critical to prevent cross-contamination, thus improving food safety and public health outcomes.

T11-09: EVALUATION OF THE INFECTIVITY OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) VIRUS AND LOW PATHOGENIC AVIAN INFLUENZA (LPAI) VIRUS FROM A BEEF SLURRY

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Highly pathogenic avian influenza (HPAI) has spread to cattle across the United States. As this is a novel viral infection of beef and dairy cattle, there are several relevant food safety research questions to be answered.

Purpose: To understand the survivability and infectivity of HPAI virus in a relevant food matrix.

Methods: Infectious HPAI and Low Pathogenic Avian Influenza (LPAI) virus were added to 100 mL of 10% beef slurry or water at 6.5 $\log_{10}$ 50% embryo infectious doses (EID_{50}) per mL of slurry or water. Samples were held at 37°C and pH 5.0 then collected at 0, 3, 7, 9 and 15 hrs. Samples were clarified through centrifugation before testing for virus genetic material using qPCR and viability by inoculating 10-day-old specific pathogen-free embryonated chicken eggs (n=4 eggs per experimental condition). Embryo survival was monitored for six days, and chorioallantoic fluid from eggs exhibiting mortality between two and six days was harvested. Hemagglutination (HA) activity was assessed using chicken red blood cells to confirm viral presence in the samples. Two complete independent trials were conducted.

Results: At 0 hrs, HPAI virus inoculated water showed no reduction in virus titer while HPAI virus inoculated beef slurry resulted in a 4-4.3 log reduction. From 3 hrs to 15 hrs, both HPAI and LPAI virus from beef slurry showed 0 titer of infectious virus from eggs with the exception of trial 1 at 7 hrs with HPAI virus. Across the entire experiment in both trials PCR Ct values averaged 22.2 ± 1.7 indicating substantial virus genetic material in all samples.

Significance: Immediately upon inoculation a beef slurry food matrix reduces HPAI virus infectivity but not LPAI virus infectivity under identical conditions. No infective virus was found at 9 hrs or later in any of the beef slurry samples.

T11-10: LISTERIA SPP. AND LISTERIA MONOCYTOGENES RESULTS FROM FSIS-INSPECTED READY-TO-EAT PRODUCTS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The Food Safety and Inspection Service (FSIS) began sampling for *Listeria* species (spp.) in addition to *Listeria monocytogenes* (*Lm*) in ready-to-eat (RTE) meat and poultry products in January 2025. FSIS made the sampling changes in response to recent listeriosis outbreaks from meat and poultry products, resulting in multiple illnesses and deaths.

Purpose: FSIS analyzed sampling results to identify risk factors associated with *Lm* contamination which can be used to inform food-safety decisions.

Methods: Samples were analyzed for non-*Lm Listeria* spp. and *Lm* following the procedures outlined in *FSIS Microbiology Laboratory Guidebook*, Chapter 8.15. Enriched samples were initially screened using the Neogen® Molecular Detection System. Positive results were confirmed using selective and differential media; *Listeria* isolates were identified and verified

with the Bruker Biotyper®.

Results: From January 17 to July 31, 2025, FSIS collected product, food contact surface (FCS), and non-food contact surface (NFCS) samples from 2,277 RTE establishments. Overall, 77/17,708 (0.43%) samples tested positive for *Lm*, 150/17,698 (0.85%) tested positive for non-*Lm Listeria* spp., and 16/17,708 (0.09%) tested positive for both. The types of non-*Lm Listeria* spp. found included *L. grayi*, *L. innocua*, and *L. welshimeri*. Samples taken from NFCS were more likely to contain *Lm* and non-*Lm Listeria* spp. (range: 1.8–11.4%) than products or FCS (range: 0.1 – 0.7%).

Significance: The results demonstrate that there were about twice as many non-*Lm Listeria* spp. positive samples as *Lm*. In addition, positive results were found at a higher rate in NFCS samples than in FCS and product samples. Testing for non-*Lm Listeria* spp. and continuing to test NFCS samples provides useful information to determine if sanitation has been effective at reducing *Lm* in establishments. FSIS can use these data to help protect public health by keeping contaminated products from reaching the marketplace.

T11-11: STARTER CULTURE EFFECTS ON SALMONELLA ENTERICA AND SHIGA-TOXIGENIC ESCHERICHIA COLI INACTIVATION KINETICS DURING SALAMI FERMENTATION

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The microbiological safety of uncooked fermented and dried salami relies greatly on achieving rapidly sufficient lactic acid fermentation to control foodborne pathogens.

Purpose: This study evaluated 3 commercial starter cultures (A, B, and C) on the inactivation kinetics of a 7-serotype *Salmonella enterica* and 7-strain mix of O157 and non-O157 Shiga-toxigenic *E. coli* (STEC) cocktail during salami fermentation.

Methods: Salami batter bearing a starter culture and pathogens cocktail was held 18 h at 2±1°C, then stuffed in edible collagen casings, clipped (22 cm length x 19 mm diameter; 14 cm length x 32 mm diameter), then fermented to a target pH of 5.0. Fermentation environment conditions were set to 35±1°C with ERH 93±1%. Sample links were prepared in duplicate over three independent trials (N=6) and tested every 2 h for pathogen survival, product pH and temperature. A Weibull model was applied to parameterize pathogen reduction dynamics, including reduction rate, time to target pH, and maximum log CFU reduction.

Results: Starter culture significantly influenced pathogen reduction kinetics for both *Salmonella* and STEC for both casing diameters. *Salmonella* reductions produced by starters A and B did not differ from each other, while starter C produced statistically lower pathogen reduction versus A and B (p=0.011: SE=0.012). Starters A and B achieved maximum *Salmonella* reductions approximately 10 h into fermentation; C required 12–14 h. Starter A exhibited the highest *Salmonella* total reduction (1.104 log CFU) whereas C produced lowest *Salmonella* reduction (0.848 log CFU). For STEC, maximum reductions did not differ across starter cultures (p=0.051). STEC reductions were similar for A (0.633 log CFU) and B (0.613 log CFU), greater than C (0.332 log CFU).

Significance: Starter culture selection plays a critical role in controlling fermentation-associated pH decline and pathogen reduction in fermented dried salami, towards broader salami safety validation.

Award Application: Developing Scientist Award

T11-12: LONG-TERM SURVIVAL OF LISTERIA MONOCYTOGENES IN COOKED PORK SAUSAGES FORMULATED WITH CLEAN-LABEL ANTIMICROBIALS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Growing demand for clean-label meat products has increased interest in nitrite removers and replacers, yet their long-term impact on *Listeria monocytogenes* survival remains poorly understood.

Purpose: To evaluate the effect of clean-label antimicrobials on the survival of *L. monocytogenes* in cooked pork sausages during extended refrigerated storage.

Methods: Ground pork was formulated with six treatments: four nitrite removers (NR1–NR4), a positive control, and a negative control. The formulations were as follows: NR1 (1.25% fruit and spice extract [FSE] + 0.94% buffered dried vinegar [BDV]), NR2 (1.25% FSE + 1.25% BDV), NR3 (1.25% FSE), NR4 (1.25% FSE + 1.56% grape pomace extract [GPE]), and the positive control (0.34% celery powder + 0.94% BDV). Samples were vacuum-sealed, cooked in a water bath to an internal temperature of 71 °C, and inoculated with *L. monocytogenes* ATCC 19115. Initial populations were enumerated on Day 0, and samples were stored under refrigeration and analyzed on Days 1, 3, 5, 7, 14, 28, 42, 56, 70, 84, 98, 112, and 126 using two independent replicates.

Results: Initial *L. monocytogenes* levels (log CFU/g) were 3.50 ± 0.22 (NR1), 3.31 ± 0.39 (NR2), 3.40 ± 0.07 (NR3), 3.37 ± 0.01 (NR4), 3.21 ± 0.14 (positive control), and 3.46 ± 0.18 (negative control). One-way ANOVA with Tukey-Kramer post hoc analysis showed that on Day 7, the negative control (4.31 ± 0.03 log CFU/g) was significantly higher than all other treatments. Similar trends were observed on Days 28 and 56. By Day 84, no significant difference was detected between positive and negative controls; however, both differed significantly from NR1, NR2, and NR4, while NR3 differed only from the negative control.

Significance: Nitrite removers and replacers demonstrated a sustained bacteriostatic effect against *L. monocytogenes* during extended refrigerated storage, supporting their potential use as effective clean-label alternatives in RTE meat products.

Award Application: Developing Scientist Award

T12-01: WATER AS A SOURCE AND TRANSMISSION PATHWAY FOR MICROBIAL CONTAMINATION AT DAIRY FARMS

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: Water is a potential source of microbial contamination in milk on dairy farms, yet regulatory monitoring primarily targets coliforms and *E. coli* despite the presence of other spoilage- and safety-relevant microbes such as *Pseudomonas* and *Listeria*.

Purpose: Here we aim to establish data on prevalence and concentration of dairy relevant microbes in dairy farm water supplies.

Methods: Between 2024–2025, water samples (n = 198) were collected from inlets and points of use on 50 dairy farms in New York State during warmer and colder months. Farms were stratified by water source (i.e., ground, surface, and municipal). Five microbial indicators, heterotrophic plate count (HPC), Gram negative bacteria (GN), aerobic spore count (ASC), yeast and mold (YM), and total coliform (CC), were quantified using membrane filtration and plate enumeration, physicochemical parameters by standard chemical methods, and *Listeria* by FDA BAM with confirmation. Season, source, and location effects were evaluated using nonparametric tests, correlation analyses with false discovery rate adjustment, and mixed-effects models (GLMMs/LMMs).

Results: All five microbial indicators were detected across dairy farm water samples, with higher median HPC and GN (2.9 and 2.4 $\log_{10}$ CFU/100 mL) than ASC, CC, and YM (0.7–1.3 $\log_{10}$ CFU/100 mL. The HPC, CC, and GN were higher in warmer than colder months (p □ 0.016) and increased with temperature (p □ 0.003), while ASC and YM showed no association (p □ 0.14). *E. coli* (16.7%) and *Listeria* (11.2%) were detected, with *L. monocytogenes* comprising 25% of *Listeria* isolates and no temperature association (p □ 0.10). Water source influenced microbial loads, with ASC, HPC, and GN differing across sources (p □ 0.028). Microbial–physicochemical correlations were generally weak (Spearman $|\rho| < 0.3$).

Significance: These findings support the need for expanded risk-based water monitoring strategies to reduce microbial contamination on dairy farms.

Award Application: Developing Scientist Award

T12-02: INFLUENCE OF ENVIRONMENTAL BACKGROUND MICROFLORA ON BIOFILM FORMATION BY LISTERIA MONOCYTOGENES ON DAIRY FLOOR SURFACES

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: *Listeria monocytogenes* and background microflora can establish persistent biofilms on floor surfaces in dairy processing facilities, increasing the risk of environmental contamination.

Purpose: This study assessed the impact of an environmental background isolate on *L. monocytogenes* biofilm formation in mixed-species biofilms under nutrient-limited conditions on two dairy floor surfaces.

Methods: Dairy environment isolates of *L. monocytogenes* and background microflora represented by *Serratia marcescens* were used to develop biofilms on 1×1 in^2 clay brick and poured concrete floor tiles (n = 3 per organism per surface per sampling point) at 22°C using dairy effluent (0.02% total solids) as the nutrient source. An initial inoculum of 7 $log_{10}CFU/in^2$ was used for both single and mixed species biofilms. Biofilms were incubated for 1, 3, and 5 days with daily medium replacement and enumerated on TSA (total counts) and MOX agar (*Listeria*).

Results: *L. monocytogenes* formed robust biofilms on both surfaces, exhibiting a consistent

increase in cell density over the 5-day period. By day 5, *L. monocytogenes* reached 5.70 ± 0.40^{a} $\log_{10}$CFU/in^2 in single-species and 6.85 ± 0.60^{b} $\log_{10}$CFU/in^2 in mixed-species biofilms on clay brick, indicating a significant increase in the presence of *S. marcescens* ($p < 0.05$). On poured concrete, *Listeria* biofilm counts increased from 6.20 ± 0.45^{a} $\log_{10}$ CFU/in^2 in single-species to 7.40 ± 0.70^{b} $\log_{10}$ CFU/in^2 in mixed-species biofilms by day 5 ($p < 0.05$). Across conditions, poured concrete supported greater overall *Listeria* biofilm, and co-culture with *S. marcescens* consistently promoted higher biofilm biomass, underscoring the potential for background microflora to enhance *Listeria* biofilm on dairy floor surfaces.

Significance: These findings show that *L. monocytogenes* forms robust biofilms under nutrient-limited conditions and that mixed species interactions with *S. marcescens* further enhance its persistence.

Award Application: Developing Scientist Award

T12-03: PRESERVING ARKANSAS PARTNERSHIP SUPPORTING NEW AND ESTABLISHED COTTAGE FOOD PRODUCERS IN FOOD PRESERVATION, SAFETY, AND REGULATORY COMPLIANCE

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Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: Cottage food and home-based businesses are growing as accessible paths to entrepreneurship, yet many producers lack knowledge of food safety and regulatory compliance. Misunderstanding processing techniques, labeling, and regulations can increase food safety risks.

Purpose: A collaborative approach with the Family and Consumer Sciences food preservation team, a local chef, and the Share Grounds: Value-Added Startup Program, provides targeted education on evidence-based preservation practices, food safety principles, and regulatory requirements. By emphasizing practical application and clarity, the program aims to build producer confidence, improve compliance, and support safe, sustainable home-based food enterprises.

Methods: In 2025, three half-day workshops focused on food preservation, safety, and regulatory practices for cottage food producers. Two sessions were hands-on in a commercial kitchen using seasonal produce, allowing participants to practice techniques and take home a product. The third was a slide-based session at a farmers' market. Topics included approved recipes, pH testing, canning methods, equipment, recipe modification, home kitchen GMPs, food safety risks, scaling, and food freedom laws, including differences between home and commercial practices. Knowledge gains were measured with pre- and post-tests, and evaluations captured feedback on content and delivery.

Results: Among 34 participants, evaluations showed measurable knowledge gains across preservation, safety, and regulatory topics. Many reported limited prior food safety and liability risks knowledge. Nearly all expressed high satisfaction and interest in future programs, highlighting hands-on learning value.

Significance: By fostering safer practices and regulatory awareness, this initiative strengthens regional food systems by supporting cottage food producers and creating sustainable economic opportunities for small-scale farmers and home-based food entrepreneurs.

T12-04: ENHANCING FOOD SAFETY AND REGULATORY UNDERSTANDING FOR NORTH CAROLINA'S LOCAL FOOD INDUSTRY USING A MULTI-TIERED TRAINING FRAMEWORK

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Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: North Carolina has a growing market for home food processors, shared-use kitchens and locally produced foods; however, food safety regulations and marketing requirements pose challenges to entrepreneurs.

Purpose: This project aims to enhance educational capacity among extension personnel and professionals to support the NC food industry through a three-tiered delivery approach.

Methods: The tiered approach included 1) a webinar series; 2) in-person workshops for food safety professionals and entrepreneurs; 3) and an online, public resource repository. The webinar series and in-person workshops were delivered in partnership with state regulators

to address food safety concerns and regulatory jurisdiction as identified from stakeholder surveys. The project will host a second series of in-person workshops for entrepreneurs, hosted in partnership with previous years' attendees. The online repository provides fact sheets, templates and regulatory resources for stakeholders.

Results: A series of seven webinars reached 172 live viewers and 9,279 recorded views. Webinar evaluations showed 94% (n=34) of respondents (N=36) strongly or somewhat agreed the content helped their understanding of food safety or regulations. A series of four workshops reached 65 professionals, where 98% (n=44), CI [89.3, 99.6] of respondents (N=45) strongly agreed the event was valuable, and 100% of respondents stated they learned new topics relevant to job responsibilities. Good Manufacturing Practices and labeling sessions were the most effective, with 96% and 92% of respondents strongly or somewhat agreeing, respectively. The repository has averaged 428 monthly views, with food safety templates being the most utilized.

Significance: This project, in partnership with regulatory agencies and local initiatives, has shown to be effective for increasing educational capacity. While currently aimed at food safety, the approach has applications for success in other training programs.

T12-05: FROM REMOTE AUDITS TO ROBOTIC INSPECTION: A LONGITUDINAL CASE STUDY ON THE EVOLUTION OF DIGITAL TOOLS IN FOOD SAFETY VERIFICATION (2018–2026)

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Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: Food safety inspections have traditionally relied on on-site presence, limiting flexibility and continuity in complex and geographically distributed food systems. Evidence from long-term operational use of digital inspection tools remains limited.

Purpose: This study evaluates the adoption of digital, immersive, AI-based, and robotic technologies to support food safety inspection and verification activities over an eight-year period, focusing on inspection practice and regulatory use.

Methods: A longitudinal, multi-phase case study was conducted from 2018 to 2026 through pilot projects in Italy and selected international contexts. In 2019, remote audits using smart glasses were piloted in 16 food companies. In 2021, mixed-reality inspections using Microsoft HoloLens were tested with Official Veterinary Services and a national dairy association. In 2023, AI-supported inspection tools for anomaly detection and decision support were evaluated. In 2024, an integrated digital inspection environment combining immersive platforms, smart glasses, and AI assistance was implemented. Current activities (2025–2026) focus on robotic systems supporting monitoring and inspection tasks. Data collection included structured observations, inspector feedback, and operational indicators.

Results: Remote and mixed-reality inspections supported audit continuity, reduced travel, and improved real-time interaction. AI-supported tools improved consistency in documenting findings and supported risk-based prioritization. Early robotic applications showed potential to standardize repetitive inspection tasks and reduce human exposure in high-risk settings. Key constraints included acceptance of digital evidence, data governance, cybersecurity, and inspector training needs.

Significance: Phased adoption of digital, AI-based, and robotic tools can support food safety verification when aligned with inspection practice and regulatory requirements.

T12-06: POWDERED INFANT FORMULA SAFETY: CAREGIVERS' KNOWLEDGE, PRACTICES, AND RISK AWARENESS

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Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: Powdered infant formula (PIF) is widely used to feed infants, yet multiple recalls have highlighted its potential safety risks. Although regulators and industry stakeholders play critical roles in ensuring the safety of PIF, caregivers also directly influence infant health outcomes through their formula preparation and handling practices, including the potential for cross-contamination during formula preparation.

Purpose: This study aimed to explore caregivers’ food safety knowledge, preparation, handling and storage practices of PIF.

Methods: An online survey was distributed via Qualtrics (October and November 2025) to a quota sample of U.S. caregivers who were taking care of an infant between 0–12 months old and were preparers of PIF for the infant. The survey was reviewed by four food safety experts. The questions included sections on caregivers’ handling, perceptions, and knowledge of PIF preparation.

Results: Out of the 714 caregivers surveyed, more than 90% reported to prepare PIF using water at temperatures below 158°F (70°C). Approximately, 10% reported leaving prepared formula at room temperature for more than 4 hours and 24% stored formula leftovers for later use. Additionally, 30% were unaware that PIF is not a sterile food product. Pediatricians

and family members were identified as the most common sources of guidance on formula preparation.

Significance: This study provides valuable insights into effective risk communication with caregivers and practical risk prevention in the home, highlighting the need for clearer, targeted guidance to reduce foodborne illness risks and better protect infants.

Award Application: Developing Scientist Award

T13-01: EVALUATING INORGANIC ARSENIC (IAS) CONCENTRATION, CONSUMPTION, AND EXPOSURE FROM INFANT RICE CEREAL BEFORE AND AFTER THE ISSUANCE OF FDA'S IAS IN RICE CEREAL DRAFT GUIDANCE

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Presentation type/Format: Technical

Category: Food Chemical Hazards

Abstract:

Introduction: In April 2016, the FDA published the draft *Inorganic Arsenic in Rice Cereals for Infants: Action Level Guidance for Industry* proposing an action level of 100 parts-per-billion (ppb) iAs in infant rice cereal (IRC). A final guidance with the same action level published in August 2020.

Purpose: This study aims to assess iAs concentration, consumption, and exposure from IRC before and after the issuance of FDA’s draft iAs in IRC guidance.

Methods: FDA compiled datasets to assess consumption, exposures, and market sales for IRC before and after the issuance of the draft guidance. This included FDA arsenic in IRC

surveys conducted in FY2012 and FY2013–2014 and data from the toxic elements compliance program (FY2010-2024). NHANES consumption data (2-day average) for children <2 years old from 2013–2014 and 2017–2018 were used, reflecting periods before and after draft guidance issuance. Circana retail sales data were collated from 2012–2023. iAs and total arsenic (As) exposures from consumption of IRC in children (<2 years) were evaluated before and after issuance of the draft guidance. In addition, iAs and As levels, IRC consumption, and market sales data were analyzed. An unbalanced nested design was used to evaluate changes in iAs and As concentrations before and after draft guidance issuance, with studies of unequal sample sizes nested within time period.

Results: On average, iAs and total As concentrations in IRC decreased after issuance of the draft guidance, although differences were not statistically significant. The proportion of IRC consumers (<2 years) decreased from 15% (90% CI [12%; 18%]) in 2013–2014 to 8.1% (90% CI [5.9%; 11%]) in 2017–2018. During this same period, estimated exposure to iAs among children (<2 years) decreased by 76% (*per capita* basis) and 56% (IRC consumer basis).

Significance: This study provides a framework for the evaluation of this draft guidance and identifies challenges associated with such an analysis.

T13-02: COMMERCIAL PLANT-BASED BURGER ANALOGUES: A POTENTIAL SOURCE OF DIETARY EXPOSURE TO ACRYLAMIDE

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Presentation type/Format: Technical

Category: Food Chemical Hazards

Abstract:

Introduction: Consumers' demand for sustainable alternatives to meat drives the increasing consumption of plant-based burgers (PBBs). Despite their benefits, concerns have emerged about PBBs chemical safety due to their complex formulations and formation of harmful compounds such as acrylamide (AA) during cooking.

Purpose: This study evaluates the contribution of PBB composition to AA content in these raw and cooked analogues.

Methods: Beef burgers (BB) and 5 PBBs produced with distinct protein sources (pea concentrate (PB), soy concentrate (SB), sweet potatoes (SPB), quinoa (QB), and lentils (LB))

were purchased locally. Samples were tested raw and after grilling to doneness (i.e., an internal temperature of 71°C, monitored using a temperature data logger). Color, moisture and protein content were assessed in raw and cooked samples using a colorimeter, the gravimetric and Dumas methods, respectively. Browning index (BI) was estimated from CIELab parameters. Free amino acids, carbohydrates, and AA content were quantified chromatographically (LCMS and HPAE-PAD). All samples were prepared and tested in triplicate, and significant differences were evaluated using ANOVA.

Results: Asparagine, the primary AA precursor, was higher in SPB, LB and QB (1102±8, 883±50, 523±47 mg/kg, respectively) than in PB and SB (<200 mg/kg). Mono- and disaccharides were generally higher in SPB, LB, and QB, with SP having the lowest level (0.07±0.01 g/kg fructose, 3.48±0.1g/kg sucrose). AA was present in all raw burgers at levels ranging from 76.3±6 (BB) to 232.0±33 (LB) µg/kg and increased during cooking (~30-400% higher), with PB exhibiting the highest level (546.3±6 µg/kg). AA in all PBBs were higher than in BB. Although SB had the lowest BI (80±14 AU) among the tested samples, it corresponded to the lowest asparagine and AA content.

Significance: This study underscores the role of formulation in the potential chemical safety of novel PBBs and provides data to inform AA exposure assessments.

Award Application: Developing Scientist Award

T13-03: MONOCYTE- AND EXTRACELLULAR-MEDIATED MECHANISMS OF LISTERIA MONOCYTOGENES TRAVERSAL OF THE PLACENTAL BARRIER

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* is a leading cause of foodborne fetal infection, yet the mechanisms enabling traversal of the human placental barrier remain incompletely defined.

Purpose: This study aimed to define monocyte-mediated versus extracellular mechanisms of *L. monocytogenes* placental barrier traversal.

Methods: A human placental co-culture barrier was established using BeWo trophoblasts and primary human placental vascular endothelial cells and validated by barrier integrity. THP-1 monocytes were infected with wild-type *L. monocytogenes*, isogenic *lap (Listeria* adhesion protein*)*, *inlA*, *inlB*, or *hly* mutants, or nonpathogenic *Listeria innocua* and assessed for transmigration. Intracellular bacterial delivery, barrier perturbation, and endothelial activation were quantified by CFU enumeration, permeability assays, tight junction

immunolocalization, and soluble VCAM-1 (sVCAM-1) ELISA, using ≥3 biological replicates.

Results: *L. monocytogenes*-infected monocytes exhibited a 4.8-fold increase in transmigration and an 8.1-fold increase in intracellular bacterial delivery versus *L. innocua* ($p < 0.0001$). Transmigration was associated with perturbation of claudin-1 and occludin tight junctions, increased paracellular permeability, and accumulation of infected monocytes at junctional breach sites. Relative to WT, *lap* and *inlB* mutants reduced intracellular delivery by ~90% and showed *L. innocua*-like transmigration, despite unchanged monocyte uptake ($p < 0.001$). *L. monocytogenes*-infected monocytes induced a 1.86-fold increase in endothelial sVCAM-1 in a LAP- and InlB-dependent manner ($p < 0.01$). In contrast, extracellular traversal required LAP and InlA, while *inlB* and *hly* mutants were minimally impaired.

Significance: These findings define distinct placental invasion routes for *L. monocytogenes* and identify LAP as a critical noncanonical virulence factor informing prevention of pregnancy-associated listeriosis.

T13-04: SOCIAL INTERACTIONS AND THE PERSISTENCE OF LISTERIA MONOCYTOGENES IN MULTI-SPECIES BIOFILMS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: The colonization of food processing facilities by *Listeria monocytogenes* compromises food safety. The composition of biofilm communities impacts its presence, but the inter-species dynamics that governs the presence of *L. monocytogenes* in multi-species biofilms remain poorly understood.

Purpose: This study evaluated the persistence of *L. monocytogenes* Lineages I and II in 37 mono- and multi-species biofilms.

Methods: Biofilm communities were re-constituted with strains to match the community composition of 6 sites of isolation in a pork processing facility. Six strains each of *L. monocytogenes* Lineages I and II were added. Biofilms were cultivated at 10°C on stainless steel coupons. Biomass was analyzed with crystal violet; viable cells were enumerated using PALCAM, LB and APT agars. The abundance of Lineage I and II was determined by qPCR with lineage-specific primers. All 31 combinations of 5 members (*Flavobacterium*, *Janthinobacterium*, *Pseudomonas*, *Serratia*, and *Carnobacterium*) were reconstituted as 1-, 2-, 3, 4-, and 5-species consortia with *L. monocytogenes*. Data from three biological replicates were analyzed using one-way ANOVA and Tukey's test.

Results: All 6 bacterial communities formed biofilms with *L. monocytogenes* with cell counts higher than 7.5 CFU/cm^2 and a biomass from 0.4 to 1.4. Cell counts of *L. monocytogenes* ranged from 4.1±0.5 to 6.8±0.4 log CFU/cm^2. Lineage I was more abundant than Lineage II (P<0.05). The abundance of *L. monocytogenes* was lower in the 3 biofilms containing *Carnobacterium maltaromaticum* than in the 3 without. In the 31 combinations, cell counts of *L. monocytogenes* in combinations with *C. maltaromaticum* were lower than in combinations without (P<0.001).

Significance: The colonization of biofilms by *L. monocytogenes* depends on the lineage and community composition. *C. maltaromaticum* antagonizes *L. monocytogenes*. These results further our understanding of the persistence of *L. monocytogenes* in food processing facilities.

Award Application: Developing Scientist Award

T13-05: FERMENTED GLUTEN DETECTION AND QUANTIFICATION IN BEER AND YOGURT MATRICES USING MASS SPECTROMETRY

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Presentation type/Format: Technical

Category: Food Allergens

Abstract:

Introduction: The inability of current analytical methods to adequately detect and quantify gluten in fermented foods is a concern for individuals with Celiac disease (CD), food manufacturers, and regulators.

Purpose: To develop a mass spectrometry (MS) method for quantitative detection of fermented gluten in two model systems (beer, yogurt) using gluten-derived target peptides.

Methods: Wheat, rye, and barley were incurred separately into beer and yogurt matrices before fermentation using malted grain or flour. Extracts were digested using trypsin or chymotrypsin to generate peptides for untargeted MS. Gluten source proteomes were used to identify peptides and only those derived from gluten proteins were considered. Abundance and quality of identifications were used to select a subset of target peptides for the novel targeted MS method. Targeted analysis allowed refinement of peptides to determine the best-performing targets for quantification.

Results: Untargeted MS identified 203 wheat, 107 rye, and 63 barley peptides (tryptic, chymotryptic) from gluten proteins in fermented beer samples (statistically validated with

false discovery rate (FDR) < 1%). For fermented yogurt matrices, tryptic and chymotryptic identifications totaled 78 (wheat), 76 (rye), and 71 (barley) (FDR < 1%). Based on abundance, up to 40 candidate target peptides, per protease, for each matrix were used to generate a novel targeted MS method. Targeted screening of replicate matrix batches provided abundance and variability measures to inform final target peptide selection of approximately 10 high-performing (sensitive, robust) target peptides per protease, grain, and matrix for inclusion in the final quantitative method.

Significance: Target peptides for the quantification of fermented gluten in a novel MS method were identified for beer and yogurt fermentations. This method bridges an analytical gap to inform labeling and adherence to current regulations, plus protect those with CD.

Award Application: Developing Scientist Award

T13-06: SYNERGISTIC EFFECT OF NATURAL ANTIMICROBIAL GLYCOLIPID AND HIGH-PRESSURE PROCESSING AGAINST ALICYCLOBACILLUS ACIDOTERRESTRIS IN APPLE JUICE

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Presentation type/Format: Technical

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: *Alicyclobacillus acidoterrestris* spores in apple juice pose a significant spoilage challenge for the juice industry. High-pressure processing (HPP) alone does not inactivate *A. acidoterrestris* spores, therefore HPP juice products must be refrigerated.

Purpose: This study aimed to evaluate the synergistic effects of HPP and antimicrobial natural glycolipid (NG) on the survival and outgrowth of *A. acidoterrestris* in apple juice.

Methods: Mixed strains of *A. acidoterrestris* were inoculated into apple juice as vegetative cells, mixed cells or spore. Inoculated apple juice was treated with HPP at 600 MPa for 3 min, NG concentrations of 0, 10, 50, or 100 ppm. Samples were stored at 45°C for 21 days and *A. acidoterrestris* was enumerated on yeast starch glucose (YSG) agar. Multiple linear regression was used to analyze the effect of several variables on *A. acidoterrestris* during storage. Results were presented as the average and standard deviation of three independent biological replicates.

Results: We first assessed growth of *A. acidoterrestris* strains isolated from different sources in different nutrient broth and determined to use YSG agar for enumeration. We also determined that headspace volume influenced post-HPP outgrowth of *A. acidoterrestris* spores, with 0% headspace inhibiting growth and 2–20% allowing growth. HPP at 600 MPa for 3 minutes significantly reduced vegetative cells but was ineffective against spores. NG at 50 ppm consistently inhibited growth across vegetative, mixed, and spore cultures at 45℃ without HPP, while 10 ppm NG required HPP for effective control in some cases. Synergistic effects between NG and HPP were observed, leading to lower viable counts during storage.

Significance: In conclusion, we find the combination of 50 ppm NG, HPP, and 5% headspace effectively controlled *A. acidoterrestris* growth in apple juice, granting future studies that can achieve shelf stability for HPP apple juice.

Award Application: Developing Scientist Award

T13-07: UNMASKING HIDDEN GLUTEN: EVALUATING THE COMPLIANCE OF GLUTEN-FREE PRODUCTS AND CONSUMER EXPOSURE IN LEBANON

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Presentation type/Format: Technical

Category: Food Fraud

Abstract:

Introduction: Despite improved regulations and growing awareness, significant gaps remain in ensuring the safety of gluten-free products.

Purpose: Our study aims to determine gluten levels in gluten-free products marketed in Lebanon and to assess the consumption frequency of noncompliant products among gluten-free shoppers.

Methods: Following a thorough market screening, a cross-sectional analysis was conducted on identified gluten-free (*n* = 115) stock-keeping units (SKUs) marketed in the country, including 54 local and 61 imported products. Gluten was quantified with the RIDASCREEN® R5 ELISA; levels >20 ppm were deemed noncompliant. A qualitative food-frequency questionnaire (FFQ) was administered to a sample of 66 gluten-free shoppers for the intake frequency of each noncompliant SKU.

Results: Eleven SKUs (9.6%) exceeded 20 ppm, while another 15 (13%) fell between the 1 ppm quantification limit of the kit and 20 ppm. Noncompliance was markedly higher in local products (18.5%) than in imports (1.6%). Forty percent of the contaminated local SKUs and the sole contaminated import bore an FSMS logo, indicating implementation gaps. Bread (6/43) and snack/bars (4/30) accounted for most infractions; a single oat-based “other grain” also tested positive. Although 87% of products were analytically compliant, the FFQ

revealed 8 daily and 11 weekly consumption events involving contaminated SKUs. Notably, 65% of respondents were celiac, and 27% purchased for celiac relatives, increasing clinical risk.

Significance: Lebanon’s retail gluten-free sector is largely compliant, yet a substantial risk persists in domestically produced breads and snack items. Strengthened regulatory surveillance, dedicated production lines for high-risk categories, and targeted consumer education must be enforced to safeguard medically vulnerable populations and uphold ethical standards.

T13-08: AN AI-BASED PUBLIC COMMENT ANALYSIS FRAMEWORK FOR FOOD SAFETY REGULATIONS: THE SALMONELLA RULE CASE STUDY

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Presentation type/Format: Technical

Category: Food Law and Regulation

Abstract:

Introduction: Public comments made on the proposed food safety regulations need a thorough analysis to ensure that opinions are considered in refining the regulations.

Purpose: We have developed an AI-based framework for the analysis large number of public comments on regulations to characterize and quantify public sentiment.

Methods: Publicly available comments (n=7,104) on the USDA FSIS "*Salmonella* Framework for Raw Poultry Products" were used for the study and analyzed using ChatGPT 5.1 Plus. We developed a team of subject matter experts, policy researchers and AI-experts to develop a stepwise framework for data analysis and prompt engineering. Duplicate comments were detected and analyzed after which they were removed to form a core dataset. This core dataset was used for thematic and summary statistics, stakeholder typology (notable stakeholders, expertise level, theme co-occurrence), sentiment analysis (polarity stance and theme stance).

Results: The comments (n=7,104) from the USDA-FSIS "*Salmonella* Framework for Raw

Poultry Products" were retrieved. Most responses were submitted by individuals (n= 5,229; 73.61%), followed by anonymous submissions (n= 1,774; 24.97%). A small proportion of responses came from organizations (n=101; 1.42%). Approximately, 84% of responses were duplicate or closely similar suggesting they came from form letter campaigns. Several themes emerged with majority of the comments (n=42) representing a supportive tone on public health protection and (n=310) science-based and data-driven standards. Expert comments identified key issues/focus areas on testing methodology, performance standards and risk-based approach, on-farm and pre-harvest controls among others.

Significance: The AI-framework enables regulatory agencies to analyze vast number of comments and taking the public sentiment into consideration for future refinement of regulations.

T14-01: ENHANCED UV-BARRIER, ANTIOXIDANT, AND ANTIMICROBIAL PROPERTIES OF TANNIC ACID-FUNCTIONALIZED PBAT/ACETYLATED THERMOPLASTIC STARCH BIOCOMPOSITE FILMS FOR SUSTAINABLE ACTIVE PACKAGING

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: The growing consumer demand for eco-friendly packaging, coupled with stringent regulations on non-biodegradable plastics, has expedited the adoption of biodegradable polymers such as poly(butylene adipate-co-terephthalate) (PBAT). While blending PBAT with thermoplastic starch (TPS) reduces costs and improves biodegradability, interfacial incompatibility and inadequate barrier properties remain key challenges.

Purpose: This study aimed to develop tannic acid–functionalized PBAT/A-TPS films that combine cost reduction and biodegradability with enhanced UV-barrier, antioxidant, and antimicrobial properties for sustainable active packaging applications.

Methods: A-TPS was blended to improve compatibility with PBAT, and tannic acid (TA) was incorporated as a multifunctional bioactive compatibilizing agent. Biocomposite films were fabricated via melt blending followed by film formation. Pork loin samples packaged with different film treatments were stored at 4°C, and three independent samples per treatment (n = 3) were analyzed at each sampling interval.

Results: The integration of TA significantly enhanced the interfacial compatibility of PBAT/A-TPS through hydrogen bonding and π–π interactions, resulting in a homogeneous film morphology. The films exhibited strong UV-blocking ability in the UV-A and UV-B regions, improved antioxidant activity, and pronounced antibacterial effects against *E. coli* and *S. aureus*. Notably, PBAT/A-TPS/TA-1.0% films effectively reduced lipid oxidation and microbial growth, maintaining bacterial counts below 6 log CFU/g and minimizing chemical changes in pork loin over 15 days of refrigerated storage

Significance: This study offers an economic and innovative approach to transform biodegradable polymers into novel active packaging materials, providing improved food protection, extended shelf life, and a viable route toward sustainable food packaging systems.

Award Application: Developing Scientist Award

T14-02: DEVELOPMENT OF AN OPTIMIZED FDA METHOD FOR DETECTION OF CYCLOSPORA CAYETANENSIS IN DIFFERENT WATER SOURCES USING NEW GENERATION DETECTION METHODS

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: *Cyclospora cayetanensis*, a coccidian parasite responsible for foodborne and waterborne outbreaks of cyclosporiasis worldwide, presents several detection challenges in water matrices due to inhibitors and turbidity-induced clogging.

Purpose: The purpose of this study was to adapt the current FDA-validated detection method for a wider range of water matrices and integrate newly-developed digital PCR to broaden the array of screening strategies.

Methods: The dead-end ultrafiltration (DEUF) protocol was used for agricultural water and wastewater. A sucrose gradient separation step was added to address high turbidity and

large pellets in wastewater. Oocysts were spiked in samples according to the FDA Microbiological Methods Validation Subcommittee (MMVS) guidelines, using 5 high-level, 12 fractional, and 5 unseeded control samples. After optimization, 80 non-spiked effluent samples were evaluated for *C. cayetanensis* using both, the FDA validated real-time PCR and the digital PCR assay.

Results: The inclusion of a sucrose gradient step successfully reduced pellet size without loss of oocysts in wastewater effluent. All high-level spiked samples were positive and all unseeded samples were negative. The fractional level yielded 6 out of 12 positive samples, in accordance with MMVS guidelines. The 80 non-spiked effluent samples were analyzed with no clogging or inhibition issues. One environmental sample tested positive for *C. cayetanensis*. The dPCR assay exhibited similar sensitivity when compared to the validated method.

Significance: This study shows the FDA method can be successfully adapted across environmental sources. Validating detection platforms provides sensitive and robust tools, improving environmental monitoring and food safety to protect public health.

T14-03: SALMONELLA POPULATIONS IN ENVIRONMENTAL SURFACE WATER EXHIBIT DAILY CHANGES AND REPEATED INTRODUCTIONS OF CLOSELY RELATED STRAINS

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Surface water contaminated with *Salmonella* poses a food safety risk; however, much is unknown about the temporal dynamics of *Salmonella* in water.

Purpose: To investigate the stability of *Salmonella* serovar populations in environmental surface water.

Methods: Modified Moore swabs were used to collect 10 L samples every weekday for 6 months at one site on a creek. *Salmonella* was isolated by enrichment. Positive samples were deep serotyped (DST) by CRISPR Sero-Seq. Isolates from samples containing serovars Agbeni, Newport, and Oranienburg, were first serogrouped with O-antigen serum, and then analyzed by whole genome sequencing.

Results: *Salmonella* prevalence was 97% (n=127/131). DST revealed a highly dynamic and

complex *Salmonella* population exhibiting frequent changes on a daily basis. The average Jaccard distance between sampling days was 0.44 (n=75). From 106 libraries, there was an average of 3.9 serovars/sample (range 1–11), with 26 serovars in total. Serovars Rubislaw, Montevideo, and Muenchen were most common (85%, 84%, 70% of samples respectively), while serovars Agbeni, Oranienburg, and Newport were sporadic. Serovar Agbeni occurred three distinct times over 21 days and all isolates (n=5) were identical. Serovar Oranienburg isolates (n=8) from three separate periods over 30 days were all closely related within 5 allelic differences. Similarly, serovar Newport isolates (n=5) from two instances 88 days apart were within two allelic differences. Contextually, a sequence type search on Enterobase showed the serovar Agbeni, Newport, and Oranienburg isolates from this study were within 15 allelic differences of clinical isolates.

Significance: This project shows rapid temporal changes in *Salmonella* populations, indicating food safety risks posed by surface water may also change quickly. The same strains of several serovars were found multiple times, suggesting that these serovars may be repeatedly introduced, likely by the same source.

Award Application: Developing Scientist Award

T14-04: MATRIX-SPECIFIC STUDIES FOR THE DETECTION OF CYCLOSPORA CAYETANENSIS IN FOOD

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: A new molecular method for the detection of *Cyclospora cayetanensis* in fresh produce, based on a mitochondrial marker (Mit1C qPCR), was recently published in the Bacteriological Analytical Method (BAM) chapter 19b.

Purpose: To evaluate the performance of the new method to detect *C. cayetanensis* in fresh produce commodities historically associated with parasite outbreaks, such as herbs and broccoli, through matrix extension studies.

Methods: Samples of 25 grams of basil, parsley, and broccoli were seeded with 5 (low seeding level) and 200 (high seeding level) *C. cayetanensis* oocysts. Unseeded produce was used as negative control. The method included washing of the produce, extraction of *C. cayetanensis* DNA, and molecular detection using a TaqMan™ assay targeting *C. cayetanensis* mitochondrial *Cox3* gene following the updated BAM chapter 19b.

Results: Positive detection rates were 83.3% (10/12), 75.0% (9/12), and 41.67% (5/12) for samples seeded with 5 oocysts in basil, parsley, and broccoli, respectively. Average C_T values were 35.0±1.30, 35.35±1.71, and 37.84±0.34 for samples seeded with 5 oocysts in basil, parsley, and broccoli, respectively ($p<0.05$). All samples seeded with 200 oocysts (n=5/commodity) were positive and all unseeded samples (n=5/commodity) were negative.

Significance: The method showed similar analytical sensitivity for *C. cayetanensis* detection in basil and parsley samples, while exhibiting reduced detection efficiency in broccoli samples. This study highlights the importance of evaluating the performance of the *C. cayetanensis* detection method in different food matrices as the USFDA advances with the implementation of such tools to strengthen regulatory activities.

T14-05: ANALYSIS OF VIRAL CONTAMINATION IN THE AGRICULTURAL AREA OF THE ALBUFERA OF VALENCIA (SPAIN) AFTER FLOODING EVENT

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: In 2024, a severe flooding event in Spain caused significant damage to wastewater treatment facilities and the sewer systems, and resulted in the dispersion of untreated sewage across surrounding agricultural areas.

Purpose: This study aims to assess the occurrence of human enteric viruses in aquatic environments and adjacent agricultural areas before and after the 2024-severe flooding event.

Methods: Samples of surface water (n=18 and 42), sediments (n =17 and 55), and vegetables (n=40) were collected before (2022–2023) and after (2025) the flooding event. First,

methodological optimization was conducted to enhance viral recovery from water and vegetable matrices. The optimized protocols were subsequently applied to quantify enteric viruses (i.e. human norovirus genogroup I and II (HuNoV GI and GII), rotavirus (RV), hepatitis E (HEV) and A (HAV) viruses, and human astrovirus (HAstV)), by RT-qPCR.

Results: Aluminum chloride precipitation and ultrafiltration proved most effective for water and vegetables, respectively. After flooding, virus prevalence in water increased in 14.2% and 20.6% for HuNoV GI and RV, respectively; while HuNoV GI was only detected in post-flooding samples. Mean concentrations values after flooding ranged from of 3.26 ± 0.50 (HuNoV GI) to 5.92 ± 0.81 (RV) $\log_{10}$(genome copies (gc)/100 mL). Same trends were observed in sediments, with a prevalence increase of 13.6% and 35.1% for HuNoV GII and RV. In vegetables, HuNoV GI was detected in tomatoes (1.36 ± 0.31 $\log_{10}$(gc/g)), aromatic plants (1.64 ± 0.90 $\log_{10}$(gc/g)) and leafy greens (1.38 ± 0.72 $\log_{10}$(gc/g); while RV was only detected in leafy greens (3.53 ± 0.01 $\log_{10}$(gc/g)).

Significance: Severe flooding represents an emerging threat that can compromise food systems by facilitating the dissemination of viruses into agricultural environments. Understanding pathogen dynamics, persistence, and recovery is crucial for designing mitigation strategies that strengthen resilience to such events.

T14-06: USFDA AND STATE LABORATORIES VERIFY PERFORMANCE OF UPDATED METHOD FOR THE DETECTION OF CYCLOSPORA CAYETANENSIS IN AGRICULTURAL WATER USING DEAD-END ULTRAFILTRATION

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Seven USFDA regulatory and six U.S. state laboratories participating in the Laboratory Flexible Funding Model (LFFM) verified competency in performing the updated FDA BAM 19c: Dead-end Ultrafiltration (DEUF) for the Detection of *Cyclospora cayetanensis* from Agricultural Water.

Purpose: Updates to the previous protocol including new reagents, supplies, PCR targets and cycling conditions were approved by the FDA Microbiological Methods Validation Subcommittee (MMVS) in 2024 based on a single laboratory validation. The purpose of this study was to verify the updated method in multiple federal and state testing laboratories.

Methods: Each laboratory received six DEUF filters; two filters each filtered with agricultural water seeded with 200 *C. cayetanensis* oocysts (high), six oocysts (low) or unseeded.

Laboratories followed the updated BAM 19c method. Key changes include the use of Nipro Eliso 25H filters, Antifoam L-30, eluting DNA in 50uL following clean-up and a Mit1C molecular target, PrimeTime Gene Expression Master Mix, and a new optimized method for PCR.

Results: All participating laboratories achieved results as expected. A total of 78 filters were tested. All unseeded filters were negative (n=26) and all filters seeded with 200 oocysts (high) were positive (n=26). Filters seeded with six oocysts (low) demonstrated a 61.5% positivity rate with 16/26 samples which is in accordance with the guidelines established by the MMVS.

Significance: Updating methods for the detection of foodborne pathogens, training testing laboratories and verifying their ability to perform these methods are critical roles of the USFDA Human Foods Program. Strengthening laboratory capacity for *C. cayetanensis* detection is imperative for public safety and protecting the nation's food supply.

T14-07: LACTOBACILLUS SALIVARIUS INHIBITS GII.4[P16] HUMAN NOROVIRUS REPLICATION IN ZEBRAFISH LARVAE

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Human gut bacteria have been shown to modulate human norovirus (HuNoV) infection, underscoring the need to characterize virus bacteria interactions.

Purpose: To evaluate the effect of *Lactobacillus salivarius* on replication of GII.4[P16] using zebrafish larvae model.

Methods: A GII.4[P16] positive stool specimen was resuspended in phosphate-buffered saline (PBS), centrifuged and supernatant stored at - 80°C. *L. salivarius* was grown overnight in de Man, Rogosa, and Sharpe broth. Bacterial cells and cell-free supernatant (CFS) were collected from overnight broth (OB). The OB, bacterial cells, and CFS were each mixed separately with virus suspension at 1:1 ratio, and 3 nL was microinjected into 3-day post-fertilization larvae. Control group was injected with PBS mixed with virus suspension. Each day post-infection (dpi), 10 larvae were pooled as a single sample, and two samples were collected daily until 4 dpi. RNA was extracted and quantified using droplet digital PCR. Two trials were conducted for each treatment group except bacterial cells (N=1) with statistical analyses performed in R. A binding assay was conducted by incubating virus suspension and bacterial cells (10^7 each) for 2 h at room temperature, then viral RNA was extracted from the pellet and supernatant following centrifugation.

Results: Zebrafish larvae injected with CFS exhibited viral replication comparable to control,

with peak levels at 2 dpi (~ 6 log viral RNA copies/larva). In contrast, no viral replication was detected in larvae injected with bacterial cells whereas larvae injected with CFS had a slight increase (0.9 log viral RNA copies/larva) on 1 dpi. Binding experiment demonstrated that 67% of virus bound to *L. salivarius*, indicating virus bacteria interaction.

Significance: These findings demonstrate that *L. salivarius* binds to HuNoV GII.4[P16] and inhibits viral replication in zebrafish larvae, highlighting its potential as a probiotic intervention for HuNoV infection.

Award Application: Developing Scientist Award

T14-08: COMPARING FOODBORNE VIRUS RECOVERY AND INACTIVATION ON POLYDIMETHYLSILOXANE (PDMS) TOPOMIMETIC ARTIFICIAL BERRY SURFACES AND FRESH BERRIES

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Polydimethylsiloxane (PDMS) replicasts (topomimetic artificial leaf surfaces) have been used to elucidate the role of the produce surface topography on foodborne virus recovery and inactivation in leafy greens, yet it is unclear if replicasts can be used as a model system for fresh berries.

Purpose: Validate that PDMS replicasts can be substituted for fresh berry surfaces to study viral recovery and inactivation.

Methods: PDMS replicasts of blueberry, strawberry, and raspberry surfaces were generated with flat PDMS replicasts (no surface topography) included as controls. Coupons of replicasts and fresh berries were inoculated with 10^5 PFU of Tulane virus (TV) in triplicate. In a one-step recovery procedure, inoculated coupons were submerged in a solution of phosphate buffered saline (PBS) with Tween 20. In a two-step recovery procedure, coupons were first submerged in a sanitizer solution (50 or 200 ppm sodium hypochlorite (SH), 20 or 80 ppm peracetic acid (PAA)) or control (ultrapure water) before transferring to the PBS solution. Solutions were collected, TV titer was determined by plaque assay, and one-way ANOVA was performed to determine significance.

Results: PDMS replicasts of berries inoculated with TV demonstrated comparable viral recovery to fresh berries with a two-step recovery procedure. SH (200 ppm) led to less than a one-log reduction in viral titer in all three types of fresh berries. However, the same sanitizer treatment and two-step recovery procedure led to a 2.45, 2.24, and 1.59 log PFU reduction in blueberry, strawberry, and raspberry replicasts, respectively. SH (50 ppm) and PAA (20 or 80 ppm) had limited efficacy against virus on fresh or replicast berry surfaces.

Significance: With similar viral recovery to fresh berries, replicasts may have utility in modeling surface adhesion of viruses to berries in viral recovery studies, yet berry replicasts are not suitable substitutes for fresh berries in viral inactivation studies.

Award Application: Developing Scientist Award

Poster Abstracts

P1-001: PHYSICAL STRUCTURE OF PEANUTS HAS A MAJOR INFLUENCE ON PEANUT ALLERGEN DESTRUCTION BY ELECTRON BEAM (EBEAM) PROCESSING

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Presentation type/Format: Technical

Category: Food Allergens

Abstract:

Introduction: Peanut allergy is one of the most prevalent and severe food allergies, accounting for 59% of deaths caused by a food allergy. Despite this, there is no established processing method that can reduce the sensitization of peanut proteins.

Purpose: To determine whether the physical structure of peanuts influence structural changes in Ara 1 and Ara 2 allergenic epitopes when exposed to eBeam.

Methods: Three peanut physical forms (whole, crushed, and powdered) were compared. Crushed and powdered peanuts were prepared by mechanical processing. Raw peanuts and purified allergenic proteins (Ara h 1 and Ara h 2) were obtained commercially. All samples were subjected to eBeam treatment at six doses including a control (0, 5, 10, 15, 20, and 30 kGy). Enzyme-linked immunosorbent assay was used to assess the effects of eBeam treatment on protein detectability. Raman spectroscopy and MALDI-TOF mass spectrometry

were used to evaluate structural and molecular modifications.

Results: Dose-dependent structural alterations were observed following eBeam treatment, with greater effects at higher doses. Treatment of purified proteins with a dose >20 kGy resulted in a 99% reduction in binding capacity compared to the control. The reduction in binding was lower for total protein extracts, likely due to protective matrix components. Compared to whole peanuts, grinding and crushing exposed allergenic proteins, making them more susceptible to damage by high-energy electrons. The structural changes induced by eBeam were evident by the physicochemical changes observed in the treated peanuts which include shifts in peptide profiles, characteristic spectral peaks, and secondary protein structure.

Significance: This study highlights the importance of the physical structure of peanuts in terms of allergen reduction by eBeam technology. This strategy has the potential for broader application to other food allergens, paving the way for safer food processing technologies.

Award Application: Developing Scientist Award

P1-002: GLUTEN QUANTITATION IN SOURDOUGH UNDER VARYING FERMENTATION CONDITIONS

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Presentation type/Format: Technical

Category: Food Allergens

Abstract:

Introduction: Gluten quantitation in fermented and hydrolyzed foods remains challenging. Accurate gluten quantitation in fermented sourdough is essential to comply with FDA's 2020 regulation on gluten-free labeling for fermented, hydrolyzed, and distilled foods.

Purpose: Gluten quantitation in fermented sourdough was evaluated by a multiplex-competitive (MC) ELISA.

Methods: Sourdough samples were prepared from rice flour by incurring 8, 20, and 100 µg/g of wheat, rye, and barley gluten, and fermented for up to 72-hours. Samples taken every 24 hours were analyzed by the MC ELISA. Five gluten-specific antibodies were utilized in the ELISA, and different quantitation strategies were implemented to quantitate wheat and rye or barley gluten. The effect of variations in fermentation conditions such as different starter cultures, higher fermentation temperature and starter weight, long-term refrigerated storage, spiking gluten after initial fermentation, and addition of salt, on quantitation were evaluated. Validation parameters including limits of detection (LOD) and quantitation (LOQ), % recovery, and % coefficient of variation (CV) were determined. Significance of differences in the % recovery values was determined by Student's unpaired *t*-test.

Results: The LOD and LOQ of the method were 1.6 and 6.7 µg/g, respectively. Analyses resulted in average gluten recoveries of 42–219% for wheat, rye, and barley gluten-incurred samples. Variations in fermentation conditions resulted in recovery of 38–195% for all

samples. Majority of the samples were within the acceptable 50-200% recovery limit (AOAC SMPR 2017.021). Samples containing 2% salt resulted in % recovery values significantly lower than their no salt counterpart ($P<0.05$). Majority of the samples resulted in a CV of <30%.

Significance: The MC ELISA appears sufficiently sensitive and accurate in quantitating intact wheat, rye, and barley gluten contamination in sourdough under several variable fermentation conditions.

P1-003: QUANTIFICATION OF WHEAT, RYE, AND BARLEY GLUTEN IN SOURDOUGH USING A MULTIPLEX-COMPETITIVE ELISA: EFFECT OF HEAT TREATMENT

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: A strict gluten-free diet is needed to avoid serious reactions in celiac disease patients. Commercially available ELISA kits can't accurately quantify gluten in fermented/ hydrolyzed foods.

Purpose: To quantitate gluten in heat-treated sourdough, using a multiplex-competitive ELISA.

Methods: Eight, 20 and 100 ppm (µg/g) wheat, rye, or barley gluten incurred sourdough were fermented for 24 or 72 hours, followed by baking for 45 min or 1 hour, respectively. Fermented non-heat-treated controls and heat-treated samples were analyzed, in 4 replicates, using a multiplex-competitive ELISA. The controls were extracted for 1 hour, and the heat-treated samples were homogenized by cryomilling before an overnight extraction. Validation parameters % recovery and % coefficient of variation (CV) were determined.

Results: Non-heat-treated wheat, rye and barley gluten incurred sourdough, resulted in average % recoveries of 30–122%, whereas fermented heat-treated samples resulted in average % recoveries of 30–213%. Cryomilling and overnight extraction improved % recovery and % CV values for heat-treated samples compared to a 1-hour extraction (average % recoveries <10% for wheat gluten incurred samples). Only six of eighteen heat-treated

sourdough samples (24-hour fermented 8, 20, and 100 ppm wheat gluten incurred samples, 72-hour fermented 100 ppm wheat gluten incurred sample, 24- and 72-hour fermented 100 ppm rye gluten incurred samples) were outside the acceptable 50–200% recovery range (AOAC SMPR 2017.021). The % CV was < 34% for all samples.

Significance: Quantification of wheat, rye, and barley gluten contamination in heat-treated sourdough by multiplex competitive ELISA may be improved by modifying the extraction time and sample homogenization.

P1-004: ALLERGEN RISK “OFF THE MENU”: GAPS IN RESTAURANT ALLERGEN COMMUNICATION IN BRAZIL AND THE CASE FOR NATIONAL REGULATION

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Brazil currently lacks a national regulation requiring standardized allergen communication in restaurant settings, relying instead on local initiatives.

Purpose: This study aimed to evaluate the presence and format of food allergy information provided on menus of Brazilian restaurants, generating empirical evidence to inform policy and practice.

Methods: A stratified sample of 317 food services was assessed in 18 cities representing all five Brazilian regions. Six of these municipalities had local regulations addressing allergen communication, with different requirements and enforcement levels. Establishments were identified in each city’s central or gastronomic area, using a 5-km buffer to select eligible restaurants. Structured interviews with owners or managers and photographic records of menus were collected. Menus were examined for the presence and type of precautionary allergen labeling (PAL; e.g., “may contain”), and for how allergen information was

communicated—categorized as directive, semi-directive, or non-directive. Less than 10% (n=30) of menus contained any PAL, predominantly referring to potential cross-contamination.

Results: A total of 231 menus were classified as non-directive (general statements or ingredient lists without clear allergen identification), eight as semi-directive (partial guidance such as colors or bold letters), and 36 as directive (explicit indications, mostly using icons for the “big 9” allergens). Among the 104 menus from cities with local ordinances, only ten complied with the established requirements.

Significance: These findings reveal a systemic lack of standardization in allergen communication in Brazilian food services. By documenting how menus fail to operationalize allergen risk communication in the absence of a national rule, this study provides an evidence base for the development of national guidelines and targeted interventions to reduce allergy-related risks in the Brazilian food service sector.

P1-005: MORAL NORMS ON THE MENU: HOW KNOWLEDGE AND ANTICIPATED EMOTIONS DRIVE FOOD ALLERGY MANAGEMENT INTENTIONS AMONG BRAZILIAN FOOD SERVICE OPERATORS

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Brazil faces a growing burden of food allergies, yet little is known about how food business operators understand risks in everyday practice.

Purpose: This study investigated how Brazilian food business operators perceive their responsibilities, moral obligations, and intentions regarding food allergy management, and how these factors relate to knowledge and anticipated moral emotions.

Methods: A total of 314 respondents from all five Brazilian regions completed a questionnaire, including major metropolitan areas and municipalities with and without specific local food allergy regulations, selected using a stratified sampling strategy. Participants answered a food allergy knowledge test, plus items measuring constructs from the Norm Activation Model (NAM)—ascription of responsibility (AR), awareness of

consequences (AC), personal norms (PN), and behavioural intention (BI)—and three anticipated moral emotions (guilt, shame, regret) in response to standardized food allergy incident scenarios.

Results: The measurement model showed excellent psychometric properties (AVE > 0.50; CFI and TLI = 0.99; RMSEA = 0.054; SRMR = 0.040), supporting the validity and reliability of the NAM. Structural equation modelling indicated that knowledge predicted AC, which in turn predicted both AR and PN, while personal norms almost fully explained BI (R^2 = 0.91), highlighting the role of moral obligation in driving intended allergy-safe practices. Knowledge was modestly higher in cities without local regulations, and NAM constructs did not differ significantly by regulatory status, suggesting that regulation alone may be insufficient to change underlying moral drivers.

Significance: These findings provide a theoretically grounded explanation of how knowledge, moral norms, and anticipated emotions shape intentions to protect allergic consumers in Brazilian food services, and underscore the need for interventions that integrate regulatory measures with targeted education.

P1-006: ALLERGEN-RISK MANAGEMENT AND PRECAUTIONARY COMMUNICATION IN PACKAGED FOODS: FIELD EVIDENCE ON LABEL INTERPRETATION AND INADVERTENT EXPOSURE AMONG FAMILIES WITH FOOD ALLERGIES IN ARGENTINA

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Purpose: To evaluate the effectiveness of allergen labeling as a risk communication tool by analyzing understanding, trust and purchasing behavior among families of children with confirmed food allergies, and its relationship with inadvertent exposure in Argentina.

Methods: A descriptive multicenter study was conducted across 18 provinces in Argentina.

Two data sources were integrated: semistructured interviews with pediatric specialists in allergy, gastroenterology and immunology, and surveys administered to families of children with a confirmed diagnosis of food allergy. Quantitative data were analyzed using descriptive statistics, and open-ended responses were examined through thematic analysis to identify failures in allergen risk communication systems.

Results: A total of 326 surveys were analyzed. Most respondents were women (93.9%) and 76.7% had higher education. Multiple food allergies were reported in 45% of children. The most frequent allergens were milk, egg, peanut, tree nuts, soy and gluten-containing cereals. Label reading at every purchase was reported by 74.2% of caregivers, while 25.5% read labels only at first purchase. Confusing or unclear allergen information was reported by 52.5%. At least one allergic reaction attributed to incorrect or unclear labelling was reported by 43.9%. Overall, 89.5% reported low trust in allergen information, increasing to 98% among those who experienced reactions. Qualitative findings linked reactions to ambiguity in precautionary statements, inaccessible terminology, legibility problems and inconsistencies within the same brand. The statements CONTAINS and MAY CONTAIN were perceived as most effective in preventing purchase.

Significance: Significant gaps exist between regulatory requirements and effective consumer understanding of allergen labeling, even among highly educated populations, highlighting the need to strengthen standardization, clarity and education in allergen risk communication.

P1-007: NOVEL AUTOMATED IMMUNOANALYSIS METHOD BASED ON NANOPARTICLES FOR GLUTEN QUANTIFICATION IN FOOD

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Category: Food Allergens

Abstract:

Introduction: Food allergies and celiac disease pose serious health risks, making accurate gluten detection essential for safe labeling. Current methods such as ELISA and lateral-flow tests face limitations in automation, processing speed, and quantitative precision, highlighting the need for rapid and reliable solutions for routine testing.

Purpose: To present a novel automated immunoturbidimetric method based on nanoparticle technology for the quantification of gluten in food and beverages.

Methods: A monoclonal antibody specific to the immunotoxic 33-mer peptide was covalently coupled to latex nanoparticles, which aggregate in the presence of gluten, generating measurable turbidity by photometry. The assay is performed on a random-access analytical platform using ready-to-use reagents, operating at 37°C, providing initial results in 10 min and enabling throughput of up to 60 samples per hour. The calibration curve was established using appropriate reference materials and optimized wavelengths. Validation followed AOAC 2024 guidelines and included both non-processed and incurred matrices, determination of LOD/LOQ, and evaluation of cross-reactivity and method robustness.

Results: The assay demonstrated a quantitative linear range up to 40 mg/kg, extendable to 200 mg/kg through automated sample predilution, with a limit of quantification (LOQ) of 2.4 mg/kg. Selectivity and interference studies showed recoveries meeting AOAC criteria without requiring additional treatment for polyphenol-rich matrices. The method exhibited strong correlation with the AOAC reference method (OMA 2012.01).

Significance: This automated approach integrates speed, accuracy, and ease of use, enabling high-throughput gluten testing and enhancing routine monitoring practices to support safer and more reliable food labeling.

P1-008: DEVELOPMENT OF A COMPETITIVE LATERAL FLOW ASSAY FOR ALLERGEN DETECTION BASED ON THERMAL-STABLE SOLUBLE PROTEINS (TSSPS) OF EDIBLE INSECTS IN PROCESSED FOODS

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Edible insect-based foods are increasingly incorporated into processed foods as powders or protein ingredients to serve as sustainable alternative protein sources. Consequently, food safety concerns related to unintended adulteration and ingredient labeling verification have emerged. In particular, insect-derived pan allergens such as tropomyosin and arginine kinase may exhibit cross reactivity with crustacean and mite allergens, necessitating rapid and reliable detection methods.

Purpose: This study aimed to develop a rapid and field-applicable competitive lateral flow assay (LFA) based on a monoclonal antibody (MAb) specific to thermal-stable soluble proteins (TSSPs) of edible insects for allergen detection in processed foods.

Methods: A monoclonal antibody specific to edible insect-derived TSSPs was purified from hybridoma supernatants and used. Gold nanoparticles were synthesized and conjugated with the purified antibody, and the optimal antibody volume for GNP stabilization was determined to be 60 μL. The competitive LFA was optimized by adjusting the GNP-antibody loading amount, test line antigen concentration, running buffer composition, and drying conditions. Analytical performance was assessed using extracts from six edible insect species and cookie samples (n = 3) spiked with 0.1–10% Tenebrio molitor larvae (TML).

Results: The developed competitive LFA reacted with six edible insect species, including *Tenebrio molitor* larvae, and showed no cross-reactivity with non-insect food allergens. The visual detection limit for TML extracts was 0.1%. In spiked cookie samples, TML adulteration was successfully detected at concentrations as low as 0.5%. The assay demonstrated stable performance with clear visual interpretation within a short analysis time.

Significance: The competitive LFA based on the MTML 5F5-1D6 monoclonal antibody enables rapid detection of heat-stable edible insect proteins associated with allergen risks in processed foods.

P1-009: RISKS AND MITIGATION OF ALLERGEN AND GLUTEN CROSS-CONTACT FROM REUSED FRYING OIL AND BREADING MIXTURES

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Cross-contact with gluten and/or food allergens during frying and breading reuse presents a significant, yet often overlooked, risk in retail and foodservice settings.

Purpose: This study aimed to evaluate transfer of allergen and gluten to food from reused frying oil and breading mixtures and strategies to mitigate cross-contact risk.

Methods: Up to 10 batches of breaded shrimp were fried in soybean oil, which was then reused to fry gluten-free foods to assess gluten cross-contact through oil reuse. Separately, up to 20 batches of cod were breaded, and the same breading mixture was reused to coat cod-free foods to evaluate seafood allergen transfer. Post-treatment mitigation strategies, such as sieving of reused oil and breading mixtures, were also examined. Levels of gluten and cod proteins were measured with allergen-specific ELISA tests. Experiments were

conducted in triplicate.

Results: Reuse of oil from frying breaded shrimp resulted in gluten transfer to gluten-free foods, with concentrations ranging from 59.4 ± 0.6 to 525.3 ± 45.6 μg/g in French fries, tater tots, and chicken bites. Filtering the oil through a 150 μm screen removed over 80% of the gluten. In the breading-reuse experiment, cod proteins accumulated to 628.7 ± 17.5 μg/g after 20 batches and was subsequently transferred to seafood-free foods at 55.8 ± 1.1 μg/g in chicken, 62.7 ± 0.5 μg/g in mushroom, and 44.3 ± 1.5 μg/g in zucchini. Although sieving reduced cod residues, about 16 μg/g remained even after sieving through a 75 μm mesh.

Significance: This study highlights the potential for gluten and allergen transfer during oil and breading reuse and underscores the importance of implementing dedicated systems and cooking media for allergenic foods to reduce cross-contact risk.

P1-010: RAPID DETECTION OF MILK ALLERGENS BY LATERAL FLOW IMMUNOASSAY IN DAIRY INGREDIENTS

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Presentation type/Format: Poster

Category: Food Allergens

Abstract:

Introduction: Milk protein represents a major food allergen requiring control in food processing. Lateral flow immunoassay has become an established rapid detection method for allergen screening because of its fast result and ease of use.

Purpose: To evaluate the performance of Neogen® Reveal® 3-D Total Milk test strip, a lateral flow immunoassay method, for milk residues in food processing.

Methods: Three samples each of milk-based raw materials and compound seasonings were analyzed. The inherent milk protein concentration in each sample was first quantified using the Total Milk ELISA kit. Simulated CIP rinse water was then prepared by serially diluting samples with deionized water. To establish the endpoint dilution for a positive reaction, rinse water samples were analyzed in five replicates per sample using the Total Milk test strip. The corresponding endpoint dilution was then re-quantified with the ELISA kit to establish the detection sensitivity of CIP water for each sample.

Results: ELISA quantification showed the total milk content in each sample as follows: skim milk powder (84,000 ppm), milk mineral salts (31.9 ppm), lactose (47.6 ppm), cream seasoning (5,630 ppm), seaweed seasoning (1,112 ppm), and barbecue seasoning (21.8 ppm). Simulated CIP rinse water prepared from these samples remained positive at the following endpoint dilutions: 1:20,000, 1:6, 1:10, 1:1,000, 1:200, and 1:3, respectively. ELISA

method reconfirmed milk allergen concentrations in these positive CIP samples as 5.3, 5.7, 4.2, 4.6, 5.1, and 6.8 ppm, indicating that the test strip can reliably detect milk residues.

Significance: The Total Milk test strips are suitable for the rapid detection of dairy ingredients. It provides a valuable tool for monitoring potential allergen cross-contact during production and verifying the effectiveness of cleaning procedures.

P1-011: QUANTITATIVE ASSESSMENT OF PHTHALATE MIGRATION FROM POLYSTYRENE MICROPLASTICS DURING SIMULATED DIGESTION USING LC–MS: IMPLICATIONS FOR DIETARY RISK

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Microplastics in food systems can release harmful additives during digestion, raising concerns about endocrine-disrupting chemical exposure.

Purpose: To quantify phthalate ester migration from polystyrene (PS) microplastics under simulated gastrointestinal conditions and assess associated health risks.

Methods: PS microplastics (□50 µm and □200 µm) were tested at concentrations of 50–1000 µg/mL using a three-phase in vitro digestion model (salivary, gastric, intestinal) with starch, protein, and lipid matrices. LC–MS analysis targeted four phthalates: bis-ethyl ester, benzyl butyl ester, bis-butyl ester, and bis-2-ethylhexyl ester. Method validation achieved $R^2 > 0.995$, LOD 0.002–0.005 µg/mL, LOQ 0.008–0.015 µg/mL, and recoveries of 92–105%. Each treatment was performed in triplicate, and statistical significance was set at $p < 0.05$.

Results: Gastric digestion showed the highest leaching, with bis-butyl ester reaching 3.58 µg/mL (200 µm) and 3.24 µg/mL (50 µm). Lipid-rich conditions elevated bis-ethyl ester to 0.808 µg/mL (200 µm) and 0.955 µg/mL (50 µm). Smaller particles exhibited greater migration ($p < 0.05$). Concentration-dependent trends revealed bis-butyl ester increasing

from 2.96 μg/mL (50 μg/mL MPs) to 7.94 μg/mL (500 μg/mL) before slightly decreasing at 1000 μg/mL (7.23 μg/mL) due to aggregation.

Significance: Microplastic ingestion can release endocrine-disrupting phthalates at physiologically relevant levels, posing risks of hormonal imbalance and metabolic disorders. These findings highlight the need for regulatory frameworks addressing chemical migration from food-contact polymers under realistic digestive conditions.

Award Application: Developing Scientist Award

P1-012: PROFICIENCY TESTING OF HEAVY METAL ANALYSIS IN FOODS

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Heavy metals such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are elements which exist naturally in the environment, soil and groundwater, and can enter the food chain and impose adverse health outcomes to humans. Robust testing methodologies for quantifying these heavy metals with reliable testing results are important. Since 2012, the Food Emergency Response Network (FERN) Proficiency Test (PT) Program organized 10 rounds of PT for heavy metal analysis in a variety of food matrices.

Purpose: Evaluate the performance of FERN laboratories and an analytical method for determination of As, Pb, Cd and Hg in food matrices through PT programs.

Methods: Heavy metals (As, Pb, Cd, Hg) in a wide range of food matrices were included in

the PT program study. Eight blind coded homogeneous samples were analyzed by 25 to 37 laboratories each round. Results reported from the laboratories were statistically analyzed according to ISO 13528 and z scores were calculated to evaluate laboratory performance. The PT data were also analyzed for evaluating the performance of the recommended method (FDA method) using harmonized analysis, including accuracy, precision (relative repeatability RSDr, relative reproducibility RSD_R), and Horwitz Ratio HorRat).

Results: Overall, the laboratories had successful performance with 94%, 88%, 87% and 93% of z-scores 2 for As (n=596), Pb (n=731), Cd (n=659) and Hg (n=349), respectively, across all PTs and a wide range of food matrices. The recoveries, RSDr, $RSD_{R,}$ and HorRat of the FDA method were: 93–119%, 1.7–4.8%, 7–26% and 0.33–0.75 for As; 97–108%, 1.7–3.3%, 5–12% and 0.27–0.41 for Pb; 100–106%, 1.7–4.0%, 4.4–10.9% and 0.17–0.51 for Cd; 99–104%, 1.9–4.1%, 5.6–13.2%, and 0.26–0.41 for Hg, respectively.

Significance: The results showed that the majority of the participant laboratories were proficient in detection and quantification of As, Cd, Pb and Hg, and the FDA method was adequate.

P1-013: MARKET SURVEY TO DETERMINE THE PREVALENCE OF MORPHINE, CODEINE, AND THEBAINE ON POPPY SEEDS

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Adverse health events have been reported to the USFDA in association with the consumption of poppy seeds or poppy seed-containing food since poppy seeds are known to contain varying amounts of opiate alkaloids.

Purpose: In this work, a market survey was conducted to inform the agency about the prevalence of opiate alkaloids on poppy seeds obtained from stores intended for use in baking.

Methods: A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of morphine, codeine, and thebaine on poppy seeds was recently multi-laboratory validated according to the FDA's Guidelines for the Validation of Chemical Methods for the FDA Foods Program. Sixty-six samples from different brands were purchased and analyzed with the method. Opiate alkaloids were extracted from poppy seeds with 80% acetonitrile, 20% water, and 0.1% formic acid by shaking. The supernatant was filtered, combined with internal standard, and analyzed via LC-MS/MS using an external

calibration curve. Internal standards were used to correct for sample matrix suppression or enhancement: deuterated morphine (morphine-D3) for morphine and deuterated codeine (codeine-D3) for codeine and thebaine.

Results: Morphine, codeine, and thebaine were either not detected (<MDL) or present in trace amounts (<10 mg/kg morphine and codeine, <15 mg/kg thebaine) in 23 out of the 66 samples. Morphine was detected in 29 samples at concentrations ranging from 10 to 312 mg/kg. Codeine was detected in 24 samples at concentrations ranging from 10 to 940 mg/kg and thebaine was detected in 28 samples at concentrations ranging from 15 to 376 mg/kg.

Significance: This data will be used, in conjunction with other data, to inform an action level for opiate alkaloids on poppy seeds for draft guidance.

P1-014: MIGRATION OF MICROPLASTICS FROM TAKE-OUT CONTAINERS

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Microplastics and associated chemicals can leach from plastics into food and water, resulting in ingestion and possible negative health outcomes.

Purpose: We analyzed 12 take-out containers and cups for bisphenols, phthalates, per- and polyfluoroalkyl substances (PFAS), and microplastics (MP) and assessed their migration into boiled or microwaved water.

Methods: One sample each of 12 commonly used take-out containers and cups was analyzed for MP, bisphenols, phthalates, and PFAS. Migration testing included three protocols: boiled water in the container for 8 min; and microwaving water in the container for 3 min and for 5 min. For MP analysis, room temperature and heated water were analyzed. Containers were pre-rinsed with MP-free water, filled with a mass-determined water volume, after which the full volume of water was filtered and analyzed by Laser Direct Infrared imaging for particle size and polymer identification. For bisphenols, phthalates, and PFAS, the containers were cryoground, extracted, and analyzed to determine their concentrations in the containers. The same water-heating protocols were followed for the migration studies.

Samples were extracted by the QuEChERS method. Bisphenols and PFAS were analyzed by LC-MS/MS and phthalates by GC-MS/MS. Isotopically labeled internal standards were used to correct for matrix effects.

Results: Bisphenols (LOD 5.0 ng/g) and PFAS (LOD 0.25–5 ng/g) were not detected in containers. Phthalates were measurable in some containers, including BBP, DBP, DIBP, DEP, DEHP, DMP, DEHA, and DEHT. The number of microplastic particles (15–500 μm) ranged from 3–40 in room temperature water, 4–327 in boiled water, 2–361 in water microwaved for 3 min, and 3–1689 in water microwaved for 5 min. No phthalates (LOD 0.5–25 ng/g) were detected in water samples.

Significance: Consumers may be exposed to microplastics from hot liquids in take-out containers. More testing is required to gauge typical consumer exposure.

P1-015: OCCURRENCE OF MYCOTOXINS IN COMPLEMENTARY FOODS SOLD IN ACCRA, GHANA

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Presentation type/Format: Poster

Category: Food Chemical Hazards

Abstract:

Introduction: Complementary foods are often given to children as a supplement to breast milk. However, mycotoxins can be detected in complementary foods due to contamination from raw materials used during processing.

Purpose: This study was conducted to assess the occurrence of mycotoxins (aflatoxin, ochratoxin A, and fumonisin B1) in complementary foods obtained from markets and shopping centers in Accra, Ghana in order to evaluate risk associated with intake of mycotoxins from complementary foods.

Methods: Seventy (70) different samples of complementary foods were collected from various markets and shopping centers and HPLC analysis was performed to determine the levels of mycotoxins (total aflatoxin, aflatoxin B1, ochratoxin A, and fumonisin B1). Additionally, information was collected on the consumption pattern of complementary foods among children aged 6 months to 24 months.

Results: About 58.4% of the study participants (children) were female and 43.1% were aged 6 to 9 months. Participants (43.1%) reported feeding their children complementary food at least once a day. The mean concentration of aflatoxins, ochratoxin A and fumonisin B1 in the complementary foods sampled were 5.13 µg/kg (1.15–16.08 µg/kg), 11.2 µg/kg (9.35–13.03

μg/kg) and 2.09 mg/kg (0.32–2.53 mg/kg), respectively. The level of aflatoxins was significantly higher than the regulatory limit (5 μg/kg). However, the levels of ochratoxin A and fumonisin B1 were within the regulatory limits of 20 μg/kg and 4 mg/kg, respectively. The results from this study shows a significant health risk associated with dietary exposure to mycotoxins particularly aflatoxins from consumption of complementary foods purchased in Accra.

Significance: The occurrence of mycotoxins in complementary foods is a food safety and public health alert. Market surveillance, product testing and enforcement of regulatory laws should be strengthened to protect children from the adverse health effects of dietary exposure to mycotoxins.

P1-016: DETECTION OF SALMONELLA ENTERICA ACROSS THE BEEF PRODUCTION WITH DIGITAL QPCR

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Current methods for detecting *Salmonella* in pre-harvest beef cattle (e.g., culture, qPCR) rely on pre-enrichment methods that increase *Salmonella* growth, but may bias results.

Purpose: The study objective was to evaluate digital qPCR as a tool for the detection of *Salmonella* from cattle feces with and without pre-enrichment and evaluate the effect of management decisions on *Salmonella* prevalence.

Methods: Fecal samples were collected from twenty animals at the following timepoints: T1 (~107 days old), T2 (~114 days old), T3 (~183 days old), T4 (~230 days old), T5 (~213 days old),

and T6 (~258 days old). Samples were mixed with a 50:50 solution of glycerol/PBS for storage at -80℃. Samples were thawed, homogenized, and divided into 2, 250µL aliquots. For each sample, one aliquot was pre-enriched in 2.5 mL of tryptic soy broth and incubated for 3 hrs at 42℃ and the other was added to 2.5 mL of PBS and stored at -20℃ until DNA extraction with the DNeasy PowerSoil Pro kit on a Qiacube Connect DNA/RNA device. An additional TSB aliquot was taken for *Salmonella* isolation. Digital qPCR was performed on a Quantstudio Absolute Q digital qPCR platform using primers for *invA* and *pagC*.

Results: The *Salmonella* prevalence based on culture was 28%, whereas digital qPCR detected *Salmonella* in all animals. We observed no statistically significant difference in detection between preenriched and non-preenriched fecal samples, though *Salmonella* concentration was higher in enriched samples.

Significance: This study significantly advances our understanding of *Salmonella* detection in cattle feces

P1-017: GENOMIC INSIGHTS INTO SALMONELLA VIRULENCE IN HOSPITALIZED CATTLE

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: WGS offers insights into a pathogen’s virulence, but few studies have leveraged this technique to explore virulence in isolates from cattle.

Purpose: Our objective was to characterize virulence genes present in *Salmonella* isolates from cattle.

Methods: 314 *Salmonella* isolates from the CSU VTH collected during routine cattle health surveillance were received at the VERO research laboratory. DNA was extracted using the DNeasy Ultraclean Microbial Kit and WGS was performed using the native barcoding kit v14 and R10.4.1 flow cells on the MinION Mk1C. Long-read sequence quality was assessed using LongQC. Genome assemblies were derived using Flye and polished by Medaka. DNA was also sent to the North Texas Genome Center for illumina sequencing. Short read quality was assessed using FastQC and MultiQC. Reads were trimmed using Trimmomatic. Pypolca was

used to further correct the polished assemblies using the short reads. Prokka was used to annotate the assemblies, which were used for pan-genome analysis using Roary. Snippy was used to identify SNPs and generate a core genome alignment. IQ-TREE was used to create a maximum likelihood phylogenetic tree that was visualized in iTOL. ABRicate was employed to search the assemblies for virulence genes using the virulence factor database. Scoary2 was utilized to detect associations between virulence genes and the metadata.

Results: A number of genes found to be specifically associated with certain serovars (*sspH1, ratB, avrA, gogB* etc.). We also identified several virulence genes significantly with housing in isolation pens (*gogB, sspH2, sodCI, sseI/sfrH, lpfD,* and *bapA)*. Four virulence genes (*ssek2, cdtB,* and *pltA, pltB*) were found to be associated with a specific farm. Pangenome analysis also revealed many genes that were associated with important host characteristics, such as fever (*tnpA*).

Significance: This study significantly advances our understanding of virulence mechanisms in *Salmonella* isolates from cattle.

P1-018: A CONCENTRATION-BASED QUANTITATIVE RISK ASSESSMENT FOR SHIGA TOXIN-PRODUCING E. COLI ON FRESH PRODUCE FROM MANURE

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Untreated manure is widely used in organic production but can also serve as a reservoir for Shiga toxin-producing *E. coli* (STEC) and extend the persistence of STEC in

manure-amended soils. Current organic standards provide limited quantitative insights into how manure management and field conditions jointly shape the risk of STEC contamination on produce. This study applies a scenario tree-based quantitative microbial risk assessment (QMRA) to model the collective contamination pathways.

Purpose: To develop a concentration-based QMRA for STEC associated with manure amendment (e.g., storage, application, run-off), and to implement the model into an interactive dashboard to support decision-making.

Methods: We used a stochastic QMRA model to estimate STEC concentration and the probability of detecting 1 Colony Forming Unit (CFU) on lettuce at harvest across modules of manure storage, field application, runoff from manure storage, irrigation, and transfer to produce. Parameters combined literature evidence, public datasets and assumptions. Global sensitivity analysis was used to identify influential parameters. All modeling and analyses were performed in R Studio.

Results: Baseline scenario considered lettuce production in Salinas Valley, CA in Dec. with untreated cattle manure applied to soil 90 days before harvest. The manure was stored in two piles of 2 m for 1 day surface applied to soil. The estimated STEC concentration on lettuce ranged from -10.8 to -2.25 log CFU/g (median = -6.52), and the probability of 1 CFU of STEC ranged from 9.10×10^{-9} to 0.951 (median = 1.61×10^{-4}). Initial STEC concentration in untreated manure was the most influential parameter.

Significance: The results provide a concentration-based QMRA that focuses on manure amendment into produce safety evaluation. The interactive dashboard turns site-specific inputs into rapid scenario estimates, supporting evidence-based food safety planning on farms.

P1-019: RECURRENT OUTBREAKS OF GENETICALLY DISTINCT STRAINS OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O157:H7 IN BEEF CATTLE AND CO-MINGLING WILDLIFE AT A RANGELAND IN CALIFORNIA CENTRAL COASTAL AGRICULTURAL REGION

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a significant cause of foodborne outbreaks in the United States.

Purpose: The infection dynamics and genomic diversity of STEC O157:H7 (Ec O157) in beef cattle and co-mingling terrestrial wildlife was characterized at an oak woodland-annual grassland rangeland location in California central coast agricultural region.

Methods: From April 2022–May 2025, livestock and wildlife fecal samples were collected and

tested using FDA BAM methods. All Ec O157-positive isolates were whole-genome sequenced, and single nucleotide polymorphism analysis was used to assess genomic relatedness.

Results: Ec O157 was detected in 14.4% (50/348) of cattle and 4.1% (7/173) of wildlife (e.g., feral pig, bobcat) fecal samples. Ec O157 was not detected in 2022, however, a different genomic strain of Ec O157 simultaneously infected both the cattle and terrestrial wildlife each subsequent year: Aug–Dec 2023 (strain A), May–Aug 2024 (strain B), and Jan–Mar 2025 (strain B and C co-circulating), with no positives in the intervening months. Ec O157 isolates were recovered from fresher feces as well as multiple aged-dried cattle and wildlife feces.

Significance: Given that cattle were confined by standard barbed wire fencing, but larger wildlife (feral pigs, bobcats) likely were not at this site, the shared strains of Ec O157 between them then suggest bidirectional transmission, with wildlife disseminating persistent strains. Ec O157 likely persisted for several months in aged-dried fecal material, enhancing the risk for environmental dissemination and continued inter-animal transmission.

P1-020: DEVELOPING CRITERIA FOR IDENTIFYING EMERGING SALMONELLA POPULATIONS OF PUBLIC HEALTH CONCERN

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: *Salmonella* is a genomically dynamic organism; the populations of greatest concern to change over time. Food safety is contingent on early identification of these populations.

Purpose: This study evaluates a framework to identify emerging genomic traits of virulence in *Salmonella* populations.

Methods: Previous work from EpiX Analytics identified higher virulence (HV) serovars and their associated genetic virulence profile. We used these results to create a framework for identifying emerging virulent *Salmonella* populations based on an analysis of 95,256 isolates. The framework considers total virulence factor (VF), exotoxin, AMR, and effector delivery system carriage, and genetic proximity to the HV profile. We applied this framework to the 20 most common serovars in beef and chicken, and two populations of *S.* Infantis: the population encoding the pESI plasmid, and the population without.

Results: *S.* Dublin was implicated as an emerging virulent serovar. Dublin isolates from 2016–2023 encode on average 108.3 unique VFs, 4.6%(95% CrI:4.1-5.5%) more than the average HV serovar, and an increase of 3.3% (CrI:2.1-4.6) over the past four years. Dublin also encoded 18.1%(CrI:16.1-19.7) more exotoxin genes, 34.1%(CrI:32.3-36) more effector delivery system genes, and 366%(CrI:354-375) more AMR genes than the average HV serovar.The framework was able to distinguish between populations at the sub-serovar level. PESI+ *S.* Infantis constitutes a higher emergence risk than pESI-Infantis, encoding 26%(CrI:25.6-26.7) more VFs, 15.7%(CrI:13.7-17.5) more exotoxin genes, and more AMR genes (6.6 vs 0.6 genes per isolate).

Significance: Industry needs to identify emerging serovars of concern before they become a public health and economic burden, but this cannot be accomplished by monitoring prevalence alone. Through this work, we've demonstrated a preliminary genetic framework which can help identify emerging *Salmonella* serovars or intra-serovar populations of concern.

P1-021: SURVEILLANCE OF ENTERIC PATHOGENS IN STUFFED FROZEN RAW BREADED CHICKEN PRODUCTS IN CANADA

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Stuffed frozen raw breaded chicken products (sFRBCP) are widely available in Canada but are excluded from the Canadian Food Inspection Agency policy aimed at reducing salmonellosis associated with FRBCP. Targeted surveillance is essential to address knowledge gaps regarding their role as a source of enteric illness and to inform the microbiological risks posed to public health.

Purpose: To characterize the microbial risk of sFRBCP to the health of Canadians and provide evidence to guide food safety interventions.

Methods: Targeted surveillance data from FoodNet Canada (FNC) weekly retail sampling of sFRBCP across four sentinel sites in Canada were analysed to determine the prevalence, concentrations, and antimicrobial resistance of enteric bacteria in these products.

Results: Multiple pathogens were found in sFRBCP, including antimicrobial-resistant bacteria carrying multidrug resistance.16% of the 1335 samples collected by FNC over 3 years were positive for *Salmonella*. The prevalence of *Salmonella* was similar to that of raw chicken

products, representing similar consumer risk, although preparation and handling practices may differ. While *Salmonella* detection decreased over time, the prevalence of *Listeria* (26%) has risen substantially in the past 2 years, surpassing levels observed in raw chicken, raising concern. *Campylobacter* was also detected despite the freezing step in producing these commodities. Close genetic linkages between *Salmonella* isolates and human cases of salmonellosis were identified, suggesting potential sources of exposure or gaps in processing controls.

Significance: The similar appearance of sFRBCP to non-stuffed FRBCP likely contribute to similar consumer handling, preparation, and consumption behaviours, contributing to a historically high burden of salmonellosis. Continued retail surveillance and integration with human illness data will help to better understand their impact on public health and antimicrobial resistance.

P1-022: A REVIEW OF TWO 2025 INVESTIGATIONS OF SALMONELLA ENTERITIDIS ILLNESSES LINKED TO SHELL EGGS

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: In 2025, FDA, CDC, and state/local partners investigated two *Salmonella* Enteritidis (SE) outbreaks linked to shell eggs sourced from two separate farms and processors on the West Coast.

Purpose: To review the course of both outbreak investigations and compare and contrast supply chains, challenges, and potential routes of contamination.

Methods: In both investigations, a case was defined as an SE infection with an isolate highly related by WGS of either 0–7 or 0–2 alleles my cgMLST and specimen collection dates of

either 3/1/2025–6/11/2025 or 1/10/2025–8/25/2025. States and CDC interviewed ill people with standard questionnaires. FDA and state partners conducted traceback analysis per peer-reviewed methodology and initiated comprehensive Egg Rule Inspections. Environmental sampling SE was conducted based on the FDA BAM.

Results: In both outbreaks, 90% of people with exposure information available reported consuming eggs prior to illness. Traceback investigations were conducted during both outbreaks and resulted in inspections at an egg farm associated with each outbreak and the related egg processing facilities, one at the same location as a farm and one off-site. In one outbreak there were sanitation issues noted and the other had extensive issues with their SE prevention plan. The outbreak strain was detected in the laying house environments of the inspected egg farms for both investigations and the off-site processing facility associated with one of the investigations. Recalls were initiated in both cases.

Significance: These investigations highlight SE contamination of shell eggs where comingled eggs from multiple farms at a packing processing facility could have been contaminated with SE through horizontal/vertical or environmental transmission. Further research into the potential risks of SE horizontal/vertical transmission is warranted to prevent potential future outbreaks

P1-023: ENHANCED SURVEILLANCE OF NON-TYPHOIDAL SALMONELLOSIS: ESTIMATING CHRONIC SEQUELAE FREQUENCY AND RISK FACTORS IN CANADA

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Non-typhoidal salmonellosis (NTS) is a key cause of foodborne disease burden. It can result in chronic sequelae such as reactive arthritis (ReA) and irritable bowel syndrome (IBS). The frequency of and risk factors for ReA and IBS are not well known among NTS cases.

Purpose: To estimate the proportion of NTS cases developing ReA or IBS and identify associated risk factors applying a prospective cohort design to an existing surveillance program.

Methods: Laboratory-confirmed NTS cases with onset dates as of January 1, 2025 (exposed individuals) are recruited from FoodNet Canada sentinel surveillance sites and non-exposed individuals from the Public Health Agency of Canada’s Foodbook Enteric Control Bank

(ECB). An online survey tool was used to capture self-reported symptoms consistent with ReA and IBS at 3, 6 and 12 months from NTS symptom onset and at the same recall periods among non-exposed individuals.

Results: Results from the initial 10 months of study indicate a total of 117 NTS cases have been enrolled in the study of which 33 have provided responses to the 3-month follow-up survey and 15 have completed the 6 month follow up survey. Of the 33 responses, 36% (n=12) report some joint pain consistent with symptoms of ReA in the 3 months since their salmonellosis. Likewise, 39% (n=13) report abdominal pain at least once a week in the previous 3 months, 77% of which experienced diarrhea alone and 23% experienced both diarrhea and constipation and 62% experienced pain or discomfort or altered bowel functioning that affected or interfered with life in general at least 50% of the time in the past 10 days.

Significance: Ongoing surveillance will improve understanding of the frequency and risk factors associated with ReA and IBS among NTS cases. This will augment burden of foodborne NTS estimates and the ability to inform food safety policy.

P1-024: ANTIBIOTIC RESIDUES AND RESISTANCE EMERGENCE IN WILDLIFE CONSUMED AS BUSHMEAT IN NIGERIA: FOOD SAFETY AND PUBLIC HEALTH CHALLENGE

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Presentation type/Format: Technical

Category: Epidemiology

Abstract:

Introduction: Foodborne hazards are evolving, with complex food safety challenges. Antibiotic residues in animals can exert selective pressure on pathogens resulting in resistance, and surveillance in wildlife can mitigate emergence.

Purpose: Assessed wildlife handlers' attitudes and perceptions regarding antibiotic residues in wild animals, risk pathways, and anthropogenic drivers of emergence at wet markets.

Methods: A cross-sectional study was conducted at 15 wet markets in Northcentral Nigeria in 2024. Structured-questionnaire was used to assess perceptions based on Health Belief Model. Flesh, fecal, and wastewater samples were collected to determine antibiotic residue levels and risk status using disc diffusion test and Traffic Light model, respectively. Also, residues were quantified using Enzyme Linked Immunosorbent Assay. Descriptive and analytic statistical analyses were performed at 95% confidence level.

Results: All recruited 196 handlers participated. However, 65.3% were consumers, 18.4% hunters, and 16.3% vendors. Only 10.3% handlers had used antibiotics on captive wildlife. Residue/resistance risk pathways were bushmeat consumption (p=0.001), contact with

contaminated wild animals (p=0.001), and contaminated wet market environment (p=0.001). Drivers of emergence were: poor regulatory frameworks (p=0.010), poor wet market biosecurity (p=0.002), and lack of risk awareness and education (p=0.001). Residues were detected in samples from 53.7% wet markets. About 69.4% of wastewater were in Class 3 (Red risk) status. Most frequently observed antibiotic residues were tetracyclines.

Significance: Wildlife serve as early warning system for antibiotic resistance. One Health approach sensitization would ensure mitigation, promote food safety, and public health.

P1-025: BEHAVIORAL AND ENVIRONMENTAL RISK FACTORS SHAPING PERSISTENCE OF CRONOBACTER SPP. IN UNITED STATES HOUSEHOLDS

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: *Cronobacter* spp. can cause invasive infections in high risk infants, but the factors that increase the likelihood of presence and persistence of *Cronobacter* spp. in homes remain understudied.

Purpose: To characterize the diversity and persistence of *Cronobacter* spp. within households throughout the continental U.S. and identify factors associated with *Cronobacter* presence.

Methods: Households (N=123) from 41 U.S. states sampled sites (N=4,015) 3 times over 9 months. Samples were processed using a modified EN ISO 22964:2017 protocol, and isolates were confirmed by WGS. Demographics, sanitation behaviors, and sample metadata were collected through a survey using Qualtrics. SNP analysis was conducted using kSNP v3.1 and the CFSAN pipeline v2.2.1, then clustered if □50 pairwise SNP differences. Maximum-

likelihood phylogenies were generated using RAxML v8.2.12 (1,000 bootstraps, GTRCAT model) and visualized in iTOL v6.8.1. Variable importance was derived from ML models. Chi-square and Fisher's exact tests, and MCMC models were used.

Results: *Cronobacter* spp. prevalence in samples (p<0.05) and households were higher in the south and summertime. Most households harbored a single species (38%), while 25%, 10%, and 0.8% contained 2, 3, or 4 species, respectively. *C. sakazakii* was most frequently isolated (n=82/236 isolates; n=48 households), especially ST4 (10 isolates). About 26% of households (n=32) containing *Cronobacter* had >1 site that was positive over multiple seasons. Most isolates were sampled from vacuum dust (30.5%), entryway floors (17.8%), and footwear (13.1%). Persistent strains (<20 SNPs isolated at two or more sampling intervals) were identified in 69 households. *Cronobacter* presence was most strongly associated with wearing outdoor shoes indoors (p<0.01), pets, region, and children.

Significance: *Cronobacter* diversity and persistence in households underscore the need to consider domestic environments in risk assessments and prevention strategies.

Award Application: Developing Scientist Award

P1-026: ESTIMATING THE BURDEN OF FOODBORNE ILLNESS FOR CAMPYLOBACTER, SALMONELLA, AND VIBRIO PARAHAEMOLYTICUS IN JAPAN, 2006–2023

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

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 2023. The physician consultation rate and the stool submission rate were estimated from telephone population surveys conducted for

whole of Japan and for Miyagi prefecture conducted in 2009 and 2016. Each estimate was introduced into the Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *V. parahaemolyticus* in whole of Japan were 3.8–13.6 million, 0.9–2.8 million, and 10-438 thousand during 2006–2023, respectively. Those estimated for whole of Japan from data on Miyagi prefecture were 0.44–1.6 million, 78–190 thousand, and 2–63 thousand during 2006–2023, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006–2023, for *Campylobacter*, *Salmonella,* and *V. parahaemolyticus*, were 760–3,200, 320–3,600 and 3–1,300, respectively.

Significance: These data reveal a significant difference in numbers and trends between our estimates of burden of foodborne illnesses and the reported foodborne disease cases associated with three pathogens. Need for continuing active surveillance system to complement the present passive surveillance is strongly suggested.

P1-027: INCORPORATING MULTI-YEAR OUTBREAKS INTO INTERAGENCY FOOD SAFETY ANALYTICS COLLABORATION (IFSAC) ANNUAL ESTIMATES OF FOODBORNE ILLNESS SOURCE ATTRIBUTION

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Multi-year foodborne disease outbreaks (outbreaks lasting > 365 days) appear to be increasing in frequency, raising public health concerns.

Purpose: Compare the epidemiology of multi- and single-year foodborne disease outbreaks caused by *Campylobacter, Listeria monocytogenes* (LM), non-typhoidal *Salmonella* (NTS), and Shiga toxin-producing *Escherichia coli* O157 (STEC).

Methods: This study analyzed Foodborne Disease Outbreak Surveillance System (FDOSS) data from 1998 to 2022. Regression and conditional forests (CF) were used to characterize differences between multi- and single-year outbreaks.

Results: During 1998–2005 1.2% of outbreaks were multi-year. This increased to 1.3% during 2006–2013, and 2.9% during 2014–2022. According to regression, LM outbreaks were more likely to be multi-year (39%; 45/115) compared to *Campylobacter* (<1%; 2/458), NTS (1%; 17/2,134) and STEC (<1%; 1/360) outbreaks. Outbreaks involving multiple states (versus one state) were also more likely to be multi-year [Odds Ratio (OR)=6.8; 95% Credible Interval (CrI)=4.1, 10.9]. According to CF, the probability of an outbreak being multi-year was greatest for multistate outbreaks linked to dairy, poultry, eggs, and seafood commodities.

Discussion: Multi-year foodborne disease outbreaks are increasing. This trend is likely driven by improved case ascertainment in outbreak investigations through Whole Genome Sequencing analytics, which facilitate the identification of temporally separated illnesses within an outbreak. The identification of specific commodities linked to multi-year outbreaks emphasizes the complexities of foodborne disease transmission and the challenges these outbreaks may pose for annual source attribution.

Conclusion: These findings highlight the importance of ongoing improvements in outbreak identification to enhance our understanding of foodborne illness epidemiology and to inform the development of effective disease reduction strategies.

P1-028: WILDLIFE-ASSOCIATED SHIGA TOXIN-PRODUCING ESCHERICHIA COLI: SPATIOTEMPORAL DYNAMICS IN CALIFORNIA CENTRAL COAST AGRICULTURAL REGION

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Wildlife species are recognized reservoirs of foodborne pathogens; however, limited data exists on the spatiotemporal variability of Shiga toxin-producing *Escherichia coli* (STEC) transmission within wildlife populations and the resulting food safety risk to raw produce.

Purpose: To determine STEC prevalence across wildlife species and assess genomic

relatedness of isolates from wildlife and other matrices.

Methods: From July 2020–May 2025, wildlife fecal samples were collected from California Central Coast agricultural region along with other matrices. Samples were presumptively assigned to host species by visual characteristics and screened for STEC per FDA BAM methods. A subset (n=174) underwent mitochondrial DNA sequencing for vertebrate host confirmation. All STEC-positive isolates were whole-genome sequenced; single nucleotide polymorphism (SNP) analysis defined strains as identical if <25 SNPs apart.

Results: Overall STEC prevalence was 13.2% (124/942), highest in feral pigs (27.3%, 45/165), cervids (22.0%, 18/82), and birds (14.9%, 15/101). Specifically, STEC O157:H7 prevalence was 1.3% (12/942). Dominant serotypes were O26:H11. WGS identified shared strains across hosts/matrices: (1) O26:H11 (7 strains): 1 within feral pigs; 2 across wildlife (e.g., feral pig/coyote); 4 involving livestock and wildlife. (2) O157:H7 (4 strains): 3 across livestock/wildlife (e.g., bobcat/coyote); 1 shared among feral pig, livestock, insects and creek water. (3) O163:H19 (1 strain): shared among deer, feral pig, and livestock. Geographic distances ranged from <10 km to 115 km.

Significance: Wildlife (feral pigs, cervids, birds) may serve as important STEC reservoirs, sharing strains with livestock and environmental matrices, including insects and creek water, across spatial distances.

P1-029: INFECTION IN INFANTS LINKED TO THE CONTAMINATION OF EXPRESSED BREAST MILK: A SCOPING REVIEW

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Expressed breast milk (EBM) has been implicated in infant infections, but the relevant bacterial hazards and routes of EBM contamination are not well defined.

Purpose: Identify the pathogens and EBM contamination scenarios that pose the greatest risk in infants to inform hazard analysis and intervention strategies for EBM safety.

Methods: A scoping review was conducted across all English language case-report studies involving a bacterial or fungal infection in an infant that was putatively linked to EBM. Four databases (PubMed, Scopus, CINAHL, and Cochrane) were queried with an electronic search protocol. Title and abstract review (n=2,584) followed by full text review (n=154) determined whether each study matched the scoping review inclusion criteria, resulting in n=40 total studies included in the review. These studies were examined by a team of trained reviewers and relevant information was recorded with a data extraction and synthesis template.

Results: EBM related infections were caused by a diversity of pathogens (n=14 species), with the pathogen most frequently reported being *S. agalactiae* (30% of studies), followed by *C.*

sakazakii (12.5%) and *S. aureus* (12.5%). Intrinsic and extrinsic contamination routes of EBM varied by pathogen. These differences have important implications for intervention effectiveness which will vary across pathogens and routes. Almost all infants with infections linked to contaminated EBM (94%) were clinically fragile, with the median birth weight and gestational age of afflicted infants being 1,080 g (Very Low Birth Weight) and 28 weeks (Extremely Preterm), respectively. Epidemiological evidence linking infections to contaminated EBM was often weak, with less than half of studies using subtyping methods to confirm the strain identity of bacterial isolates.

Significance: This provides evidence to strengthen hazard analysis and intervention prioritization with respect to the microbiological safety of EBM.

P1-030: FOODBORNE BACTERIAL ILLNESS OUTBREAKS LINKED TO NON-DAIRY FERMENTED FOOD PRODUCTS — UNITED STATES, 2011–2023

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: The foodborne illness burden linked to fermented products in the United States is not well understood. Fermentation is not accounted for in common categorization schemes.

Purpose: To quantify the burden of foodborne bacterial outbreaks linked to non-dairy fermented foods (NDFF) in the U.S. from 2011–2023.

Methods: The U.S. Foodborne Disease Outbreak Surveillance System (FDOSS) was queried for bacterial outbreak reports with a known vehicle and date of first illness between 2011–2023. Outbreaks attributed to multiple foods or dairy products were excluded. All foods associated with the remaining outbreaks were reviewed to identify possible NDFFs. Keywords (ferment, pickle, starter, culture) were also used to string-search FDOSS comment fields for review. SAS, R, and RStudio were used for analysis. A p-value <0.05 was considered statistically significant.

Results: Thirty-nine bacterial NDFF outbreaks were identified, resulting in 509 illnesses, 132 hospitalizations, and four deaths. The leading cause of NDFF outbreaks was *Clostridium botulinum* (47%), while most illnesses were attributed to *Salmonella enterica* (64%). A total of 64% NDFF outbreaks were single-state; however, multistate NDFF outbreaks resulted in over three times as many illnesses (386 multistate vs. 123 single-state). Multistate NDFF outbreaks (median: 15 illnesses, range: 4–104) were also significantly larger than single-state outbreaks (median: 3 illnesses, range: 2–18). Single-state NDFF outbreaks were primarily linked to vehicles categorized as “other aquatic animals” (seal oil, whale, walrus). Multistate NDFF outbreaks were primarily linked to “pork” (charcuterie meats, deli ham, salami sticks) or meat/dairy alternatives (tempeh, cashew cheese).

Significance: This study highlights the public health impact of outbreaks associated with NDFFs. Preventive controls during the manufacture of fermented foods and safe food handling by the consumer are important to prevent foodborne illness.

P1-031: FOODBORNE DISEASE OUTBREAKS CAUSED BY HEPATITIS A VIRUS—FOODBORNE DISEASE OUTBREAK SURVEILLANCE SYSTEM, UNITED STATES, 1998–2024

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: Hepatitis A virus (HAV) causes foodborne disease outbreaks in the United States, but its epidemiology has not been fully described.

Purpose: To provide the first national summary of foodborne disease outbreaks caused by HAV and inform prevention efforts.

Methods: Foodborne disease outbreaks caused by HAV and reported to the Foodborne Disease Outbreak Surveillance System (FDOSS) during 1998–2024 were characterized by severity, food sources, location of food preparation, and contributing factors. Food sources were characterized by importation status and method of preparation.

Results: During 1998–2024, 113 outbreaks caused by HAV were reported to FDOSS. These resulted in 3,089 illnesses, 637 hospitalizations, and 17 deaths. Of the 113 outbreaks, 8 (7%) were multistate, 45 (40%) had an identified food source, 7 (6%) were caused by imported foods (fruit or shellfish), and 68 (60%) resulted from consuming food prepared at restaurants.

Of the 45 outbreaks with identified food sources, fruits were the most common source (10, 22%), followed by leafy greens (4, 9%) and shellfish (4, 9%). Leafy greens caused the most illnesses (1,031). Contributing factors were reported for 51 outbreaks (45%); most identified contamination from HAV-infected food handlers (37, 73%).

Significance: Over 27 years of surveillance data, HAV caused a substantial number of outbreaks in the United States with common food sources identified as fruits, leafy greens, shellfish, and imported foods. This highlights increased risks of widespread exposures when contaminated foods are distributed and the need for holistic approaches to outbreak prevention. Prevention strategies include routine education on hygiene and sanitation standards in restaurants to prevent contamination from infected food handlers.

P1-032: U.S. TRENDS IN V. CHOLERAE INFECTIONS ATTRIBUTED TO SEAFOOD AND WATER EXPOSURES, 2017–2024

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Presentation type/Format: Poster

Category: Epidemiology

Abstract:

Introduction: *Vibrio cholerae* (*Vc*) is a species of pathogenic bacteria of increasing prevalence in the United States; however, etiologic, demographic, and clinical characteristics of infections are not fully explored.

Purpose: We aim to characterize the epidemiology of non-toxigenic *Vc* in the United States, with a focus on seafood products and water exposures, to inform prevention and control.

Methods: We queried the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system for reported non-toxigenic *Vc* cases during 2017–2024. We classified modes of transmission and summarized patient demographics, clinical characteristics, and outcomes using descriptive statistics.

Results: From 2017–2024, 2,610 *Vc* cases were reported to COVIS, including 2,281 (87%) foodborne, 108 (4%) non-foodborne, and 221 (8%) with undetermined transmission route. More cases (1,492, 57%) were culture independent diagnostic test (CIDT) positive with no culture confirmation than culture confirmed (1,118, 43%). Overall, 1,319 (51%) cases were male, 1,758 (81%) were non-Hispanic, and 1,808 (80%) were white. Underlying conditions were reported for 1,085 (52%); 893 (39%) were hospitalized and 83 (3%) died. Cases were most often reported from non-coastal states (1,035, 40%). Among foodborne cases with

available seafood consumption history, shrimp (572, 37%) and fish (522, 35%) were most commonly reported; oysters (359, 23%) were frequently consumed raw (77%). Among non-foodborne cases with available water exposure history, skin contact with freshwater was most frequent (23, 41%).

Significance: *Vc* infections are predominantly foodborne and increasingly detected by CIDT without further culture confirmation. *Vc* is unlike other *Vibrio* species, as cases are most often reported by non-coastal states and, among non-foodborne cases, freshwater exposure is more common than salt and brackish water. These findings underscore opportunities for public health education and intervention.

P1-033: EFFECT OF BACTERIOPHAGES AGAINST SALMONELLA BIOFILMS WITH AND WITHOUT SOILING OF EGG CONSTITUENTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* is a significant concern during egg processing and preparation. Egg constituents such as egg yolk, white and a combination of egg yolk and white may enhance *Salmonella* biofilms on egg processing and preparation surfaces.

Purpose: Our study examined the effect of bacteriophages (n=2) against monospecies *Salmonella* biofilms of *S.* Singapore (n=2), *S.* Enteritidis (n=2), *S.* Schwarzengrund (n=1) and *S.* Typhimurium (n=2) on glass, stainless steel and wood with and without soiling of egg yolk, white and a combination of egg yolk and white.

Methods: *Salmonella* biofilms with and without soiling were grown on coupons at 25°C for 24h and were treated with bacteriophage LW1 and LW2 (multiplicity of infection at 0.01), respectively, at 25°C for 24h. *Salmonella* numbers were enumerated on thin agar layer medium. All assays were conducted in triplicate with independently grown cultures. *In silico* analysis determined the presence/absence of phage receptors, prophages and biofilm associated genes in *Salmonella*.

Results: Soiling did not significantly (p □ 0.05) influence biofilm cell numbers or

bacteriophage efficacy. Both bacteriophages significantly ($p \leq 0.05$) reduced *Salmonella* numbers by ~ 0.9 – 3.5 log CFU/cm^2 on surfaces under various soiling conditions. *S.* Singapore lacked phage receptors associated with the *rfb* operon which may have contributed to the highest reduction of *Salmonella* cells following treatment with both bacteriophages on stainless steel and glass irrespective of soiling conditions. Lack of these receptors may show they are receptive to these bacteriophages while inducing resistance to others. Wood generally exhibited minimal reduction of *Salmonella* numbers after treatment with bacteriophages.

Significance: Both bacteriophages show potential as biocontrol in commercial and home kitchen settings for reducing *Salmonella* biofilms on egg-related surfaces.

P1-034: DEVELOPMENT OF A PHAGE COCKTAIL FOR CONTROLLING SALMONELLA IN POULTRY

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Antimicrobial resistance is an increasing challenge in controlling foodborne pathogens, highlighting the need for effective alternatives to antibiotics. Bacteriophages (bacterial viruses) offer a promising strategy for pre-harvest intervention in poultry production.

Purpose: This study aimed to develop and optimize a broad-host-range bacteriophage cocktail targeting poultry-relevant *Salmonella* serovars, particularly *Salmonella* Enteritidis (SE) and *Salmonella* Kentucky (SK).

Methods: Fifty-one bacteriophages were screened against a panel of thirty *Salmonella* strains isolated from poultry litter. Selected phages were characterized based on lytic activity, host range, and receptor usage. Phage combinations were evaluated for enhanced efficacy, and encapsulation via spray drying was assessed to improve pH stability and enable targeted release. Experiments were conducted at 25°C and 41°C to simulate environmental and poultry conditions.

Results: Thirteen phages demonstrated strong lytic activity across both temperatures and were shortlisted as candidates. Seven phages were further characterized, with receptor usage primarily involving *BtuB* and *OmpC*. Five phages were tested in all combinations against SE and SK, and a four-phage cocktail showed the highest and most consistent bacterial reduction at both temperatures. The top-performing phage at 25°C was successfully

encapsulated, showing only a 0.18 log reduction in titer after 250 days at 4°C while retaining lytic activity upon reconstitution.

Significance: This study demonstrates the feasibility of developing a stable and effective phage cocktail for pre-harvest control of *Salmonella*. This approach has the potential to reduce pathogen load in poultry and barn environments, minimize contamination during processing, and enhance food safety.

P1-035: APPLICATION-READY PHAGE BIOCONTROL OF MDR SALMONELLA INFANTIS ON CHICKEN BREAST WITH NO DETECTABLE QUALITY TRADE-OFFS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Chicken meat is the most consumed meat worldwide, and contamination with multidrug-resistant (MDR) *Salmonella* Infantis (SI) remains a major food-safety risk.

Purpose: To evaluate 4-phage-cocktail stability across temperatures and on chicken meat, validate anti-SI activity on chicken and assess effects on quality indicators.

Methods: A phage cocktail was (i) tested for stability in SM buffer or formulated with lactic acid or with chicken protein plus trehalose at 0, 7, and −20°C, with titers quantified by spot assay; and (ii) screened for host range across *Salmonella* serovars by spot test. The cocktail was applied to chicken breast by spraying, marination, immersion, and immersion with 400 ppm peracetic acid (PAA) and phage recovered was quantified at 0.5 and 18 h. Phage persistence after spraying was assessed during storage at 4°C for 15 days. Efficacy was tested on chicken artificially contaminated with SI (4 log CFU/mL) and tested on meat

rinsates using SureCount qPCR. Quality was monitored at 4°C for 14 days by testing pH, color, and aerobic plate counts (APC). Data were analyzed by two-way ANOVA with Dunnett's test (p<0.05).

Results: Excipients did not significantly improved stability at the tested temperatures. The cocktail lysed SI, Heidelberg, Agona, Enteritidis, and Typhimurium. All application strategies preserved phage viability except immersion with PAA, where phage was undetectable after 0.5 h. Phage titers remained stable with an initial of 6.22±0.30 and at day 14, 6.01±0.06 logPFU/mL on chicken at 4°C. Compared with the control, one phage spray treatment reduced SI by 1.19 log CFU/mL after 24 h (p<0.005). No significant differences were observed in pH, color, or APC.

Significance: These results support the feasibility of a phage cocktail as a targeted, quality-neutral intervention to control MDR SI in chicken meat and identify compatible application methods for industrial implementation.

Award Application: Developing Scientist Award

P1-036: SURVIVAL OF ALIARCOBACTER BUTZLERI ON RAW POULTRY UNDER DIFFERENT PACKAGING ATMOSPHERES AND PERACETIC ACID TREATMENTS WITH DIFFERENT ORGANIC MATTER LEVELS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Aliarcobacter butzleri* (Ab) is an emerging foodborne pathogen frequently associated with poultry products and represents a potential risk to consumer health.

Purpose: To evaluate the survival of Ab on raw poultry under aerobic and anaerobic storage conditions and to assess the efficacy of peracetic acid (PAA) treatments in the presence of varying organic matter (OM) levels.

Methods: Fresh chicken thighs (236.5 ± 4.8 g) were artificially inoculated with Ab (ATCC 49616) at 6 log CFU/mL of rinsate. Samples were stored at 4°C for 14 days under aerobic or anaerobic atmospheres to monitor Ab survival and aerobic plate counts (APC). In a separate experiment, contaminated thighs were immersed for 90 s in PAA (500 ppm) containing 0, 15, or 30 g/L OM. Ab populations were enumerated using NRJ and mCCDA-CAT agar. Data are reported as mean log CFU/mL from two independent replicates and analyzed using two-way ANOVA ($p < 0.05$).

Results: No significant differences in Ab survival were observed between aerobic and anaerobic conditions, with reductions of 0.71 and 1.82 log CFU/mL after 14 days, respectively. APC increased from an initial 2.13 ± 0.05 to >7.2 log CFU/mL under both atmospheres. PAA treatments did not significantly reduce Ab levels regardless of OM concentration, with counts in PAA-treated samples (5.64 ± 0.23 log CFU/mL) comparable to water-treated controls (5.38 ± 0.01 log CFU/mL). Although both selective media produced similar Ab counts, mCCDA-CAT supported greater background microbiota growth, complicating Ab detection.

Significance: These findings demonstrate the persistence of Ab on raw poultry and its limited susceptibility to PAA under high organic load conditions, highlighting the need for improved intervention strategies to control this emerging pathogen in poultry processing.

Award Application: Developing Scientist Award

P1-037: EFFECTIVE REDUCTION OF SALMONELLA LOAD AND SEROVAR COMPLEXITY IN CHICKEN PARTS THROUGH ANTIMICROBIAL INTERVENTION

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: In-plant antimicrobial interventions, specifically peracetic acid, have been shown to effectively reduce *Salmonella*, but prevalence increases after whole carcasses are cut into parts.

Purpose: This study sought to assess *Salmonella* prevalence, quantity, and serovar complexity during broiler processing, specifically comparing skin-on, bone-in parts (whole and cut wings) with skinless, boneless parts (whole breast or tenders).

Methods: Four-pound part rinses were collected in 12 plants in three states, from both pre- and post-intervention as well as at the beginning and end of a shift (n = 1499). *Salmonella* prevalence and enumeration was performed with the bioMérieux GENE-UP assay. CRISPR-SeroSeq was performed on *Salmonella*-positive samples.

Results: In breasts, 33.1% (124/375) of pre-intervention samples were *Salmonella*-positive, which was reduced to 18.1% (68/375) post-intervention (X^2 (1, N = 750) = 21.176, p = <0.05).

Wings were lower with 20.6% (77/374) pre-intervention and 9.9% (37/375) post-intervention (X^2 (1, N = 749) = 15.86, p = <0.05). A Wilcoxon rank-sum test indicated no significant difference in *Salmonella* load after antimicrobial application in tenders (W= 4023.5, p = 0.5773). However, a significant difference was observed in wings (W = 1722, p = 0.03193). Deep serotyping results revealed that 24.2% (30/124) of tenders and 6.5% (5/77) of pre-intervention wings contained multiserovar populations, which was reduced to 5.9% (4/68) and 2.9% (1/35) respectively. Overall, eight serovars were detected with serovar Infantis the most prevalent (62.8%; 191/304), followed by serovar Typhimurium (22.0%; 67/304), and serovar Enteritidis (16.1%; 49/304).

Significance: These data demonstrate that antimicrobial interventions used during broiler processing reduce *Salmonella* prevalence and complexity in both tenders and wings and reduce load in wings but not tenders. Further, *Salmonella* incidence is higher in tenders than in wings.

Award Application: Developing Scientist Award

P1-038: OPTIMIZING PKA-BASED ORGANIC ACID COMBINATIONS TO ENHANCE ANTIMICROBIAL EFFICACY AT PH 6

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Lactic and acetic acids are commonly used to inhibit *Listeria monocytogenes* and *Salmonella enterica* in meat; however, at pH 6, which is common for meat preservation, much higher concentrations are needed for effective inhibition.

Purpose: To evaluate organic acid combinations at pH 6 to minimize total acid needed for effective inhibition.

Methods: Treatments included formic, lactic, acetic, propionic, and butyric acids tested individually. For combinations, acids were selected and formulated based on their pKa values to maximize the active fraction at pH 6. To minimize sensory effects, butyric acid was limited to 50 mg/L. Untreated growth and sterility controls were included. All assays were performed in triplicate. MICs were determined by OD600 measurements and supplemented with iodonitrotetrazolium chloride (INT) assays for colorimetric detection of bacterial viability.

Results: The MIC of lactic acid was 70 g/L for both pathogens, while the MICs of acetic, propionic, formic, and butyric acids were higher for *L. monocytogenes* (18, 14, 22, 16 g/L,

respectively) than for *S. enterica* (9, 7, 11, 2 g/L, respectively). Formic and propionic acids were avoided due to corrosiveness and meat quality issues, whereas lactic and acetic acids were selected for their practicality and established use in the meat industry. Butyric acid (pKa 4.82) was included as a synergist due to its high fraction of undissociated acid at pH 6. Adding 50 mg/L butyric acid reduced the lactic acid MIC from 70 g/L to 56 g/L for both pathogens. For acetic and butyric acids combination, the acetic acid MIC decreased from 18 g/L to 10 g/L for *L. monocytogenes* and from 9 g/L to 6 g/L for *S. enterica,* compared to when acetic acid was used individually.

Significance: Synergistic organic acid combinations enhance antimicrobial efficacy at lower concentrations and can be applied for food and meat preservation.

Award Application: Developing Scientist Award

P1-039: EFFECT OF ORGANIC ACID COMBINATIONS ON CLOSTRIDIUM PERFRINGENS GROWTH INHIBITION DURING EXTENDED COOLING OF COOKED UNCURED BEEF

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Option 1.2 of the FSIS Revised Appendix B 2021 guidelines requires cooked meat products without validated antimicrobials to be cooled as such: 48.9-26.7°C in □1 hour (Phase 1), 26.7-12.8°C in □5 hours (Phase 2), cooling to 4.4°C (Phase 3). Clean-label antimicrobials often contain organic acids, but the combinations necessary for *Clostridium perfringens* growth inhibition are relatively unknown, requiring product validation to extend Phase 1.

Purpose: To compare the efficacy and synergistic effects of active components (lactate, propionate, acetate) found in clean-label antimicrobials during extended cooling.

Methods: Ground beef (Trial 1-71% moisture, pH 6.1, 1.0% NaCl; Trial 2-74% moisture, pH 6.3, 1.0% NaCl) was formulated with sodium lactate (0.11-3.39%), sodium acetate (0-1.05%), and/or sodium propionate (0-1.05%) selected to build a response surface model. Formulations

were inoculated with 2-3 log spores/g of three strains of *C. perfringens*, portioned into 25 g, cooked to 73°C, and cooled in 3, 4, or 5-hours for Phase 1 and 5-hours each for Phases 2 and 3.

Results: Formulations with ≥1% lactate, ≥0.25% acetate, and ≥0.25% propionate inhibited *C. perfringens* growth (<1-log CFU/g increase) when Phase 1 was ≥5 hours for Trial 1 and ≥3 hours for Trial 2. In formulations containing 1% lactate with either 0.25% acetate and 0.75% propionate or 0.75% acetate and 0.25% propionate, *C. perfringens* growth was inhibited during ≥5-hour Phase 1 for Trial 2. Decreasing lactate to 0.11% with 0.5% acetate and 0.5% propionate led to an average increase in *C. perfringens* populations of 1.23±0.01 log CFU/g with a 4-hour Phase 1 for Trial 2. Formulations without organic acids had >2-log CFU/g increases when Phase 1 was ≥3 hours in both trials.

Significance: Study results suggest clean-label antimicrobials with ≥1% lactate, ≥0.25% acetate, and ≥0.25% propionate inhibit *C. perfringens* growth with a ≥3-hour Phase 1 in cooked beef with ≥74% moisture, pH ≥6.3, and salt ≥1%.

P1-040: CLEAN-LABEL ANTIMICROBIALS FOR INHIBITING SPOILAGE MICROORGANISMS ISOLATED FROM PROCESSED MEAT SYSTEM

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Spoilage microorganisms, including lactic acid bacteria (LAB) and yeast, reduce the shelf life of processed meats. Clean-label antimicrobials are increasingly used to meet consumer demand for natural preservation.

Purpose: To evaluate the inhibitory efficacy of clean-label antimicrobials against LAB and yeast from meat snack sticks.

Methods: Treatments included Flavoset 8300L (T1; 0.15–0.25%), Flavoset 5400L (T2; 0.5%), and combinations of 0.15% T1 with 0.05–0.1% T2. Solutions in MRS or PDB media (pH 5.0) were inoculated with one LAB strain and two yeast strains. Growth at 30°C (LAB) and 25°C (yeast) was monitored at 600 nm using an accelerated data platform. Growth curves were fitted to the modified Gompertz model to estimate μmax and lag time. MIC was the lowest concentration preventing growth. Significance was determined by one-way ANOVA ($P < 0.05$).

Results: All antimicrobials significantly inhibited growth ($P < 0.05$). MIC for all isolates was

0.25% T1, fully inhibiting LAB and yeasts. At 0.15% T1, yeasts were completely inhibited, while LAB showed reduced growth (μmax = 0.06 h^{-1}; lag = 13.7 h) vs. control (μmax = 0.10 h^{-1}; lag = 8.4 h). Combination treatments achieved complete inhibition. At 0.5% T2, LAB and yeast 2 were fully inhibited; yeast 1 showed strong reduction (μmax = 1.8 h^{-1}; lag = 5.3 days) vs. control (μmax = 9.1 h^{-1}; lag = 1.1 days).

Significance: Natural flavor-based antimicrobials effectively inhibit spoilage LAB and yeasts in meat snack sticks, offering a promising clean-label solution for shelf-life extension.

P1-041: EFFICACY OF NATURAL FLAVOR FOR INHIBITING THE GROWTH OF SPOILAGE CAUSING LACTIC ACID BACTERIA ASSOCIATED WITH MEAT PRODUCTS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Lactic acid bacteria (LAB) are major spoilage organisms in meat systems, reducing shelf life. With growing sustainability demands, extending shelf life of processed meats is critical.

Purpose: To evaluate the effectiveness of a natural flavor system in controlling spoilage LAB in fresh and processed meats.

Methods: Antimicrobial solutions (0.1–0.2% Flavoset 5400L) and controls were prepared in MRS broth (pH 6.0), aliquoted into 100-well plates, and inoculated with LAB strains *(Leuconostoc mesenteroides, Lactobacillus sakei, L. curvatus, L. plantarum*). Plates were incubated at 30°C on an accelerated data platform. Optical density at 600 nm was measured every 30 min. Growth curves were analyzed using the Gompertz model to estimate lag phase (days) and maximum growth rate (μmax, h^{-1}). MIC was the lowest concentration preventing

growth. Treatment performance was compared by one-way ANOVA ($P < 0.05$).

Results: All antimicrobials significantly inhibited growth ($P < 0.05$) vs. control. MIC for all isolates was 0.2% Natural Flavor. At 0.16%, *L. mesenteroides* and *L. curvatus* were completely inhibited, while *L. plantarum* and *L. sakei* showed significant reduction. For example, 0.16% Natural Flavor reduced growth rate and extended lag time for *L. plantarum* (μmax = 5.4 h^{-1}; lag = 2.02 d) and *L. sakei* (μmax = 1.9 h^{-1}; lag = 2.9 d) compared to controls (*L. plantarum*: μmax = 8.3 h^{-1}; lag = 0.4 d; *L. sakei*: μmax = 6.9 h^{-1}; lag = 0.2 d).

Significance: Natural flavor systems effectively inhibit spoilage LAB in processed meats, offering a clean-label solution for shelf-life extension.

P1-042: EFFECTS OF PIMENTA ESSENTIAL OIL AND TRANS-CINNAMALDEHYDE ON SALMONELLA HEIDELBERG AND MICROBIOME SUCCESSION IN REFRIGERATED GROUND TURKEY

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* Heidelberg (SH) is a multidrug-resistant foodborne pathogen frequently linked to poultry products. Beyond pathogen reduction, interventions that modulate the meat microbiome during refrigerated storage may improve food safety and shelf stability.

Purpose: To evaluate effects of two plant-derived antimicrobials (PDAs), Pimenta essential oil (PEO) and *trans*-cinnamaldehyde (TC), on SH survival and microbiome dynamics in ground turkey stored at 4°C.

Methods: Fresh ground turkey was assigned to 4 treatments: negative control (NC; no SH), SH control (SC), SH+PEO (2.0% v/w), and SH+TC (1.0% v/w). Except for NC, samples were inoculated with ~4 log CFU/g SH and stored at 4°C for 0, 3, and 8 days. Three samples per treatment per sampling day were analyzed, and the experiment was repeated twice. SH and total viable counts (TVC) were enumerated at each sampling day and analyzed using one-way ANOVA. Microbial composition was profiled using 16S rRNA sequencing; α- and β-diversity were analyzed using Wilcoxon test and PERMANOVA, and differentially abundant taxa were

identified using ANCOM-BC2.

Results: PDAs reduced SH and TVC compared to SC during storage (P<0.05). Microbiome analysis showed minimal community divergence on day 0, followed by treatment-dependent shifts over time. By day 3, TC reduced the relative abundance of psychrotrophic spoilage-associated genus *Carnobacterium*. By day 8, treated samples exhibited distinct microbial community structures and altered α-diversity, with TC suppressing late-stage spoilage taxa, *Janthinobacterium*. The relative abundance of *Lactobacillus*, *Akkermansia*, *Bifidobacterium*, and *Bacteroides* was reduced in all treatments on day 8. These shifts correlated with SH reductions, indicating that PDAs redirect microbial succession in addition to exerting antibacterial effects.

Significance: PDAs can be used to steer microbial ecology in refrigerated ground turkey, providing dual benefits for food safety and storage stability.

Award Application: Developing Scientist Award

P1-043: CONTROL POST-LETHALITY LISTERIA SPP. CONTAMINATION IN RTE BEEF BOLOGNA USING LAURIC ALGINATE VIA SPRAY LETHALITY IN CONTAINER (SLIC) TECHNIQUE

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Post-lethality contamination with *Listeria* spp. remains a critical food safety concern in ready-to-eat (RTE) products. SLIC with lauramide arginine ethyl ester (LAE) offers a unique and practical intervention to mitigate pathogenic risks to the consumer.

Purpose: This study evaluated the efficacy of LAE solutions applied via SLIC to control *Listeria innocua* on beef bologna.

Methods: Six pounds of beef bologna logs were surface inoculated with approximately 4 log CFU/pack of *Listeria innocua* ATCC 33090 and 33091 cocktail. After 10 min of *Listeria* attachment, antimicrobials were sprayed into the bags; following by vacuum sealing immediately. Treatments included LAE alone or in combination with a smoke condensate along with negative control. Two different spray volumes were also investigated, delivering 22 ppm and 44 ppm LAE, to mimic spray volume variation in production. Each treatment was sampled in triplicate. Treated products were stored under refrigeration (4°C) and monitored for *Listeria* growth for 63 days.

Results: All antimicrobial-treated samples achieved greater than 2-log reductions of *Listeria innocua* within the first week. This reduction met USDA Alternative 2 requirement. There is no difference in log reduction observed between LAE alone and LAE combined with liquid

smoke. There is also no difference between the levels of LAE delivered, indicating spray variation did not compromise antimicrobial efficacy. During shelf life, the negative control exhibited rapid growth, increased from 4 to 5 log CFU/pack shortly after one week. LAE-only treatments extended shelf life by four weeks. The combination of LAE with smoke provided an additional week of shelf-life extension beyond LAE alone.

Significance: LAE applied via SLIC provides an effective post-processing intervention to control *Listeria* spp. LAE can also be paired with another inhibitor to maximize product shelf life.

P1-044: ANTIFUNGAL ANTIMICROBIALS SYSTEM FOR INHIBITING MOLDS ISOLATED FROM PROCESSED MEAT PRODUCTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Mold contamination in dry-cured meats is a major spoilage risk, driving demand for clean-label solutions to ensure quality and safety.

Purpose: Evaluate the antifungal properties of natural, clean-label antimicrobials against molds isolated from processed meat systems.

Methods: Antimicrobial solutions were prepared in acidified Potato Dextrose Broth with no antimicrobial, 0.3 %, 0.4% and 0.5% of natural flavor. The pH of the solution was adjusted to 6 and aliquoted to 100 well plates inoculated with isolated molds from the processed meat products, included one *Penicillium* spp. and six *Aspergillus* spp. Growth curves were generated using accelerated data generation platform (ADGP) at 25°C for 14 days. Lag time (days) and maximum growth rate (μmax; h-1) were modeled using a Gompertz function. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which no growth occurred. Data were analyzed using

ANOVA (p □ 0.05).

Results: Antimicrobial treatments significantly inhibited mold growth compared to the untreated control ($p < 0.05$). Control samples exhibited a higher growth rate (μ_max = 0.7–1.9 h^{-1}) relative to all antimicrobial treatments ($p < 0.05$). MIC of the natural flavor system was determined to be 0.5% for four out of six *Aspergillus* spp. For the remaining two species, sub-MIC levels (0.2%) resulted in a significant ($p<0.05$) extension of the lag phase, increasing from 0.92 to 3.67 days for one species and from 1.32 to 13.46 days for the other. All treatments prolonged the lag phase and reduced the growth rate of *Penicillium* spp. at 0.2%, with lag time increasing from 0.65 days in the control to 4.5 days under treatment conditions.

Significance: This study shows a natural flavor system effectively inhibits mold in processed meat using accelerated data analysis.

P1-045: EVALUATION OF CLEAN-LABEL BUFFERED VINEGAR AS AN ALTERNATIVE TO CONVENTIONAL SODIUM ACETATE FOR SHELF-LIFE EXTENSION IN READY-TO-EAT COOKED PORK

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Sodium acetate, widely used for microbial control in meat preservation, is being banned by premium retail chains, creating a need for clean-label alternatives

Purpose: Evaluated the efficacy of a clean-label vinegar intervention compared to conventional sodium acetate in maintaining shelf-life performance of ready-to-eat (RTE) cooked pork.

Methods: Pork was prepared using two seasoning blends: a control seasoning mix containing 6% sodium acetate and a clean label blend containing 3% vinegar. Seasoning blends were applied to the product. Pork was cooked, vacuum-packaged, and stored under

refrigeration throughout the shelf life (n=32). At each sampling interval, analyses were performed in duplicate. Physicochemical parameters (pH, water activity, moisture, and salt) were measured. Microbial growth was monitored over 95 days at 15-day intervals for Aerobic Plate Count (APC), Lactic Acid Bacteria (LAB), and yeasts/molds. Spoilage was defined as APC 6 log CFU/g or sensory defects. Data were analyzed using ANOVA ($p < 0.05$).

Results: Both the clean-label treatment and the control exhibited comparable physicochemical profiles pH (control 6.47; treatment 6.31), a_w (0.673 vs. 0.6405), and salt (~1%). Initial APC counts were ~1.5 log CFU/g for both treatments. APC remained below 3 log CFU/g through Day 95. LAB increased from ~0.8 log at Day 0 to ~2 log by Day 30 across both groups. After Day 30, both APC and LAB remain constant showing no signs of spoilage till Day 95. Yeasts and molds were undetectable.

Significance: Clean-label vinegar can replace sodium acetate in RTE meat while maintaining shelf life and meeting retailer standards.

P1-046: INHIBITION OF SALMONELLA GROWTH IN FRESH GROUND BEEF, PORK AND POULTRY BY A NOVEL VINEGAR-BASED PRESERVATION SYSTEM UNDER ABUSIVE TEMPERATURE CONDITIONS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Fresh ground meat (pork and chicken) can support growth of *Salmonella* under abusive temperature storage conditions. Consumers demand natural and clean label food protection system that can control foodborne pathogens.

Purpose: Determine the antimicrobial efficacy of a Vinegar Oil™ against *Salmonella* in ground pork and chicken under abusive temperature storage.

Methods: Fresh ground pork and chicken were prepared in the meat laboratory and portioned into (i) no treatment control (Cont), (ii) Vinegar Oil, 1.0% (VO-1.0%), or (iii) Vinegar Oil, 1.5% (VO-1.5%). Five-gram portions of the meat were weighed into vacuum pouches (6.35 by 7.6 cm), sealed and stored frozen until use. Bags were thawed and inoculated with Nalidixic Acid-resistant *Salmonella* to obtain ca. 3.5 log CFU/g. The bags were subjected to sinusoidal temperatures (10°C to 40°C within 6 h, for 36 h). *Salmonella* was enumerated every 12 h. Statistical analysis and model building was performed in JMP Pro version 16.1.0 (SAS Institute Inc., NC, USA), at $P < 0.05$.

Results: Initial *Salmonella* population was 3.58±0.02, 3.51±0.16 and 3.58±0.06 in Cont, VO-1.0% and VO-1.5%, respectively in ground pork and 3.73±0.06, 3.70±0.04 and 3.71±0.04, respectively in ground chicken. Subjecting *Salmonella*-inoculated ground pork and ground

chicken (Cont) to abusive sinusoidal temperatures resulted in growth to 3.68±0.37 and 4.59±0.25 log CFU/g within 18 h and 4.49±1.17 and 5.74±0.07 log CFU/g within 36 h. However, reduction (*P*□0.05) in *Salmonella* population was observed, with 2.20±0.17 and 2.84±0.06 log CFU/g in ground pork and chicken (containing VO-1.0%), respectively within 18 h. Higher reduction in *Salmonella* population was observed in ground meats containing higher concentration of Vinegar Oil (1.5%) and extended exposure to abusive temperatures.

Significance: Novel Vinegar Oil demonstrated significant *Salmonella* inhibition in ground beef, pork and chicken when subjected to extended abusive temperature conditions.

P1-047: EXTENSION OF REFRIGERATED SHELF LIFE OF FRESH GROUND BEEF, PORK AND POULTRY USING A NOVEL VINEGAR-BASED PRESERVATION SYSTEM

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Fresh ground meat (beef, pork and chicken) has short microbial shelf life and results in food waste. Consumer interest in natural and clean label food protection system has increased.

Purpose: Determine the antimicrobial efficacy of a Vinegar Oil™-based solution against spoilage microorganisms in ground beef, pork and chicken.

Methods: Fresh beef chuck, pork shoulder and chicken thighs (boneless, skinless) were obtained from a local wholesale store, diced and divided into three equal portions; no treatment control (Cont) and the other two portions were sprayed uniformly with Vinegar Oil at either 1.0% (VO-1.0%) or 1.5% (VO-1.5%). Each portion is manually mixed, and ground (3.18 mm). The product was portioned (454 g), distributed in a plastic tray, over-wrapped with polyvinyl chloride (PVC) film and stored under refrigeration (7.2C) for up to 14 days. General microbial population (APCs) of the ground meat was evaluated on days 0, 7 and 14. Three independent replications, with one sample (454 g) was used. Statistical analysis was performed in JMP Pro version 16.1.0 (SAS Institute Inc., NC, USA), at $P < 0.05$.

Results: APCs were 3.73±0.30, 2.93±0.18 and 2.76±0.14 log CFU/g (Cont, VO-1.0% and VO-1.5%, respectively) in ground beef, 5.44±0.23, 4.95± 0.10 and 4.79±0.07 log CFU/g in ground pork and 5.45±0.26, 5.01±0.42, and 4.21±0.15 log CFU/g in ground chicken,

respectively on day 0. Refrigerated storage of ground beef, pork and chicken resulted in growth (P□0.05) to 6.36±0.13, 7.18±0.30 and 8.32±0.18 log CFU/g in Cont product after 14 days. Similar storage resulted in reduction (P□0.05) in APCs of ground beef (to 1.80±0.12 and 1.18±0.15 log CFU/g in VO-1.0% and VO-1.5%, respectively), ground pork (to 3.71±0.10, 3.56±0.26 log CFU/g) and ground chicken (to 4.57±0.40, 4.33±0.45 log CFU/g).

Significance: Novel Vinegar Oil demonstrated significant microbial inhibition and enhanced microbiological shelf life (refrigerated) of ground beef, pork and chicken.

P1-048: NO-SODIUM DRY VINEGAR EXTENDS SHELF LIFE OF GROUND BEEF, PORK, AND TURKEY

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Category: Meat, Poultry and Eggs

Abstract:

Introduction: With growing interest in low-sodium preservation strategies, no-sodium dry buffered vinegar offers a clean-label option to suppress spoilage under refrigeration. Ground meats are highly susceptible due to increased surface area and microbial redistribution during grinding.

Purpose: This study evaluated the efficacy of no-sodium dry buffered vinegar versus a no-antimicrobial control in extending shelf life and reducing spoilage microorganisms in ground meat.

Methods: Beef, pork, and turkey were prepared as control (no antimicrobial) and with 0.5% IsoAge D300 (no-sodium dry vinegar). Vinegar was added after coarse grinding, followed by fine grinding. Products were packed in chubs (250g), stored at 38°F for 21 days, and analyzed for aerobic plate counts (APC), lactic acid bacteria (LAB), and psychrotrophs. Shelf life was defined as APC 6.0 log CFU/g or onset of sensory spoilage (off-odor). Experiments were performed in duplicate and data were analyzed by ANOVA ($p<0.05$).

Results: In ground beef, baseline APC was ~2.0 log CFU/g; addition of the no-sodium dry vinegar produced an immediate ~1-log APC reduction post-mix. The control exceeded 6.0 log CFU/g with off-odor by Day 14, while the vinegar treatment extended shelf life by 7 days. In ground pork, baseline APC was ~3.0 log CFU/g; controls reached 6.0 log by Day 11, whereas the vinegar treatment remained significantly lower ($p<0.05$), measuring ~4.5 log at Day 16 and not crossing 6.0 log until Day 16 (+5 days). In ground turkey, baseline APC/psychrotrophs were ~4.3 log CFU/g; controls exceeded 6.0 log by Day 6, while the vinegar treatment extended shelf life by 6 days. Across species, psychrotroph level mirrored APC, and LAB counts remained comparatively low throughout refrigerated storage.

Significance: A no-sodium, dry vinegar at effectively extends refrigerated shelf life by 5–7 days across ground beef, pork, and turkey providing a sodium-free, clean-label solution for ground meat preservation.

P1-049: VINEGAR INTERVENTION STRATEGIES FOR SHELF-LIFE EXTENSION IN FRESH CHICKEN BREASTS AND THIGHS

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Category: Meat, Poultry and Eggs

Abstract:

Introduction: Fresh poultry products are highly perishable and supports rapid microbial growth during refrigerated storage.

Purpose: The objective of this study was to determine the impact of 1.5% liquid vinegar on microbial stability and shelf life of vacuum-packaged fresh chicken cuts compared to no-antimicrobial control.

Methods: Chicken breasts and thighs were marinated with no antimicrobial control and1.5% IsoAge 240L, combined with their respective marinade, and vacuum-tumbled. After marination, samples were portioned into 100-g units and vacuum-sealed in plastic pouches.

Baseline physicochemical properties, including pH, water activity, and moisture content, were measured post-marination. Packaged samples were stored under refrigeration and monitored for aerobic plate counts (APC) and lactic acid bacteria (LAB) until spoilage, defined as 6 log CFU/g or the presence of sensory defects such as off-odor or slime. Experiments were carried out twice and microbial data were analyzed using ANOVA with significance set at $p<0.05$.

Results: Baseline physicochemical measurements showed pH 6.02 in the control and pH 5.74 in the vinegar treatment. For thighs, the control exceeded 6 log CFU/g APC by Day 9 with noticeable off-odor, whereas the vinegar treatment maintained significantly lower counts ($p<0.05$), at approximately 3.5 log CFU/g at the same time point, and delayed spoilage until Day 19, providing an additional 10 days of shelf life. For breasts, the control started at 4.5 log CFU/g on Day 0, while vinegar achieved an immediate 1-log APC reduction post-marination. Control samples exceeded 6 log CFU/g by Day 7, whereas the vinegar treatment extended shelf life by approximately 5 days compared to control.

Significance: Clean label vinegar at 1.5% effectively extended the refrigerated shelf life of fresh chicken breasts and thighs by suppressing spoilage organisms and delaying sensory deterioration, offering a clean-label solution for fresh poultry applications.

P1-050: SPOILAGE SUPPRESSION IN BACON USING CLEAN-LABEL ANTIMICROBIALS: SHELF LIFE AND LAB CHALLENGE STUDIES

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Lactic acid bacteria (LAB) dominate spoilage in refrigerated, vacuum-packaged bacon and drive slime formation and off-odors during extended storage.

Purpose: This study quantified the dose-response efficacy of a natural flavor antimicrobial on LAB suppression and shelf life in bacon under uninoculated and LAB-challenged conditions.

Methods: Brines were injected into bacon to yield: No antimicrobial control and natural flavor (Flavoset 5400L) at 0.13%, 0.16%, 0.20%, 0.22%, plus 0.20% + 0.35% hardwood smoked sugar. Bacon was packaged as two stacked slices (mid-overlap contact surface - 25g) and

vacuum-sealed. For the challenge, 25g samples were inoculated with ~2.0 log CFU/g *Lactobacillus sakei* + *Leuconostoc mesenteroides* cocktail prior to packaging. Storage was refrigerated; sampling continued to 135d. Sampling was performed for APC and LAB for uninoculated sets; LAB for inoculated sets. The spoilage criterion was APC or LAB 6.0 log CFU/g and/or visible slime. Experiments were conducted in duplicate and data were analyzed using one-way ANOVA ($p < 0.05$).

Results: In the uninoculated bacon, control LAB and APC increased, with LAB reaching 6.0 log CFU/g by Day 70 and showing presence of slime. All antimicrobial treatments were significantly lower than control ($p < 0.05$) across time points. 0.13% reached 6.0 log by Day 90 (extending shelf life by 20 days), whereas 0.16%, 0.20%, 0.22%, and 0.20% + hardwood smoked sugar prevented outgrowth, maintaining LAB and APC ~4.0 log CFU/g through Day 135 (extending shelf life by >65 days). For the LAB-challenged bacon, control exceeded 6.0 logs and spoiled by Day 49. 0.13% and 0.16% crossed 6.0 log by Day 84. 0.20% and 0.22% remained ~4.0 log and stable through at least Day 90 (no slime), demonstrating efficacy of natural flavor antimicrobial to prevent spoilage LABs.

Significance: Natural flavor-based antimicrobials effectively extend the refrigerated shelf life of bacon by suppressing LAB growth and spoilage.

P1-051: ASSESSMENT OF ANTIMICROBIAL SYSTEMS FOR CONTROLLING LISTERIA MONOCYTOGENES AND SPOILAGE BACTERIA IN HOT DOGS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Listeria monocytogenes* is a major post-lethality contamination concern in ready-to-eat meats, while lactic acid bacteria drive spoilage, creating a need for effective antimicrobial strategies.

Purpose: This study evaluated vinegar and vinegar-based antimicrobial systems for controlling *L. monocytogenes* and spoilage microorganisms in hot dogs.

Methods: Four formulations were tested: control (no antimicrobials), 1.8% IsoAge 325L (potassium-buffered vinegar), 2–3% Nourishield 2000L (vinegar-fermentate), and 0.9%

Nourishield D4010 (vinegar-natural flavor). Packaged samples (100g) were either uninoculated for shelf life or surface inoculated with a five-strain *L. monocytogenes* cocktail, vacuum sealed and stored at 38°F for 135 days. Uninoculated samples were analyzed for aerobic plate counts and lactic acid bacteria, and inoculated samples were analyzed for *Listeria* counts. Antimicrobial success was defined as preventing □2 log CFU/g *Listeria* outgrowth; spoilage was □7 log CFU/g or visual deterioration. Data were analyzed by one-way ANOVA ($p < 0.05$).

Results: Initial *L. monocytogenes* levels ranged from 2.5 to 3.5 log CFU/g across all treatments. By day 28, control samples exhibited significant *L. monocytogenes* proliferation, reaching approximately 6.5 log CFU/g and exceeding 3 log CFU/g outgrowth ($p < 0.05$) relative to treated products. In contrast, hot dogs formulated with 2–3% vinegar-fermentate and 0.9% vinegar-natural flavor significantly suppressed *L. monocytogenes* growth ($p < 0.05$) throughout the 135-day storage period. In uninoculated samples, all antimicrobial treatments prevented spoilage for the entire storage duration.

Significance: The findings highlight the potential of vinegar-fermentate and vinegar-natural flavor to enhance food safety and extend shelf life by effectively controlling *L. monocytogenes* outgrowth and spoilage microorganisms in hot dogs stored at temperature of 38°F.

P1-052: EFFICACY OF VINEGAR FOR CONTROL OF SPOILAGE MICROORGANISMS AND LISTERIA MONOCYTOGENES IN READY-TO-EAT CHICKEN STORED UNDER REFRIGERATION

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Post lethality contamination and spoilage remain limiting factors for shelf life and safety of ready-to-eat (RTE) poultry products.

Purpose: To evaluate the effectiveness of a clean label liquid vinegar in controlling spoilage microorganisms and *Listeria monocytogenes* in RTE chicken during refrigerated storage at 4°C and 7°C.

Methods: RTE chicken was formulated with no antimicrobial (control) and 1% vinegar (IsoAge 200L), packaged (25g), and stored at 4°C or 7°C. Samples were evaluated for aerobic

plate counts (APC) and lactic acid bacteria (LAB) throughout storage. Spoilage threshold was defined as 6.0 log CFU/g microbial growth or visible signs of spoilage. Separate samples (25g) were surface inoculated with 2.5 log CFU/g *L. monocytogenes* prior to packaging and enumerated during storage at 4°C and 7°C. Product failure for *Listeria* was defined as 2.0 log CFU/g outgrowth. Experiments were performed in duplicate and data were analyzed using one-way ANOVA, with significance set at $p < 0.05$.

Results: At 4°C, control samples exhibited sensory spoilage by Day 18, with APC and LAB counts of approximately 5 log CFU/g. Vinegar–treated samples remained acceptable through Day 31, with significantly lower APC and LAB counts (~3 log CFU/g at Day 18 and 4.5 log CFU/g at Day 31; $p<0.05$). At 7°C, control samples reached spoilage levels (~7 log CFU/g) by Day 14, while vinegar significantly delayed spoilage until Day 26 ($p<0.05$). For *L. monocytogenes*-inoculated samples stored at 4°C, control treatments exceeded a 2 log CFU/g outgrowth by Day 17, whereas vinegar–treated samples remained <2 log CFU/g until Day 31. Under 7°C storage, the control exceeded this threshold by Day 6, while vinegar delayed outgrowth until Day 15, providing 14 and 8 additional days of *L. monocytogenes* suppression at 4°C and 7°C, respectively.

Significance: These findings support the use of vinegar as an effective clean label intervention to enhance safety and quality of RTE poultry products.

P1-053: BALANCING CLEAN LABELS AND SHELF LIFE IN FRESH GROUND PORK

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Category: Meat, Poultry and Eggs

Abstract:

Introduction: The rising demand for healthier, clean-label foods is prompting the meat industry to reduce traditional antimicrobials, such as lactates and diacetates. Although these ingredients extend shelf life, reducing them can increase spoilage and food waste, highlighting the need for effective alternative preservation strategies.

Purpose: To assess the efficacy of clean-label antimicrobials for two package types, aerobic or vacuum-sealed, on the shelf life of ground pork stored at 4.4°C.

Methods: Three ground pork treatments were prepared: a negative control [no antimicrobial], 1.05% Vinegar [Verdad® N8], and 0.55% Cultured Dextrose [Verdad® N10] + 1.05% Vinegar. For each formulation, 50 g portions were vacuum-packaged, or 100 g portions were tray-packaged with overwrap and stored at 4.4°C. At each sampling interval, three replicates of each treatment and packaging condition were diluted in Buffered Peptone Water and spread-plated on both de Man, Rogosa, and Sharpe agar (MRS) for lactic acid bacteria (LAB) and tryptic soy agar (TSA) for aerobic bacteria. MRS plates were incubated at 30°C for 48 h, and TSA plates were incubated at 35°C for 48 h.

Results: Across treatment and packaging types, initial aerobic and LAB populations ranged from 4.1–4.3 and 3.9–4.1 log CFU/g, respectively. By day 3, the negative control samples exceeded 6 log CFU/g under both packaging types. All antimicrobial-containing treatments significantly ($p < 0.05$) extended the shelf life compared with the negative control. Cultured dextrose and vinegar combination increased ground pork shelf life by 10 days in overwrapped samples and by 13 days in vacuum-packaged samples.

Significance: This study demonstrates that clean-label antimicrobial solutions can significantly extend the shelf life of fresh ground pork under both aerobic and vacuum packaging, providing the meat industry with effective alternatives to conventional preservatives while maintaining product safety and quality.

P1-054: INVESTIGATING THE EFFECT OF DEEP-CHILL, TYPE OF MICROBIAL STRAIN, AND ANTIMICROBIAL ON THE GROWTH OF L. MONOCYTOGENES IN RTE HOT DOGS

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Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Listeria monocytogenes (Lm)* can grow during refrigerated storage of ready-to-eat (RTE) meats, necessitating effective hurdles beyond chill temperature alone. USDA-FSIS guidance limits post-lethality *Lm* outgrowth to □2 log CFU/g over the shelf life of RTE meat and poultry products. Buffered vinegar systems have shown promise for controlling *Lm* in cooked meats, but there is limited information on their performance under deep-chill conditions.

Purpose: To quantify *Lm* log outgrowth in RTE hot dog sausages as a function of storage regimen, *Lm* strain cocktail, and Verdad® N9A concentration.

Methods: Hot dogs (~30 g), formulated with Verdad® N9A (0, 1.0, 1.5, or 2.0%) were surface inoculated (~3 log CFU/g) with one of two cold-adapted *Lm* strain cocktails (*Lm1* & *Lm2*), vacuum-packed, and stored under three regimens: 4.4°C for 120 days; –2°C for 4 weeks

followed by 4.4°C for 13 weeks; or –2°C for 8 weeks followed by 4.4°C for 9 weeks. *Lm* populations (n=3) were enumerated on Modified Oxford agar at defined intervals and expressed as log CFU/g, and log outgrowth was calculated as the change from the initial inoculation level.

Results: At 4.4°C, untreated controls reached a 2-log outgrowth on days 13 (*Lm1*) and 26 (*Lm2*). Deep-chilling at 2°C delayed 2-log outgrowth to 39 d (*Lm1*) and 45 d (*Lm2*) after 4 wk of storage, and to 60 d (*Lm1*) and 70 d (*Lm2*) after 8 wk of storage at 2°C, before transfer to 4.4°C. With 1.0% Verdad® N9A, 2-log outgrowth occurred on days 60 (*Lm1*) and 100 (*Lm2*) under continuous 4.4°C, and on days 74 (*Lm1*) and 111 (*Lm2*) following 4-wk deep-chilling. Only *Lm1* reached 2-log by day 120 after 8 wk deep-chilling. Verdad® N9A at 1.5% maintained 1-log outgrowth over 120 d across all regimens.

Significance: Integration of deep-chill storage with 1.5% Verdad® N9A can maintain *Lm* log outgrowth at regulatory 2-log CFU/g benchmarks in RTE hot dogs, supporting its use as part of a multiple-hurdle safety strategy.

P1-055: MAPPING MICROBIAL ECOLOGY IN A SMALL PASTURE POULTRY OPERATION

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Ensuring food safety throughout the poultry production continuum is vital, as *Salmonella* and *Campylobacter* are significant causes of foodborne illness and linked to poultry consumption. Moreover, poultry products can become contaminated at the farm and during processing.

Purpose: This study evaluated the microbial ecology of a poultry operation from flocks of pasture-poultry farms to carcasses processed at a single small plant.

Methods: A total of 800 samples, including boot swabs (BS=160), rehang (RH=320), and post-chill (PC=320) carcass rinses were collected from two flocks (F1 and F2) of pasture-poultry farms (n=8) processed in a small plant over a 6-month period. *Salmonella* and *Campylobacter* were isolated by culture methods and quantified with BAX®. The isolates from flock 2 were further serotyped. Aerobic bacteria (AC) and Enterobacteriaceae (EB) were enumerated from the carcass rinses, and the data was analyzed with Chi square and ANOVA in JMP.

Results: *Salmonella* prevalence was similar between the flocks (F1=82%, F2=78%). In F1, prevalence reduced from 91% (BS) to 65% (PC) and 100% (BS) to 59% (PC) in F2. In contrast, *Campylobacter* differed between F1 (77%) and F2 (37%), with a downstream reduction from BS (F1=100%, F2=78%) to PC (F1=50%, F2=14%). *Salmonella* load slightly reduced (0.8 to 0.5 $\log_{10}$

CFU/ml) from RH to PC, while *Campylobacter* increased (2.8 to 3.1 log CFU/ml). The most common *Salmonella* were *S*. Kentucky (68%), *S*. Alachua (11%), and *S*. Senftenberg (8%), while *C. jejuni* was the most isolated *Campylobacter*. On average, AC and EB significantly reduced from respective 6.1 and 5.6 log CFU/ml at RH to 3.7 and 2.7 log CFU/ml at PC.

Significance: The study showed significant reduction of both pathogens from farm to final product, yet contamination remains, raising concerns about the efficacy of antimicrobial intervention at processing. Further studies will evaluate correlation in serotypes and strains between the farms and the processing plant.

Award Application: Developing Scientist Award

P1-056: EVALUATING PRE-HARVEST SALMONELLA SEROTYPE PERSISTENCE OVER MULTIPLE BROILER HOUSE CYCLES

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: An improved understanding of *Salmonella* persistence in pre-harvest broiler production is needed to determine on-farm sampling frequencies and improve food safety in chicken products.

Purpose: To determine efficient sampling schedules for broiler houses, this study screened *Salmonella* serotypes during different broiler flock cycles from the same houses and used this information to evaluate the incidence of serotypes Enteritidis (SE) and Typhimurium (ST) over time.

Methods: In total, 409 broiler houses were screened (May to December 2024) for *Salmonella* prevalence using the BAX Sal assay and via qPCR assays to determine SE and ST prevalence. Each flock was assigned a risk score as follows: 0 if the flock was *Salmonella* negative, 1 if *Salmonella* was detected but was not SE or ST, 2 if SE or ST was present, and 3 if both SE and ST were detected. Different flocks from the same broiler house were categorized as consecutive or non-consecutive based on sample date.

Results: In total, 196 houses were screened for more than one flock: 270 consecutive flocks

(127 houses, including those sampled three times) and 182 non-consecutive flocks: 106 with one and 76 with two intervening and not sampled broiler flocks (91 houses, including 22 houses that also have consecutive flocks). For consecutive and single skip flocks, risk score was the same 61% and 57%, respectively. For non-consecutive 2-skip flocks, only 37% retained the same risk score. *Salmonella* risk score was more likely to decrease when there were two intervening flocks, rather than between consecutive or single skip flocks (Chi-Square *p*-value = 0.02).

Significance: These results show that presence of highest risk *Salmonella* serotypes is less likely to change between successive flocks than when there are two intervening flocks. This information is useful for developing more meaningful pre-harvest broiler house testing strategies.

Award Application: Developing Scientist Award

P1-057: TEMPERATURE-DEPENDENT MICROBIAL VOC SIGNATURES FOR EARLY DETECTION OF SALMONELLA IN EGGS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Early detection of microbial contamination in eggs is vital for improving food safety and hatchery biosecurity.

Purpose: This study investigates volatile organic compounds (VOCs) released from eggs as potential early indicators of *Salmonella* Enteritidis (SE) contamination.

Methods: We compared VOC patterns from SE-inoculated and control eggs stored at 17°C and 4°C to identify temperature-dependent signals relevant to pre-hatchery incubation and commercial storage. Specific Pathogen-Free (SPF) eggs were divided into eight treatment groups (control vs. SE; yolk vs. albumen; 17°C vs. 4°C). Eggs were inoculated with sterile phosphate-buffered saline (PBS) or PBS containing SE (100 µL, 10^4 CFU/mL) and stored over three days. VOCs were collected daily, from the same eggs, using a sorptive extraction device and analyzed by GC×GC–MS, with compounds identified through the NIST database. Egg breakouts confirmed SE presence through plating on selective agar. Data were evaluated

using principal component analysis (PCA) and one-way ANOVA.

Results: Temperature strongly influenced VOC patterns. Eggs at 17°C produced higher VOC levels regardless of SE status, while those at 4°C showed minimal change. PCA revealed clear differences between SE-inoculated and control yolk samples at 17°C, indicating that warmer conditions support SE activity and distinct VOC emissions. At 4°C, SE remained largely inactive, producing weaker or overlapping VOC profiles.

Significance: These findings suggest that VOC profiling could serve as a rapid, non-invasive approach for early detection of bacterial contamination in eggs. Ongoing replicates aim to validate these preliminary results and refine the use of microbial VOCs as practical biomarkers for monitoring egg safety and reducing pathogen transmission risks.

P1-058: CONTROLLING SALMONELLA AND IMPROVING EGG SAFETY THROUGH AERATED WATER-BASED EGG WASH TREATMENTS

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* Enteritidis (SE) is a leading cause of egg-associated foodborne illness. Standard water washing may not sufficiently reduce SE on shells or prevent transferring to internal membranes and wash water. Practical antimicrobial upgrades are needed to improve egg safety.

Purpose: Evaluate aerated water (AW), alone and with peracetic acid (PAA), for reducing SE on eggs and wash water.

Methods: Fresh eggs (n=60) were spot inoculated with SE at ~9 log CFU/egg. Treatments (32°C, 5 min) included: unwashed control; tap water (TW); AW (~30 mg/L dissolved O_2); TW with PAA (80 ppm; TP); and AW with PAA (AP). Eggs and corresponding wash water were sampled for SE immediately (Day 0) and after refrigerated storage (Days 1, 3, 7). Three eggs per treatment/time were analyzed for SE on the shell surface and internal contents. Data were evaluated in a completely randomized design; significance set at $P < 0.05$.

Results: SE reductions were treatment and time dependent. On the egg surface, tap water

washing reduced SE counts from 6.5 to 3.97 log CFU/egg by Day 7. Aerated water (AW) produced significantly greater surface reductions, decreasing SE from 5.42 log CFU/egg at Day 0 to enrichment positive by Day 7; though, viable cells remained detectable on the internal membrane. Together, these results indicate that reducing surface contamination alone is insufficient to mitigate food safety risks. However, AP provided comprehensive control with surface SE nondetectable by Day 3 and internal contamination reducing to enrichment negative through Day 7. AP also rendered wash water enrichment negative, whereas TW, AW, and TP samples remained positive at various times.

Significance: Combining aeration with PAA delivers concurrent SE control on the shell surface, internal membranes, and wash water, supporting AP as an effective, organic compatible intervention to improve egg safety.

Award Application: Developing Scientist Award

P1-059: EGG-PRODUCTION HOUSING SYSTEMS IMPACT ON THE EGGSHELL MICROBIOME AND SAFETY

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Category: Meat, Poultry and Eggs

Abstract:

Introduction: This study investigated the influence of two different egg production housing systems, conventional cage house (CH) and aviary (cage-free house, CFH), on the eggshell microbiome and the presence of antimicrobial resistance (AMR) genes over a full production cycle.

Purpose: The goal was to determine if housing systems affect the safety of eggs by influencing the presence of pathogens and AMR genes on the eggshell.

Methods: 120 and 240 eggs from CH and CFH, respectively, were collected. The eggs were washed individually, and pooled wash samples (3 washes/genomic sample). Whole Genome Sequencing (WGS) was performed to analyze the microbiomes and AMR genes. Samples were analyzed for: taxonomy (Kraken2 and Bracken 2.5), α and β diversity (QIIME2), gene family and metabolic pathway abundance (HUMAnN3), differential abundance analysis (ANCOM), and antibiotic resistance genes (MEGAHIT, ABRicate, ResFinder).

Results: WGS revealed significant differences in microbial diversity (pairwise PERMANOVA, $p < 0.05$), composition, and evenness between housing systems, with greater Pielou's evenness in CH than CFH. Bray–Curtis Principal Coordinate Analysis revealed distinct microbial communities in CH and CFH samples. The ANCOM analysis identified 38 differentially abundant species, including *Staphylococcus*, *Corynebacterium*, *Fusarium*, *Acinetobacter*, and

Pasteurella. Of note, *Pasteurella multocida* (Fowl Cholera) was more abundant in CH, whereas *Staphylococcus argenteus* (formerly, *S. aureus* CC75) was higher in CFH ($p<0.05$). CFH-floor samples contained more AMR genes than CH, dominated by tetracycline resistance genes, and multidrug resistance genes were detected in both systems, particularly mid-cycle.

Significance: WGS analysis of eggshell wash samples showed a significant difference in microbial diversity, AMR gene prevalence, and the number of different AMR genes between house systems. Pathogenic (non-foodborne) bacteria showed significant differences in abundance.

P1-060: FECAL CONTAMINATION DETECTED ON EGGSHELLS USING PORTABLE FLUORESCENCE IMAGING AND EXPLAINABLE DEEP LEARNING

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Fecal contamination on chicken eggshells poses a food safety risk as a vector for pathogens like *Salmonella*. Traditional visual inspection often fails to detect dried fecal residue on eggshells.

Purpose: To combine portable fluorescence imaging and an explainable deep learning framework to enhance detection of fecal contamination on eggshells.

Methods: A total of 120 eggs (60 brown; 60 white) were inoculated with 10uL of one of four inoculum treatments: sterile water (control) or fecal suspensions containing 1%, 2%, or 10% chicken feces. Each egg was inoculated at two sites (n=240 samples). Fluorescence images were acquired using a portable Contamination and Sanitization Inspection (CSI) device capable of dual mode excitation at 405nm and 365nm wavelengths. A dataset of 240 images

was partitioned into training (120), validation (60), and testing (60) sets, with data augmentation expanding the training set to 3,600 images. Nine deep-learning architectures, including Convolutional Neural Networks (CNNs) and Vision Transformer (ViT), were evaluated for classification performance. EigenCAM and Attention Rollout were applied to visualize the model's decision-making process.

Results: Classification performance varied by egg color and excitation wavelength. For brown eggs, the 365nm excitation suppressed background fluorescence, allowing the lightweight MobileNet model to achieve the highest level of accuracy (90.0%). Conversely, for white eggs, the 405nm excitation yielded the best results, where the ViT Base 384 model achieved a 93.3% accuracy level by preserving fine spatial details. Explainable AI visualizations confirmed these models accurately localized fluorescence signals in the 600 to 720 nm range emitted by fecal porphyrins and were not background artifacts.

Significance: Portable fluorescence imaging combined with optimized deep learning models provides a rapid, nondestructive, and easily interpretable method for detecting fecal contamination on eggs.

P1-061: QUANTIFICATION OF CAMPYLOBACTER COLI USING DIGITAL PCR, QPCR, MOST PROBABLE NUMBER, AND DIRECT PLATING IN PURE CULTURE AND POULTRY RINSATE

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Accurate quantification of *Campylobacter* spp. in poultry matrices is critical for food safety risk assessment. Few studies have investigated assays, including digital PCR (dPCR), for quantifying *Campylobacter* in poultry and poultry products.

Purpose: This study evaluated dPCR, qPCR, and automated Most Probable Number (MPN) and compared these methods to direct plating on Campy-Line agar (CLA) for enumerating *Campylobacter coli* in pure culture and poultry rinsate.

Methods: Serial dilutions of gentamicin-resistant *C. coli* were prepared in phosphate-buffered saline (PBS) and post-chill poultry rinsate. DNA was extracted from each dilution using the Qiagen PowerSoil Pro kit, and the dPCR targeted the *cae* gene with FAM-labeled probes. BAX Q7 real-time PCR and TEMPO most probable number (MPN) followed manufacturer protocols. Each concentration-method combination included three biological

and two technical replicates (N = 6). A linear mixed-effects model assessed method and matrix effects, accounting for random variation across inoculation levels.

Results: Detection methods significantly influenced log estimates ($p < 0.001$), with MPN yielding the highest recovery (9.87 and 9.96 log), followed by direct plating (8.39 and 7.89 log), dPCR (7.97 and 7.58 log), and qPCR (7.75 and 6.85 log) in pure culture and poultry rinstae, respectively. No significant differences were found based on sample type ($p = 0.229$). Method performance varied by inoculum level, where the culture-based methods provided accurate quantification at high loads, while molecular assays improved detection at intermediate levels (4 CFU) but were constrained at low levels (1–2 CFU).

Significance: These results indicate that method choice strongly influences enumeration. These findings highlight the need for matrix-specific validation and consideration of detection limits in method selection for *Campylobacter* testing.

P1-062: RAPID DETECTION AND IDENTIFICATION OF HIGHLY PATHOGENIC SALMONELLA AT 10 CFU/ML IN MICROTALLY POULTRY CARCASS MITTS USING HYGIENA'S BAX® SYSTEM REAL-TIME PCR SOLUTIONS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Current regulatory sampling of whole poultry carcasses and parts relies on a rinse-based method to determine the presence of *Salmonella*. New sampling methods, such as the MicroTally® Mitt, have since been developed and are gaining acceptance in the industry. Along with a shift to quantification, these combined approaches can provide the poultry industry with a more comprehensive strategy to reduce *Salmonella* contamination in raw poultry.

Purpose: The purpose of this study was to evaluate poultry sampling mitts inoculated at 10 CFU/mL (equivalent to a 400 mL carcass rinse) using rapid real-time PCR assays to detect *Salmonella* species and assess pathogenicity through multiplex detection of virulence markers.

Methods: Mitts from post-chill poultry carcasses were inoculated with a cold-stressed *Salmonella* Typhimurium culture at 10 CFU/mL. Following inoculation, mitts were divided into

two sets (n = 10 per set) and enriched with either 200 mL of MP media or 200 mL of BPW media. All samples were incubated at 42°C for 4–24 hours and analyzed by real-time PCR in triplicate to generate 30 technical replicates per enrichment.

Results: MP enrichments yielded 100% positive results for *Salmonella* species and detection of HPS-B, HPS-A and HPS-X virulence targets at 4 hours, whereas BPW enrichments yielded 100% positive results at 5 hours. Consistent results were obtained as incubation extended, and all samples were confirmed positive following the USDA FSIS MLG 4.15.

Significance: This study demonstrates that the BAX System Real-Time PCR assays support limits-based testing by reliably detecting *Salmonella* species at 10 CFU/mL and differentiating pathogenicity levels of public health significance.

P1-063: POTENTIAL LEVEL-BASED PERFORMANCE STANDARDS THAT FOCUS ON RISKY HIGH-LEVEL SALMONELLA IN CHICKEN PARTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: While prevalence of *Salmonella* in raw poultry has decreased over the last decade, rates of salmonellosis have not decreased proportionally, suggesting a reason to move beyond prevalence-based performance standards.

Purpose: We explore potentially new, level-based performance standards that better link performance categories to high-level, high-risk poultry parts, which could incentivize controlling risky, high-level contamination.

Methods: We built upon existing prevalence-based standards and 3-class sampling plans. We defined “m” as the limit of detection of 1 CFU/30 mL rinsate, “c” as 7.7%, and “M” as 100 CFU/mL. An establishment was Category 3 if one or more samples collected during a 52-week window exceeded M; Category 2 if it had no M failures and more than 7.7% of samples had detectable *Salmonella*; else, Category 1. We used RStudio and USDA-FSIS verification sampling data for chicken parts from the most recent 52-week window (Mar 2024 – Mar 2025) to categorize the establishments.

Results: Out of the 460 establishments that were analyzed, 26 (5.7%) were Category 3, 128 (27.8%) were Category 2, and 306 (66.5%) were Category 1. Compared to current FSIS prevalence-based categories (11.0%, 20.0% and 69.1% in Categories 3, 2, and 1, respectively), these level-based standards produced a lower proportion of Category 3 and similar Category 1 performance. We also explored different combinations of thresholds for level-based performance standards.

Significance: We propose logic for potential level-based performance standards that could better align with risk and identify thresholds for these standards where current industry performance is similar to existing performance. There is a need for a follow-up assessment of the potential public health impact of these standards.

P1-064: POTENTIAL INDUSTRY RESPONSE TO POTENTIAL LEVEL-BASED STANDARDS SHOWS THAT REDUCING HIGH-LEVEL SALMONELLA REDUCES ESTIMATED PUBLIC HEALTH RISK IN CHICKEN PARTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Our group has explored potential level-based performance standards for raw poultry to incentivize reducing higher-risk contamination, but the public health benefit of adopting such standards is unknown.

Purpose: We assess how performance improvements under level-based performance standards could affect public health, thereby guiding science-based decision making among stakeholders.

Methods: Using the USDA-FSIS verification sampling data from March 2024 to March 2025, we estimated an overall *Salmonella* level by fitting a censored $\log_{10}$-scale normal distribution. Baseline salmonellosis risk was obtained using our previous quantitative microbial risk assessment. We assumed that industry improves performance by targeting high-level contamination, reducing the Category 3 percentage by half. The new *Salmonella* level distribution was used in our risk assessment to calculate the new salmonellosis risk relative

to the baseline risk.

Results: The risk assessment predicted that the baseline *Salmonella* level (μ=-5.04, σ=2.56 $\log_{10}$CFU/mL of parts rinse) had a salmonellosis risk of 2.1 illnesses in 1 million servings. The assumed industry performance improvement decreased establishment Category 3 frequency from 8.9% to 4.6%, which is modeled by decreasing the *Salmonella* level standard deviation from 2.56 to 2.37. Applying the new *Salmonella* level distribution (μ=-5.04, σ=2.37 $\log_{10}$CFU/mL of parts rinse) in the risk assessment, the new salmonellosis risk is 1.1 case in 1 million servings, which is 52% of the baseline risk.

Significance: The study found that improving performance under level-based performance standards could decrease the public health risk by about 50%, which would meet the Healthy People 2030 goal of *Salmonella* reduction of 25%. The findings encourage future studies assessing how the industry responds to different performance standards.

Award Application: Developing Scientist Award

P1-065: TEMPERATURE-DEPENDENT EFFECTS OF SALT AND BACKGROUND MICROBIOTA ON SALMONELLA GROWTH IN RAW GROUND CHICKEN

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Consumption of contaminated poultry products is a major source of foodborne salmonellosis. Despite its public health relevance, limited information is available on *Salmonella* population dynamics at low temperatures in raw chicken in the presence of natural background microbiota.

Purpose: To characterize changes in *Salmonella* population density in raw ground chicken as a function of salt concentration and background microbiota levels under refrigeration and temperature abuse conditions.

Methods: Raw ground chicken breast was inoculated with a five-strain *Salmonella* cocktail (*S.* Braenderup, *S.* Enteritidis, *S.* Enteritidis, *S.* Typhimurium, and *S.* Hadar). A full-factorial design [3 temperatures (4, 8, and 12°C) × 2 background microbiota levels (low and high) × 2 salt concentrations (0.5 and 1.5% w/w)] with two replicates per treatment was used. *Salmonella* and psychrotropic aerobic plate counts (APC, background microbiota) were

enumerated over 20 days.

Results: The initial *Salmonella* level was 3.8 ± 0.2 log CFU/g. Initial APC levels were <1 log CFU/g for low microbiota and 3.60 ± 0.07 log CFU/g for high microbiota. No *Salmonella* growth was observed at 4°C, with a slight decline over time, particularly at higher APC and salt levels. At 8°C, limited growth occurred and was greater at lower APC and salt concentrations. At 12°C, both *Salmonella* and APC exhibited classical growth curves, with higher background microbiota associated with lower maximum *Salmonella* populations.

Significance: These findings demonstrate that temperature, salt content, and background microbiota interact to influence *Salmonella* behavior in raw poultry, highlighting the protective role of competing microbiota and the increased risk associated with temperature abuse.

P1-066: PERSISTENCE AND CROSS-CONTAMINATION OF SALMONELLA FROM RAW CHICKEN ON DOMESTIC SURFACES

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Although there are studies describing *Salmonella*'s cross-contamination and survival in the poultry industry, they are usually conducted with locally isolated strains and do not consider the dynamics in house kitchens.

Purpose: To describe *Salmonella*'s survival on cutting boards and its transfer from chicken to fresh vegetables through the cutting board.

Methods: *Salmonella* was searched for and quantified in 98 raw chicken pieces from retail. From the isolated strains, six were selected by growth rate and mixed into a cocktail for further experiments. Raw chicken was inoculated and put into contact with a cutting board for 30 s, 3 min, and 30 min to measure the transfer rate. The inoculated cutting board was left exposed for 6 days to determine survival, and biofilm production was measured in a 96-well plate. Lettuce leaves were pressed against the inoculated cutting board to measure

transfer. Experiments were done thrice at room temperature, and quantifications were made on XLD Agar.

Results: The 71 positive chicken pieces ranged from less than 1 log CFU/g (limit of quantification) to 3.4 log CFU/g. From an inoculated chicken (6.79±0.18 log CFU/piece) 1.5±0.6% was transferred after 30 s of contact to the cutting board, 3.4±1.1% at 3 min, and 3.8±0.9% at 30 min; there was no difference (p=0.940) in *Salmonella*'s transfer between 3 min and 30 min of contact. *Salmonella* showed its ability to survive on the cutting board although its numbers decreased from the initial inoculation of 4.87±0.03 log CFU/surface to 1.30±0.43 log CFU/surface after six days. The decrease over time correlates with the weak or not-biofilm-forming characteristic of the studied strains. Nevertheless, 0.2–2.1% of *Salmonella* from the inoculated cutting board was transferred to lettuce, which might represent a risk of disease to consumers.

Significance: The study evidences the capacity of autochthonous *Salmonella* strains to transfer from raw chicken to ready-to-eat leaves.

Award Application: Developing Scientist Award

P1-067: SIMULATION OF QUANTIFICATION-BASED 3-CLASS SAMPLING SCHEMES FOR SALMONELLA MONITORING USING INTENSIVE COMMERCIAL POULTRY SAMPLING DATA

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* risk in poultry is dose-dependent, yet regulatory monitoring largely relies on presence/absence data despite growing availability of quantitative measurements.

Purpose: To characterize *Salmonella* load distributions from intensive commercial sampling and evaluate quantification-based 3-class sampling schemes as potential regulatory scenarios.

Methods: A one-year sampling study across 10 large poultry plants generated N = 2,879 part samples (breasts, tenders, thighs, wings, leg quarters, whole legs). *Salmonella* concentrations were expressed as $\log_{10}$ CFU/mL. Three scenarios were simulated:

Scenario 1: <10 CFU (Acceptable), 10–<100 CFU (Marginal), 100 CFU (Reject)
Scenario 2: <50 CFU, 50–<100 CFU, 100 CFU
Scenario 3: <100 CFU, 100–<1,000 CFU, 1,000 CFU

Results: Mean loads (± SD) were highest for thighs (0.40 ± 0.64) and tenders (0.24 ± 0.57), followed by breasts (0.19 ± 0.50) and wings (0.11 ± 0.35); leg quarters (0.02 ± 0.12) and whole

legs (0.02 ± 0.15) were lowest. Distributions were right-skewed, indicating sporadic high-load events. Across scenarios, Category 1 predominated (N = 54), but Scenario 1 produced more Category 2 (N = 14) and 3 (N = 4) outcomes. Products with higher loads (thighs, tenders, breasts) contributed most to Category 2 and 3, while leg quarters and whole legs remained almost exclusively Category 1.

Significance: Quantitative *Salmonella* data from intensive sampling can inform establishment classification under simulated verification frameworks. Incorporating load-based metrics may enhance risk-based monitoring by capturing product-specific contamination and process control performance.

P1-068: SIMULATION OF QUANTIFICATION-BASED 3-CLASS SAMPLING SCHEMES FOR SALMONELLA MONITORING USING USDA-FSIS POULTRY VERIFICATION DATA

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Current poultry *Salmonella* regulatory frameworks rely on prevalence results despite the dose-dependent nature and variable pathogenicity of salmonellosis risk.

Purpose: To evaluate long-term *Salmonella* prevalence and load patterns in poultry products (whole carcasses and parts) and simulate alternatives for quantification-based 3-class sampling schemes as potential regulatory monitoring frameworks.

Methods: Publicly available FSIS *Salmonella* verification records (Raw Poultry Sampling | Food Safety and Inspection Service) with approximately 12 years were analyzed as an observational study. Analyses were restricted to chicken parts and whole carcasses (N ~ 150,000), excluding processed, comminuted, and incomplete records. *Salmonella* loads were expressed as log CFU/ml, including imputed values for censored or missing values. Three 3-class sampling scenarios (Acceptable/Marginal/Unacceptable) were simulated using concentration thresholds of <10/10–<100/□100 CFU, <50/50–<100/□100 CFU, and <100/100–<1000/□1000 CFU. Weekly marginal and reject counts were aggregated by establishment and evaluated using 52, 26 and 13-week rolling windows to assign plants to Categories 1–3.

Results: Annual prevalence peaked at 24.6% in 2015 and stabilized at 8.0–10.5% from 2019–2025. Mean loads remained low (0.022–0.055 log CFU) but increased modestly after 2021 despite stable prevalence. Seasonal prevalence was consistent (10.6–10.9%). Chicken parts showed higher prevalence than whole carcasses. Simulated categorization varied significantly across scenarios ($p < 0.05$), indicating sensitivity to threshold selection.

Significance: Quantitative *Salmonella* data provides information beyond prevalence alone and materially affects establishment potential classification outcomes. Incorporation of load-based metrics could strengthen risk-based regulatory monitoring and risk-based food safety decision-making.

P1-069: SALMONELLA INFANTIS RISK IN BROILERS: AN EXPERIMENTAL COMPARISON OF PLASMID-BEARING AND ANCESTRAL S. INFANTIS, AND S. INFANTIS CO-INFECTED WITH S. KENTUCKY

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* Infantis is an emerging food-safety hazard in poultry and is increasingly associated with human illness.

Purpose: This study compared persistence and dissemination of plasmid-bearing vs. ancestral (plasmid-negative) *S.* Infantis, and plasmid-bearing *S.* Infantis co-infected with *S.* Kentucky, in broilers.

Methods: A controlled trial included 450 broilers in 18 pens, assigned to 3 treatment groups: G1: plasmid-positive *S.* Infantis; G2: plasmid-negative *S.* Infantis; and G3: plasmid-positive *S.* Infantis plus *S.* Kentucky. Within each pen, directly challenged and horizontally exposed birds were assessed. Boot-sock samples (n=6 pens/treatment) were collected on days 5, 8, 14, 35, and 41; ceca (n=24 birds/treatment/timepoint) were collected on day 20 and day 41/42 to estimate prevalence and $\log_{10}$ MPN/g loads. Analyses used exact tests and regression/mixed models (α=0.05).

Results: Boot-sock prevalence differed among treatments on days 5 and 8 (P<0.001) and day

35 (*P*=0.009); on day 5, 0/6 pens were positive in G2 vs 6/6 in G1 and G3. All recovered isolates were serogroup C1. Cecal prevalence was 24/24 at day 20 in all groups and remained high at day 41/42 with no treatment effect at end of cycle (*P*=0.98). At day 20, treatment differences in cecal loads depended on challenge status (interaction *P*=0.019); in directly challenged birds, G3 loads were lower than G2 (1.45 vs 3.60 log10 MPN/g; *P*=0.010). At day 41/42, loads did not differ by treatment (*P*=0.15) but were higher in directly challenged than contact birds (*P*=0.005). *S.* Infantis persisted through grow-out, and coinfection modulated early intestinal loads without eliminating end-cycle carriage.

Significance: Boot-sock plus cecal quantification enables a risk-based benchmarking of contamination pressure and evaluation of multi-hurdle measures that limit horizontal transmission before processing.

P1-070: GEOGRAPHIC AND TEMPORAL PATTERNS OF SALMONELLA SEROVARS IN U.S. POULTRY PRODUCTS AND THEIR POTENTIAL LINK TO HUMAN ILLNESS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Despite extensive food-chain controls, salmonellosis remains a leading cause of foodborne illness in the U.S., with poultry as a major source. Characterizing the patterns of *Salmonella* serovars in poultry and their association to human illness may inform risk-based regulatory strategies.

Purpose: Assess the correlation of *Salmonella* serovars isolated from poultry carcasses and CDC-reported human illnesses.

Methods: FSIS routine poultry processing data (2015–2023) were downloaded and sorted by species, region (Midwest, Northeast, South, West), and serovar. CDC surveillance data on annual *Salmonella* serovar incidence in human illness (2015–2022) were downloaded. Descriptive statistics was calculated, and serovar richness was quantified using the Shannon

index. Spearman's correlation was used to assess associations between the top 20 poultry-related serovars and corresponding CDC illness incidences.

Results: A total of 3,650 chicken and 101 turkey *Salmonella* positives were reported by FSIS. Across all regions, *S.* Kentucky predominated (47.8%), followed by *S.* Infantis (14.9%), *S.* Enteritidis (12.0%), and *S.* Typhimurium (9.9%) among isolates from 2015 to 2023. Salmonellosis had a downward trend (P= 0.078) from 2015 to 2022, averaging 6,459 cases per year. The top 5 human illness serovars were Enteritidis (19.2%), Newport (11.8%), Typhimurium (10.5%), Javiana (8.6%), and I 4,[5],12:i:- (5.9%). Shannon indices for poultry isolates declined (P= 0.041) over time, indicating reduced serovar richness likely driven by increasing dominance of a few major serovars. Only *S.* Braenderup, *S.* Heidelberg, and *S.* Reading correlated with CDC-reported human illness (ρ= 0.72, 0.76, and 0.81, respectively; P< 0.05).

Significance: Poultry serovar profiles only partially align with those causing human illness, suggesting other sources contribute to disease burden. Findings highlight the need for targeted interventions and integrated surveillance to high-risk predominant serovars.

P1-071: IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL BIOFILM PROFILES IN QUEBEC PORK SLAUGHTERHOUSES

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Pork meat promotes the growth of pathogenic and spoilage bacteria, which can cause foodborne illnesses or reduce product shelf life. In slaughterhouses, these bacteria can be found as biofilms, whose resistance to cleaning and sanitation procedures represents a major challenge for food safety.

Purpose: To identify and characterize biofilms present in Quebec pork slaughterhouses.

Methods: Two slaughterhouses were selected. A total of 21 surfaces at equivalent locations in each facility (3 at slaughter, 6 at evisceration and 12 at butchering) and one pork loin, were sampled after cleaning procedures using a prototype sonication sponge. Aliquots (100 µL of PBS 1X) from each sample were plated on nine different agar media. Isolated strains were identified using 16S rRNA Sanger sequencing. Further analyses will include metagenomic profiling and characterization of strains for biofilm-forming capacity, heat resistance, antimicrobial susceptibility (Kirby-Bauer method), and disinfectant resistance.

Results: Slaughterhouse A showed a significantly higher number of isolated strains than slaughterhouse B (670 vs 284), when all processing steps were combined (2-way ANOVA, p=0.0106). No significant differences were observed among processing steps within the same slaughterhouse (2-way ANOVA, p=0.1725). Distinct bacterial family distributions were observed between facilities. In slaughterhouse A, dominant families shifted from Microbacteriaceae at slaughter to Enterobacteriaceae at evisceration, and to Pseudomonadaceae at butchering. In slaughterhouse B, Microbacteriaceae and Micrococcaceae were the dominant families. In the finished product, Pseudomonadaceae and Moraxellaceae predominated in both slaughterhouses.

Significance: This study provides novel insights into bacterial biofilms in Quebec pork slaughterhouses, supporting identification of critical contamination points and optimization of control strategies to improve pork safety, quality, and shelf life.

Award Application: Developing Scientist Award

P1-072: THERMAL INACTIVATION OF SALMONELLA DURING PORK FAT RENDERING FOR LARD PRODUCTION (60 TO 93.3°C)

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Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Industrial lard production from raw pork fat requires high temperature rendering to eliminate pathogens such as *Salmonella*. However, excessive and prolonged heating (>76.6°C) may negatively impact product quality through increased lipid oxidation. Literature has limited validated data describing thermal lethality of *Salmonella* specifically in rendered pork fat matrices.

Purpose: This study evaluated the instantaneous thermal lethality of pork fat rendering to achieve a 6.5-log reduction of *Salmonella*, consistent with USDA Appendix A lethality performance standards.

Methods: Comminuted pork fat was inoculated with a four-strain thermotolerant *Salmonella enterica* (Typhimurium ATCC 700720, Typhimurium ATCC 14028, Newport ATCC 6962, and Montevideo clinical isolate) cocktail (10^8 CFU/g) associated with *Salmonella* outbreaks. Samples were vacuum-packaged and processed in a circulating water bath at temperatures ranging from 60 to 93.3°C. After rapid chilling with ice, *Salmonella* counts were determined, and log reductions were calculated. Lipid oxidation was evaluated using peroxide value analysis.

Results: Processing at 82.2°C and above, specifically at 87.7 and 93.3°C achieved an

instantaneous >6.5-log reduction of *Salmonella*. No additional hold time was applied once target temperatures were reached. However, processing at temperatures of 76.6°C and below resulted in a <3-log *Salmonella* reduction. No tested temperature resulted in increased peroxide values, indicating no adverse impact on product quality under study conditions.

Significance: These findings provide validated thermal lethality data for *Salmonella* inactivation/reduction in pork fat rendering. The results support animal fat processors in designing effective time–temperature processing parameters that ensure product safety while maintaining quality and meeting regulatory expectations.

P1-073: EVALUATING CARNOBACTERIUM SPP. AS A SURROGATE FOR SALMONELLA TYPHIMURIUM DT104 IN DROËWORS PROCESSING

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Droëwors is a traditional South African dried beef sausage produced without a thermal lethality step. Achieving the USDA-FSIS-mandated 5-log reduction of *Salmonella* is challenging, and the ground, high-fat matrix requires product-specific lethality data and reliable non-pathogenic surrogates.

Purpose: To evaluate *Salmonella* reduction during droëwors processing, assess the efficacy of buffered vinegar and liquid smoke, and determine the suitability of *Carnobacterium* spp. as a non-pathogenic surrogate.

Methods: A droëwors formulation based on commercial processor recommendations was prepared using beef batter (8% fat). Batter was inoculated with either a 3-strain cocktail of *Salmonella* Typhimurium DT104 or a 4-strain cocktail of *Carnobacterium* spp. Treatments included a control and an antimicrobial blend containing IsoAge 325L and Flavoset 5400L

(Kerry Ingredients) at manufacturer-recommended minimum levels. Sausages were stuffed into 22-mm sheep casings and dried at 75°F and 55% RH for 12 days. Samples were collected on days 0, 2, 4, 6, 8, 10, and 12 for microbial enumeration and water activity (a_w). Experiments were conducted in triplicate using independently inoculated batches; data (mean log CFU/sample or a_w ± SD) were analyzed using repeated-measures ANOVA with Holm–Sidak post hoc comparisons ($P < 0.05$).

Results: Initial *Salmonella* populations (7.53 log CFU/sample) declined by 3.5 log in controls and by >6.0 log in antimicrobial-treated samples by day 12, achieving the 5-log reduction requirement (1.48 log CFU/sample). *Carnobacterium* spp. were more resistant, declining only to ~4.8 log (control) and ~4.4 log (treated) by day 12. Final a_w for all samples ranged from 0.56 to 0.60.

Significance: Buffered vinegar and liquid smoke enabled droëwors to meet USDA-FSIS lethality requirements. Greater resistance of *Carnobacterium* spp. supports its use as a conservative surrogate.

P1-074: PREVALENCE OF HYBRID SHIGA TOXIN-PRODUCING ENTEROAGGREGATIVE ESCHERICHIA COLI AMONG MEAT SAMPLES IN THE UNITED STATES

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: In 2011, an outbreak of hybrid Shiga toxin-producing *Escherichia coli* (STEC)-enteroaggregative *E. coli* (EAEC) caused 3,842 illnesses and 53 deaths in Germany. The hybrid strain was an EAEC that had acquired a Shiga toxin (*stx*) gene. Food safety regulatory bodies now include the AggR gene of EAEC as an indicator of disease-causing STEC. Studies of cattle in the United States (U.S.) suggest they may be colonized by similar *E. coli*.

Purpose: Determine if U.S. meat products require routine screening for AggR to prevent outbreaks of disease.

Methods: PCR detection of the AggR gene was validated in inoculated beef and pork enriched in modified tryptic soy broth containing casamino acids, then FSIS regulatory broths of beef (n=818; 494 with *stx*), pork (n=1,039; 425 with *stx*), and poultry (n=128; *stx* unknown) were screened for the gene. If AggR was present, the cooccurrence of *stx* was confirmed using Bio-Rad iQCheck-STEC test kits. AggR/*stx* containing broths were cultured by plating onto *E. coli* ChromAgar with all colonies picked and screened for both genes.

Results: The PCR screening test was found to detect AggR^{+} *E. coli* in broths inoculated with as few as 20CFU/325g of ground beef or ground pork. Only one beef enrichment broth (0.12% of beef, 0.05% of all samples) was found positive for AggR and it was also positive for *stx*, however colonies of *E. coli* isolated from the positive broth were found to be identical to the control strain and determined to be a lab contaminant.

Significance: No AggR suggestive of a potential hybrid STEC was found in representative U.S. meat samples and little risk appears currently present. Routine screening for AggR is not necessary but should be revisited periodically to ensure hybrid STEC have not emerged as a contaminant of raw U.S. meat products.

P1-075: STRAIN SELECTION INFLUENCES THE PERFORMANCE OF AN ENRICHMENT-BASED SALMONELLA QUANTIFICATION METHOD

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Quantification of *Salmonella* in raw meat is an important tool in risk management, but the development of fast, accurate and user-friendly methods remains a challenge.

Purpose: We assessed the impact of strain selection on the performance of a *Salmonella* quantification method utilizing enrichment and Assurance® GDS PCR.

Methods: We previously generated a linear regression model that predicts *Salmonella* concentration in raw beef. In an advance upon past standards, we verified this model using current industry practices by directly inoculating 375g raw beef samples (N=33) with between 1–100 CFU/g of individual strains of *Salmonella* and estimating the CFU concentration. Accuracy was evaluated by the mean log difference between the model predictions and the known *Salmonella* concentration of the inoculum. Performance was also assessed by the confidence interval of model predictions.

Results: The model performance varied among different strains of *Salmonella*. Relatively slow-growing strains that were used in model construction showed a mean log difference of -0.15 (*S.* Dublin; N=12) and 0.45 (*S.* Enteriditis; N=9). A relatively fast-growing strain that was not used in model construction produced a mean log difference of 1.41 (*S.* Typhimurium;

N=12). The model estimates had an average 95% confidence interval of 0.24 log.

Significance: This data suggests that enrichment-based *Salmonella* quantification methods perform best on strains that were used for model construction. Strain selection is therefore an important consideration in risk tolerance when developing *Salmonella* quantification methods.

P1-076: COMPARISON OF SALMONELLA SEROTYPE ABUNDANCE AND COMPLEXITY IN BEEF CATTLE ORGANS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella enterica* can be carried in non-gastrointestinal organs in cattle, including lymph nodes (LNs) that can contaminate ground beef, and the relationship between *Salmonella* in LNs and other tissues is not well understood.

Purpose: Because *Salmonella* serotypes differ in human health impact, this study employed deep serotyping to determine the prevalence and complexity of serotypes in three types of LNs plus lungs, liver, and colon in beef cattle.

Methods: At harvest, tissue from three LNs (mesenteric, mediastinal, and subiliac), livers, and lungs were collected along with colonic contents from 151 beef cattle carcasses. Samples were selectively cultured for *Salmonella* and positive samples (n = 322) were deep-serotyped by CRISPR-SeroSeq to determine the identity and number of serotypes in each sample, yielding 318 libraries. Generalized linear mixed-effects modeling was used for statistical

comparisons between samples.

Results: Over half (81/151; 53.6%) the animals carried *Salmonella* at two or more body sites, with seven positive in all six. Multiple serotypes were found in 124/318 samples, representing 77/151 animals (50.9%). Serotype complexity was highest in mediastinal LNs (avg. 2.2 serotypes/sample). The five most prevalent serotypes (present in over 20% of samples) were Mbandaka/Lubbock, Muenchen, Cerro, Montevideo, and Anatum. Models demonstrated evidence of tissue tropism; Mbandaka/Lubbock occurred more frequently in livers, lungs, and mediastinal LNs ($p<0.01$), while Muenchen appeared most tropic to the colon and mediastinal and subiliac LNs ($p<0.01$).

Significance: *Salmonella* is frequently carried in multiple tissues in pre-harvest cattle and often comprises multiple serotypes that may assort differently between tissues. Determining the prevalence and complexity of serotypes across tissues deepens knowledge of how *Salmonella* resides and moves within an animal.

Award Application: Developing Scientist Award

P1-077: EVALUATION OF MICROORGANISM RECOVERY USING AN ALTERNATIVE MANUAL SAMPLING SWAB DEVICE IN COMPARISON WITH N60 SAMPLING FOR BOXED BEEF CUTS IN BRAZILIAN BEEF INDUSTRY

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The use of less labor-intensive sampling methods increases convenience and uniformity during sample collection, while enabling reliable microbiological analyses. The USDA has already recommended the use of sampling devices (MicroTally® swabs) for FSIS inspectors in the beef industry. Confirming the efficacy of this sampling method will support the broader adoption of faster and more robust practices in the global beef industry.

Purpose: To evaluate performance of sample collection methods for the recovery of total aerobic mesophilic and Enterobacteriaceae microorganisms in beef cuts, comparing a sample collection device with the reference N60 excision method.

Methods: Samples were collected in a commercial beef plant. Natural contamination of aerobic mesophiles and Enterobacteriaceae was assessed using 28 samples collected from boxed beef cuts with both sampling methods. Each sample was collected from a different batch. From the sample boxes, meat pieces were first collected to compose the N60 portion, and then manual sample devices were applied (1 swab across 5 boxes, 45 s per box). Buffered peptone water (BPW) volumes of 975 mL of was added to 325 g of excised meat, and 200 mL was added to swab samples. Neogen Petrifilm® Aerobic and Enterobacteriaceae count plates were inoculated with 1 mL of homogeneized samples. A paired *t*-test was conducted to determine statistical differences between methods ($p < 0.05$).

Results: The *t*-test showed no significant difference between the swab and the N60 reference method for Enterobacteriaceae recovery ($p > 0.05$). A significant difference ($p < 0.05$) was observed for aerobic mesophiles, with higher recovery achieved when using the swab sampling method.

Significance: The use of manual sampling devices enabled fast, convenient and reliable recovery of aerobic mesophiles and Enterobacteriaceae microorganisms from boxed beef cuts, with results comparable or superior to the N60 excision method.

P1-078: ANTIMICROBIAL RESISTANCE PATTERN OF CAMPYLOBACTER SPP. RECOVERED FROM CHICKEN MEAT SAMPLES OF RETAIL GROCERY STORES

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Campylobacter* spp. are commonly associated with poultry and poultry products. Moreover, drug-resistant campylobacters are a serious public health threat because they further complicate foodborne infections in humans.

Purpose: To determine the phenotypic resistance patterns of *Campylobacter* isolates recovered from retail chicken meat products.

Methods: The antibiotic susceptibility tests of 21 *Campylobacter* isolates derived from raw poultry products at retail against nine different antibiotics were conducted using the NARMS broth microdilution method, and Minimum Inhibitory Concentration (MIC) breakpoint values were considered based on CLSI, 2024. Data were analyzed using R, and the odds ratio and 95% confidence limits, based on antimicrobial resistance of bacterial isolates, were calculated for different chicken parts (wings, thighs, drums, breasts, and tenders) and production types (conventional, organic, and antibiotic-free). The level of significance was set to 0.05.

Results: Overall, 52% (11/21) of total *Campylobacter* isolates were resistant to at least one antibiotic tested, while none of the isolates were multi-drug resistant. There was no significant difference (p>0.05) in bacterial isolates having antimicrobial resistance based on different parts and production types. The percentage of *Campylobacter* isolates resistant to at least one antibiotic from conventional, organic, and antibiotic-free production types were 50% (4/8), 50% (3/6), and 57% (4/7), respectively. Similarly, the percentage of bacterial isolates resistant to at least one antibiotic from drums, wings, thighs, breasts, and tenders were 60% (3/5), 57% (4/7), 50% (3/6), 50% (1/2), and 0% (0/1), respectively.

Significance: The results suggest a potential public health threat because >50% isolates were resistant to at least one antibiotic. Future studies could be directed to determine the genetic characteristics of antimicrobial resistance and quantify the resistance traits.

P1-079: GROWTH AND SURVIVAL OF ALIARCOBACTER BUTZLERI IN GROUND CHICKEN UNDER REFRIGERATION AND FROZEN STORAGE CONDITIONS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Aliarcobacter butzleri* is an emerging poultry-associated pathogen whose behavior under cold storage conditions remains poorly characterized.

Purpose: To evaluate the growth kinetics and survival of *A. butzleri* in ground chicken and in vitro systems under refrigerated and frozen storage conditions.

Methods: *A. butzleri* ATCC 49616 was inoculated into ground chicken breast (850.0 ± 0.4 g batches) formulated with 0.5% or 1.5% (w/w) NaCl and into *Brucella* broth for in vitro assays. Samples were stored at 4, 8, and 12°C for 20 d and sampled every 24–48 h. Frozen samples were stored at −18°C and analyzed after 1, 3, 5, 7, 14, and 20 d. Populations were enumerated using selective *Campylobacter* agar for chicken samples and blood-supplemented trypticase soy agar for broth, while background microbiota were quantified using aerobic plate counts. Growth under refrigeration was modeled using primary and competitive models to estimate μmax, and frozen storage effects were assessed using log

reductions.

Results: Temperature was the only significant factor influencing *A. butzleri* behavior under both individual and competitive conditions ($p < 0.05$). In ground chicken, μmax values were negative at all temperatures ($p < 0.0373$), with greater decline at 12°C (□0.46 ± 0.04) than at 4°C (□0.26 ± 0.04). In vitro growth occurred at 8°C (μmax = 0.62 ± 0.05) and 12°C (μmax = 0.44 ± 0.05), while no growth was observed at 4°C (μmax = □0.22 ± 0.05; $p < 0.0031$). Under competitive conditions, μmax values were more negative at 12°C (□0.60 ± 0.05) than at 4°C (□0.30 ± 0.05; $p < 0.0156$). Salt had no significant effect ($p > 0.05$). Frozen storage resulted in significantly greater reductions in vitro (2.78 ± 0.95 log CFU/mL) than in ground chicken (1.29 ± 0.81 log CFU/g; $p < 0.0008$).

Significance: *A. butzleri* can survive in ground chicken during abusive storage and remain viable during freezing due to matrix protection, highlighting its importance in poultry food safety risk assessments.

Award Application: Developing Scientist Award

P1-080: ASSESSING MEAT AND POULTRY SAFETY PRACTICES AND KNOWLEDGE GAPS AMONG FARMERS' MARKET VENDORS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Farmers' markets in the United States continue to grow as consumers seek fresh, locally produced foods. As meat and poultry offerings become more common in these settings, ensuring safe handling and processing practices becomes increasingly important.

Purpose: As meat and poultry sales increase in these settings, consistent and science-based food safety practices are essential, yet small-scale vendors often operate with limited training and oversight. This study assessed the knowledge, attitudes, and practices of meat and poultry vendors at Kentucky farmers' markets.

Methods: Thirty-three vendors meat and poultry vendors completed surveys covering demographics, handling practices, food safety knowledge, perceived risks, and training preferences. Statistical analyses, including descriptive statistics and Chi-square tests or Fisher's Exact test, were performed using SPSS software.

Results: Findings indicated that most demonstrated general awareness of temperature hazards including 82% recognized that inadequate chilling can promote pathogen growth, and 76% stored poultry in a freezer after packaging. However, critical knowledge gaps were

evident. Although 73% identified major contamination points during raw poultry processing, only 12.8% correctly named *Salmonella* and *Campylobacter* as the primary pathogens associated with raw poultry. Handling practices also varied. Only 46% used electrically powered coolers during transport, and 15% transported poultry in coolers without ice. Despite these issues, 78% believed their products were safer than supermarket poultry.

Significance: Overall, the findings reveal notable gaps in pathogen awareness and temperature control practices. These results highlight the need for practical and targeted training to strengthen vendor knowledge, improve handling behaviors, and support the safe expansion of meat and poultry sales at farmers' markets.

P1-081: OPTIMIZING BACTERIAL WHOLE GENOME SEQUENCING FOR FOOD SAFETY: BEST PRACTICES FOR LONG-READ SEQUENCING

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Short-read sequencing (SRS) has long been considered the gold standard in bacterial whole genome sequencing. However, short reads often result in more fragmented assemblies, which may limit the resolution of mobile genetic elements and repetitive regions. Long-read sequencing (LRS) has been repeatedly recognized for producing more complete genome assemblies, however concerns exist given the higher error rate of some LRS technologies.

Purpose: This study determined a streamlined workflow to establish best practices for producing high-accuracy bacterial genomes using LRS technology.

Methods: The sample pool consisted of thirty isolates, with ten strains each of *Salmonella enterica*, *Listeria monocytogenes*, and *Cronobacter sakazakii*, sequenced using the Oxford Nanopore GridION. Data were processed using both Dorado high accuracy (HAC) and super accuracy (SUP) basecalling models. Assembly quality and accuracy was benchmarked against SRS assemblies using checkM and QUAST. cgMLT and SNP comparisons were also performed to ascertain which approaches resulted in the highest quality and most accurate genome

assemblies.

Results: SUP increased read accuracy (Q20) by on average 65% over HAC. LRS produced assemblies with 8 times fewer contigs and 12.5 times higher N50 values than SRS. While differences in basecalling produced no significant changes in contig numbers or N50 values, these values were impacted by library prep kit. LRS-only assemblies only differed from SRS assemblies by an average of 1.6 cgMLST alleles and 1 SNP difference on average.

Significance: This optimized workflow results in LRS analyses that are indistinguishable from the same analysis performed with SRS (Illumina), while producing superior assemblies. This can allow for more cost-effective and rapid whole genome sequencing, thus improving food safety by facilitating superior source tracking and more comprehensive genomic characterization of pathogens.

P1-082: IMPACT OF PARALOG MERGING ON INTEGRATIVE SOURCE ATTRIBUTION MODELS OF VIBRIO PARAHAEMOLYTICUS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Paralogs, created by gene duplication events, could benefit the niche adaptation of organisms and encode genomic variation relevant to source attribution. Therefore, merging paralogs during bioinformatics analyses may affect the downstream analysis, potentially impacting the inferences for food safety.

Purpose: This study evaluates the impact of paralog merging on source attribution of *V. parahaemolyticus* using various genomics tools integrated with machine learning models.

Methods: Assemblies of *V. parahaemolyticus* (n= 6,227) were collected from the National Center for Biotechnology Information database. Quality control was performed using CheckM (v1.2.2), yielding 176 environmental, 960 seafood, and 853 clinical isolates for further analysis. Six pangenome pipelines, combining three annotation tools (Bakta (v1.9.4), PGAP (v6.4), and Prokka (v1.14.5)) with two pangenome tools (Panaroo (v1.5.1) and Roary (v3.13.0)), along with core genome Multi Locus Sequence Typing (cgMLST) and whole genome Multi-Locus Sequence Typing (wgMLST) analyses using chewBBACA (v3.3.10), were performed both with and without merging paralogs. Outputs from these pipelines were used to build Random Forest-based source attribution models in Python (v3.12.5). Sensitivity, Specificity,

Area Under the Receiver Operating Characteristics (AUROC), and Area Under the Precision Recall (AUPR) were used to assess model performance.

Results: Models based on pangenome pipelines without merging paralogs showed higher sensitivity, specificity, AUROC, and AUPR values than those with merging paralogs, indicating improved source attribution performance when paralogs were retained. However, the performance of MLST-based models was not affected by paralog merging.

Significance: This study is an initial attempt to evaluate how paralogs influence the source attribution of *V. parahaemolyticus*, highlighting that paralog retention can preserve source-discriminatory genomic information in pangenome analyses.

Award Application: Developing Scientist Award

P1-083: DEVELOPMENT OF A DATABASE FOR INFB SEQUENCE-BASED SUBTYPING AND IDENTIFICATION OF CRONOBACTER

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Cronobacter*, a genus of bacteria within the *Enterobacteriaceae* family capable of causing severe infections in infants, is of concern to the dairy industry because outbreaks of *Cronobacter* are often linked to exposure via powdered infant formula.

Purpose: The purpose of this study was to establish a database and standardized method for *infB* sequence-based subtyping and classification of *Cronobacter* isolates to support the identification and characterization of *Cronobacter* within dairy products and processing environments.

Methods: A database of DNA sequences spanning a 441-nucleotide region within the *infB* gene was curated from (i) sequences available at the *Cronobacter* PubMLST database, (ii) *Cronobacter* whole genome sequence (WGS) data available on NCBI Pathogen Detection, and (iii) *Cronobacter* isolates previously characterized in our laboratory. The *infB* database was then incorporated into the Food Microbe Tracker online platform

(www.foodmicrobetracker.com).

Results: The 362 *infB* allelic types (ATs) included in the *infB* database as of January 13, 2026 were derived from over 3,400 isolates and span all seven *Cronobacte*r species. The three species with the most *infB* ATs were *Cronobacter sakazakii*, *C. dublinensis*, and *C. malonaticus*. As a proof of concept, we used this database to characterize 93 environmental isolates of *Cronobacter* obtained from nine different states across the U.S. These isolates represented 30 *infB* ATs and 5 species. All ATs identified among this environmental dataset were represented in the original database, suggesting that the initial data comprehensively covers *Cronobacter* diversity.

Significance: Although recent advances have increased the use of WGS for bacterial identification in industrial settings, limitations, such as cost, remain. By offering a more rapid and affordable alternative, the *infB*-based method described here is a promising tool for initial *Cronobacter* identification and characterization.

Award Application: Developing Scientist Award

P1-084: ARTIFICIAL INTELLIGENCE-GUIDED DEVELOPMENT OF SPECIES-SPECIFIC REAL-TIME PCR ASSAYS FOR DETECTION OF DOMINANT BACTEROIDES SPECIES IN THE HUMAN GUT

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Accurate species-level identification of dominant *Bacteroides* species in the human gut is essential for food- and health-related microbiome studies, yet closely related species are often misclassified by sequencing-based methods.

Purpose: This study aimed to develop and validate a species-specific real-time PCR method for detecting and quantifying six dominant *Bacteroides* species using machine learning-derived genetic markers.

Methods: Species-specific markers were identified by integrating pangenome analysis with supervised machine learning. Real-time PCR assays were developed and evaluated using pure cultures and human fecal samples, and results were compared with shotgun metagenomic sequencing.

Results: Pangenome analysis of 600 *Bacteroides* genomes generated 279,528 gene clusters, which were analyzed using machine learning-based feature selection. This approach

identified six species-specific marker genes encoding enzymes or transport-related proteins involved in gut carbohydrate and sulfur metabolism (*ksgA*, *asrA*, *sasA*, *gacA*, *nag6S*, and *susD*). Real-time PCR demonstrated 100% analytical specificity, with no cross-reactivity against non-target *Bacteroides* species or other intestinal bacteria. The analytical sensitivity of each assay reached a limit of detection of 10^1 CFU/mL, and standard curves showed strong linearity across 10^1-10^7 CFU/mL (R^2 □ 0.99). When applied to fecal samples from healthy adults (n=6), real-time PCR-based detection showed an overall concordance rate of 89% with shotgun metagenomic sequencing. Discrepancies were primarily attributed to misclassification of closely related *Bacteroides* species due to high sequence similarity of conserved marker genes.

Significance: This machine learning-guided real-time PCR approach provides a rapid and accurate tool for species-level detection of dominant *Bacteroides* species, supporting improved food safety-related microbiome analysis and public health risk assessment.

P1-085: AN EXPANDED FDA BOTANICAL DNA REFERENCE LIBRARY AND MARKER EVALUATION FRAMEWORK FOR PLANT SPECIES IDENTIFICATION

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Identification of plant-derived foods and dietary ingredients remains a persistent challenge, driven by the immense diversity of plant taxa and the limited availability of authenticated genomic reference data.

Purpose: Here we focused on expanding DNA reference libraries from voucher specimens to increase those found in foods. Also, we developed a computational tool to automatically find suitable genetic markers to distinguish plant species from genome skimming.

Methods: We sequenced and assembled chloroplast genomes for 110 vouchered plant specimens at the Smithsonian National Museum of Natural History. R is used for computational analysis.

Results: We expand the USFDA's Botanical DNA reference library (GenomeTrakrCP; BioProject PRJNA325670) by spanning 48 families and 28 orders, including 16 orders not previously represented in the database. Nearly one-third of the taxa represents new additions to GenBank, including plants used as foods and dietary supplements or that might be containments of these. Evaluation of commonly used barcode loci showed high above-genus-level discrimination (90%, 77/85 genus) but lower species-level resolution (57%, 63/110 species), showed the limitations of these markers for discrimination in complex plant groups. These findings motivated the development of a pipeline that quantitatively assesses the discriminatory power of novel individual genes, intergenic regions, and their combinations automatically. Using novel multi-locus combinations, intergenic regions can distinguish 73 species. Additionally, 5 *Prunus* species that were difficult to distinguish using classic barcoding loci were targeted, and one new locus (*rpl32 to trnL-UAG*) and 11 loci combinations were identified for barcoding these species.

Significance: Our results establish both an expanded genomic reference foundation and an evidence-based strategy for improving species discrimination beyond classic plastid markers.

P1-086: SPROUT: A COMPUTATIONAL AND TARGETED SEQUENCING APPROACH FOR MIXED-PLANT DNA IDENTIFICATION WITH ANGIOSPERMS353

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The identification of plant species from mixed samples is crucial in food and dietary supplement safety and traditional methods face several potential challenges.

Purpose: This study introduces a novel metagenomic pipeline using the Angiosperm353 target sequencing to offer the taxonomic identification of angiosperm DNA in mixed samples.

Methods: We assembled short pair-end reads using existing workflows to assemble contigs from enriched DNA (HybPiper) for each mixed sample. Using a reference database of

selected representative Angiosperms353 sequences from 871 taxa, we apply phylogenetic inference to categorize contigs and variance in phylogenetic distance across genes to identify the presence of likely taxa in mixed plant samples.

Results: Pipeline performance was evaluated using in silico mixed-read libraries, including 100 mixtures of varying complex mixtures, 48 skewed species proportions samples, 36 reduced read depths samples, as well as 11 mock libraries constructed from plant blends. For in silico mixtures, prediction accuracy ranged from 98.1–99.6% with precision of 92.9–100%, depending on pipeline parameter thresholds. Systematic exploration of thresholds cutoffs revealed predictable tradeoffs between sensitivity and precision, with optimal performance maintained across mixtures of three to ten species. Tests with skewed compositions and low-yield inputs showed that false negatives were not significantly increased when minor contributors were represented by more than ~20,000 mapped reads. In mock tissue mixtures, family- and order-level predictions largely matched expected composition, which reached 90.7% accuracy and 98.0% precision, with reduced resolution primarily attributable to reference database limitations.

Significance: Our method offers a potential framework for scientific and practical applications in plant species identification, including plant product authentication and detection of food fraud and adulteration.

P1-087: GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF BACILLUS CEREUS ISOLATES FROM HUMAN CLINICAL CASES IN SLOVENIA

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: In 2022, European Union member states reported 127 foodborne outbreaks attributed to *Bacillus cereus* toxins. Although foodborne illness is typically associated with *B. cereus sensu stricto* (*s.s.*) and biovar Emeticus, the recovery of biovar Thuringiensis from clinical cases has raised concerns.

Purpose: To characterize the genotypic and phenotypic diversity of 47 *B. cereus* group isolates recovered from human clinical cases by the Slovenian National Laboratory of Health, Environment, and Food between April 2024 and July 2025.

Methods: Genomic DNA was extracted using the QIAGEN DNA Blood Mini Kit; libraries were prepared with the Rapid Barcoding Kit and sequenced on Nanopore MinION R9 flow cells. Genome assembly, polishing, and quality assessment were conducted with Flye (v2.9.5), Medaka (v1.3.2), and QUAST (v5.2.0). Taxonomic assignment and virulence gene detection were performed using BTyper3 (v3.4.0), while phylogenetic relationships were resolved using

kSNP4 (v4.1.0). Maximum-likelihood phylogenetic trees were constructed with RAxML (Galaxy v8.2.12) and visualized in iTOL (v6). Cytotoxicity was assessed using Caco-2 cells exposed to 15% (v/v) culture supernatant, followed by WST-1 metabolic activity measurement.

Results: Isolates with good quality of assemblies (n=43) were classified as *B. cereus s.s.* (n=25, including 9 biovar Thuringiensis), *B. mosaicus* (n=12, including 1 biovar Emeticus), *B. mosaicus/luti* (n=2), and *B. toyonensis* (n=4). BTyper3 detected *nhe* genes in 86% of 43 isolates, *hbl* in 65%, *cytK-2* in 60%, and *sph* in 86%, while *cesABCD* was present in only one isolate (2%). Isolates from group IV (*B. cereus s.s.*) exhibited the highest average cytotoxicity (0.556±0.317), whereas group II showed the lowest cytotoxicity (0.121±0.007).

Significance: *B. cereus s.s.* predominated among tested isolates and included 9 biovar Thuringiensis strains, six of which were closely related (>99% average nucleotide identity) to commercial biopesticide strains.

Award Application: Developing Scientist Award

P1-088: ISOLATION AND CHARACTERIZATION OF BACILLUS CEREUS ISOLATED FROM KITCHEN SPONGES

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Kitchen sponges are commonly used to clean dishes after food consumption. Studies previously indicated the presence of multiple microorganisms but also of common food pathogens such as *Campylobacter* spp., *Escherichia coli* and *Salmonella* spp. Interestingly, *Bacillus* spp. were shown to be part of the core microbiota of kitchen sponges but the prevalence and hazard of the food pathogen *Bacillus cereus sensu lato* in kitchen sponges is unknown.

Purpose: This study evaluates the presence of and characterizes viable *B. cereus s.l.* in kitchen sponges collected from Belgian households (Flanders region).

Methods: One hundred kitchen sponges were collected from 82 households from Flanders (Belgium). Enumeration of *B. cereus s.l.* was performed following ISO 7932, which includes

culture on the selective mannitol egg yolk polymyxin (MYP) agar medium. The total aerobic microbial count was determined by culture on plate count agar (PCA), following ISO 4833-1. 27 isolates, selected from a subset of the sponges positive for *B. cereus s.l.* (n = 17 from 38) were retained for characterization by whole-genome sequencing (Illumina NovaSeq X platform, S4 PE150 XP). Their (putative) virulence repertoire was screened and the isolates were compared using multi-locus sequence typing (MLST) and core-genome MLST (cgMLST).

Results: The characterized isolates displayed multiple (putative) virulence genes, including enterotoxin genes. Twelve different genetic lineages (MLST) were detected, of which previously encountered in outbreaks. Interestingly, cgMLST demonstrated a clonal isolate in two sponges collected from the same household at different time points.

Significance: These results indicate that kitchen sponges are a potential facilitator for cross-contamination in (domestic) kitchens and in the development of (toxico) infection.

Award Application: Developing Scientist Award

P1-089: ISOLATION AND GENETIC CHARACTERIZATION OF A CRONOBACTER SAKAZAKII STRAIN RECOVERED FROM SPICE POWDER

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Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Cronobacter sakazakii* is an opportunistic, Gram-negative, rod-shaped, facultative anaerobic, foodborne bacterium that can cause life-threatening meningitis and necrotizing enterocolitis in neonates, elderly, and immunocompromised individuals. It can survive in extreme dry conditions and has been linked primarily with contaminated powdered infant formula. However, it has also been isolated from a wide variety of foods. WGS has been established as a comprehensive technique for advanced bacterial typing to analyze bacterial strains isolated from foodborne outbreak and sporadic cases. Here, we report the draft genome sequence of a *C. sakazakii* strain, recovered from spice powder.

Purpose: The major objective of this study was to identify a *C. sakazakii* strain isolated from a spice powder sample by performing WGS analysis.

Methods: A *C. sakazakii*-like bacterial strain from spice powder sample was recovered and analyzed. Preliminary ID was accomplished by VITEK 2, real-time PCR, and MALDI-TOF MS analysis, following FDA's BAM techniques. WGS was completed on an Illumina MiSeq system, using a Nextera XT DNA library preparation kit and a 250-bp paired-end read MiSeq Reagent v2 kit (500-cycle), following manufacturer's suggested protocols.

Results: MALD-TOF MS analysis revealed species identification with high confidence value

(>99%) for the *C. sakazakii* strain SRL-124. Genome sequence of SRL-124 strain was 4,603,544 bp in length, distributed in 40 contigs. WGS analysis revealed the sequence type as ST4 for the genome of *C. sakazakii* strain SRL-124.

Significance: *C. sakazakii* ST4 strain has been associated with most serious meningitis clinical cases caused by the *Cronobacter* strains worldwide. WGS can be used for precise strain identification of *C. sakazakii*.

P1-090: GLOBALLY INTEGRATED LISTERIA AND CRONOBACTER WGS STRAIN TRACKING USING OXFORD NANOPORE TECHNOLOGIES' LONG-READ SEQUENCING

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Reduced cost and faster turnaround of next-generation sequencing (NGS) now enable its routine use in high-throughput microbiology labs. Laboratories in Madison (Wisconsin, USA) and Nantes (France) conducted a proof of concept (POC) to implement harmonized methods for *Listeria* and *Cronobacter* identification and strain tracking using long-read sequencing and internal bioinformatics pipelines. Building on a successful 2025 *Salmonella* tracking POC, adding *Listeria* and *Cronobacter* broadens support for food industry supply-chain investigations.

Purpose: To demonstrate a reliable end-to-end NGS workflow to identify and globally track *Listeria* and *Cronobacter* isolates from the food industry.

Methods: Three replicates of a reference strain and three wild-type strains were grown on chromogenic agar in both locations. DNA from isolated colonies was extracted, purified, fragmented, and normalized following internal/manufacturer protocols. Libraries were prepared using the Native Barcoding Kit 24 V14 and sequenced on an R10.4.1 flow cell using a GridION from Oxford Nanopore Technologies until each barcode produced □300 Mb of

data. FASTQ files were analyzed using internally validated pipelines for identification and single nucleotide variant (SNV) calling.

Results: De novo assembled genomes of the reference species *Listeria monocytogenes* ATCC 19114 differed by <11 SNVs between replicates; wild-type *Listeria* differed by <3 SNVs. The reference species *Cronobacter turicensis* DSM 18703 differed by <1 SNV between replicates, and wild-type *Cronobacter* differed by 0 SNVs. Assemblies produced 5 contigs per genome and >30X coverage was achieved for 98% of genomes. These results demonstrate high accuracy and reproducibility.

Significance: This harmonized NGS system enables identification and tracking of *Listeria* and *Cronobacter* across labs in Europe and North America without requiring international pathogen shipment and the associated documentation, uncertainty and delay.

P1-091: CHARACTERIZING THE ANTIBIOTIC RESISTANCE GENES, VIRULENCE FACTORS, AND PATHOGENS IN THE HOUSEHOLD ENVIRONMENT OF THREE GLOBAL CITIES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The built microbiome has the potential to harbor pathogenic microbes which may pose a risk to human health. In this study, we aimed to characterize the presence of clinically relevant foodborne pathogens across household surfaces in three global cities – Tucson (AZ, USA), Mysore (Karnataka, India), and Dubai (UAE).

Purpose: Assess the presence of foodborne pathogens within the microbiome of household surfaces and how it varies between cities and across different locations within the house.

Methods: Ten samples were collected from ten household surfaces in each city, including the kitchen, bathroom, and living spaces. Samples were sequenced using Oxford Nanopore technology. The microbiome for each surfaces was characterized, medically relevant pathogens were identified using Kraken2 software and in-house scripts, and antibiotic resistance genes (ARGS) and virulence factors (VFs) were also characterized for all samples.

Results: All the alpha diversity parameters utilized (e.g., Shannon, ANOVA, p=0.0014) found significant diversity between the three countries with homes in Mysore showing higher microbial diversity compared to Dubai and Tucson. *Escherichia coli* and *Salmonella enterica* were found on kitchen counters in Dubai and Tucson and within kitchen sinks in Dubai, Tucson, and Mysore. *Listeria monocytogenes* was found in kitchen sinks in Tucson and Dubai. *Campylobacter jejuni* was found in the toilet bowl in Tucson but not in the kitchen in any of the three cities. The ARG and VF profiles differed across the three cities (PERMANOVA, p<0.001), with diverse core ARG and VF profiles in each location. ARGs and VFs can be picked up by foodborne pathogens present, increasing virulence and risk to household occupants.

Significance: Characterizing the presence of pathogens in the built environment is essential to assess the risk of illness presented to household occupants and to inform food safety practices and guidelines in residential spaces.

P1-092: GENOMIC INSIGHTS INTO IMPORTED RETAIL FOOD AS A SOURCE OF ANTIBIOTIC-RESISTANT ESCHERICHIA COLI IN PRISTINE AREAS FREE FROM AGRICULTURAL ACTIVITY

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Food can introduce antibiotic-resistant bacteria into novel environments, potentially shaping environmental circulation and human exposure in pristine areas free from agricultural activity.

Purpose: Identify contribution of imported meat and produce to antibiotic resistance (AR) by comparing AR genes and mobile genetic elements (MGEs) in *E. coli* from food, water, and human sources.

Methods: Meat and produce were collected from retail markets in Kasane, Botswana, along with river water and human fecal samples from food handlers and patients in 2022. *E. coli*

was isolated and whole-genome sequencing (Illumina NextSeq) was performed on multidrug-resistant *E. coli* isolates. AR genes, MGEs, and predicted pathogenicity were analyzed in 24 genomes (meat: 8, produce: 4, water: 6, human 6) using comparative genomic approaches.

Results: Phylogenetic analyses revealed interspersed clustering of isolates from food, water, and humans into two distinct clades. Isolates across sources showed high predicted pathogenicity (0.91–0.95), with ExPEC- and EPEC-like profiles in food and humans and EPEC/EHEC-like strains isolated from water. Food-handler isolates carried more plasmid-associated AR genes than patient isolates, with resistance profile overlapping those in food isolates, including *β*-lactamase, tetracycline, sulfonamide, and quinolone resistance determinants. IncF-family plasmids were shared across all sources, whereas food isolates showed the greatest plasmid diversity, including non-IncF replicon types and Col-type plasmids. Integrons were detected only in food isolates including both meat and produce. Within food sources, meat isolates carried more plasmid-associated AR genes than produce isolates.

Significance: Imported food, particularly meat, is an important route of AR introduction into human-environment interfaces and food handlers are a key population for food-mediated exposure.

Award Application: Developing Scientist Award

P1-093: ANTIMICROBIAL RESISTANCE PROFILES AND GENOMIC CHARACTERISTICS OF HYBRID STEC/ETEC ISOLATED FROM ANIMAL FECES, BEEF AND PORK MEAT IN SOUTH KOREA

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Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The emergence of hybrid *Escherichia coli* strains associated with large outbreaks, together with the increasing prevalence of multidrug resistance (MDR), highlights the urgent need to better understand antimicrobial resistance mechanisms in pathogenic *E. coli*.

Purpose: This study aimed to investigate antimicrobial resistance profiles and genetic characteristics of hybrid STEC/ETEC isolated from animal feces, beef and pork meat in South Korea between 2012 and 2022.

Methods: Virulence factors, antimicrobial resistance genes, and horizontal gene transfer were analyzed using whole-genome sequencing (WGS). Antimicrobial susceptibility testing was conducted to assess phenotypic resistance and to compare it with genomic

characterization. Resistance genes were identified using ResFinder(v4.7.2), while mobile genetic elements and plasmid origins were analyzed using MGE(v1.0.2) and PlasmidFinder(v2.0.1), respectively. All analyses were performed in biopython using the default parameters.

Results: All hybrid STEC/ETEC strains carried both *stx* and *est* genes. WGS analysis revealed that *stx* genes were chromosomally encoded, whereas *est* genes were plasmid-encoded. Among the 63 isolates, 40 (63.5%) exhibited resistance to at least one antimicrobial agent, and 36 showed MDR, defined as resistance to three or more antimicrobial subclasses. Resistance was most frequently observed to β-lactam antibiotics, tetracyclines, and phenicols. Most antimicrobial resistance genes were plasmid-encoded and commonly co-localized with *est*-carrying plasmids in MDR hybrid strains.

Significance: These findings indicate that hybrid STEC/ETEC strains from animal and food sources serve as important reservoirs of antimicrobial resistance genes, posing potential risks to public health and emphasizing the need for continuous surveillance.

P1-094: GENOMIC SURVEILLANCE OF ANTIMICROBIAL RESISTANCE, PLASMIDS, AND VIRULENCE DETERMINANTS IN SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IN THE UNITED STATES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The ability of Shiga toxin-producing *Escherichia coli* (STEC) to acquire resistance and virulence determinants via mobile genetic elements complicates food safety mitigation strategies.

Purpose: This study utilized large-scale genomic surveillance to evaluate the distribution and co-occurrence of antimicrobial resistance (AMR), plasmid replicons, and virulence in STEC isolates across the USA.

Methods: We retrieved 11,290 STEC genome assemblies from NCBI and verified their quality

with CheckM. Using ResFinder, PlasmidFinder, and VFDB databases, we screened for AMR, plasmids, and virulence genes. We then analyzed these features across O157 and non-O157 serovars from both clinical and environmental sources, using GraphPad Prism for statistical analysis.

Results: Of 11,290 STEC genomes analyzed, clinical sources (n=10,744) far exceeded environmental ones (n=546). Non-O157 serovars (n=7,166) also outnumbered O157 (n=3,197). Furthermore, we found 2,716 multi-drug-resistant isolates (≥3 AMR classes), with aminoglycoside (n=6,478), sulfonamide (n=3,552), tetracycline (n=2,820), and beta-lactam resistance genes (n=1,349) being the most common. Plasmid profiling was dominated by IncFIB (AP001918) (n=9,444) and ColRNAI (n=5,657), with frequent detection of IncB/O/K/Z (>4,000), as well as resistance-associated IncI1 (n=766) and IncX4 (n=824) replicons. We found distinct AMR clustering on the Col (BS512) plasmid (n=121), which consistently carried *mdf(A)*, *ant(3")-Ia*, *tet(B)*, *mph(A)*, and *sul2*. Adherence and type III secretion virulence genes were present in all genomes; however, Shiga-toxin distribution across the 11,290 genomes was heterogeneous, led by *stx1B* (n= 7,148) and *toxB* (n= 6,460), with *stx2* variants appearing in roughly (n= 4,742) of isolates and *cnf1* being rare (n=28).

Significance: Our findings demonstrate the significant burden of plasmid-mediated multidrug resistance in STEC in the USA, underlining its importance as a long-term public health threat.

Award Application: Developing Scientist Award

P1-095: COMPARATIVE GENOMIC ANALYSIS REVEALS CLADE, SOURCE, AND REGION-SPECIFIC PATTERNS IN SALMONELLA ENTERICA SEROVAR RUBISLAW IN THE U.S.

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella enterica* serovar Rubislaw is an important cause of human salmonellosis that is considered endemic to the Southeastern U.S. Yet, little is known about the reservoirs of *S.* Rubislaw, making control strategies challenging.

Purpose: This study characterized the population structure of *S.* Rubislaw and evaluated associations between phylogenetic clades, isolation sources, geographic regions, sequence types, pangenome composition, and AMR profiles to provide insight on potential sources of *S.* Rubislaw in the U.S.

Methods: Genome assemblies of *S.* Rubislaw isolated from the United States (n = 2504) were downloaded from the Pathogen Detection Database. kSNP4 was used to assign isolates to phylogenetic clades based on core SNPs. Multilocus sequence typing (MLST) was done using PubMLST database. Antimicrobial resistance genes (ARG) were identified using ResFinder. A pangenome analysis was executed with Panaroo and genome-wide associations were calculated in Scoary.

Results: SNP-based phylogenetic analysis generates 7 distinct clades with significant associations between isolation type, MLST type, and geographic region (χ^2 test, $p < 0.001$). Clade 7 predominated with around 25% of all isolates including *S*. Rubislaw CFSAN029938

used by EPA for agricultural water testing; pangenome analyses revealed substantial accessory genome diversity, driven by mobile genetic elements (plasmid) as well as stress response genes and cell envelope modifications. ARGs were considerably rare in the dataset, with just 2 isolates having >1 ARG detected, evenly distributed across clades except clade 3, and significant AMR gene difference found only in isolation type among clinical and environmental samples (Welch *t*-test, *p*-value < 2.2e-16).

Significance: This study provides novel genomic insights for *S.* Rubislaw, highlighting clade-specific ecological and geographic patterns that are relevant for risk assessment and surveillance efforts relevant to food safety and public health.

Award Application: Developing Scientist Award

P1-096: MACHINE LEARNING-BASED IDENTIFICATION OF HOST-ASSOCIATED GENOMIC FEATURES IN SALMONELLA ENTERICA SEROVAR DUBLIN

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella enterica* serovars pose a significant public health threat due to their high morbidity and mortality, compounded by rising antimicrobial resistance. Among these, *S. enterica* serovar Dublin, a cattle-adapted serovar, is increasingly prevalent in dairy cows, being a zoonotic pathogen, presenting a notable risk to human health.

Purpose: This study aimed to investigate the genomic characteristics of *S.* Dublin isolates from humans, animals and environmental sources to identify host-associated genetic features using machine learning (ML) analysis.

Methods: A total of 3,573 *S.* Dublin genomes, combining publicly available sequences and newly sequenced isolates, were analyzed. The principal coordinates analysis, *t*-distributed stochastic neighbor embedding and phylogenetic tree were used to examine genomic relatedness. For supervised ML, Decision Tree, Random Forest, Support Vector Machine (SVM), Multi-Layer Perceptron, and XG Boost were applied. Host-associated genes were

identified with permutation importance analyses.

Results: The clustering analysis and phylogenetic tree could not distinguish isolates by hosts, whereas supervised ML achieved. Among the various supervised ML models examined, SVM showed the best performance in classifying *S*. Dublin genomes by host source. Notably, host-specific genetic features were identified by permutation importance analyses including the *rhaR* gene, which regulates L-rhamnose metabolism, supporting bacteria to survive in sugar limited environment; the *ydjM* gene, which encodes inner membrane protein; and the IS3 family transposase ISSty2, which drives genetic variability, impacting virulence and resistance.

Significance: These findings demonstrate that *S.* Dublin has adapted to specific hosts through the acquisition of key genetic traits. Understanding these host-associated genetic features can help reduce zoonotic transmission and persistence and may provide targets for controlling *S*. Dublin in both animals and humans.

P1-097: IN-SILICO EVALUATION AND SIMULATION OF PERFORMANCE OF SALMONELLA VIRULENCE MOLECULAR TARGETS UNDER REALISTIC SEROVAR PROFILES IN FOOD PRODUCTS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Virulence assays supporting *Salmonella* control should be robust in multiple products.

Purpose: In-silico performance evaluation of proposed *Salmonella* virulence targets in meats.

Methods: We estimated *Salmonella* serovar proportions on beef, pork, chicken, turkey, and meats combined from 63,197 FSIS isolates (NCBI 2016-2023). Serovars were predicted (SeqSero2) and isolates annotated for eight previously proposed highly pathogenic *Salmonella* (HPS) virulence genes (CD-HIT 2D). We calculated Sensitivity *Se* and Specificity *Sp* for 5 HPS genes proposed elsewhere to detect 18 serovars of concern (SoC) or 40 SoC+ serovars to watch (SoC+StW). We simulated corresponding yearly virulence false negatives (FN) in a beef facility.

Results: SoC+StW serovars represented 65.3% of Beef isolates [n= 11,247; top 3: *S.*Montevideo(2,065; 18.4%), *S.*Dublin(1,132;10.1%), *S.*Anatum(1,048; 9.3%)], 56.3% in Pork [n=12,890; *S.*Anatum(1,802, 14.0%), *S.*Infantis(1,104, 8.6%), *S.*I 4,[5],12:i:-(932, 7.2%)], 59.1% in Chicken [n= 33,261; *S.* Infantis(8,726, 26.2%, *S.*Enteriditis(5,626, 16.9%), *S.*Typhimurium(3,142, 9.4%)], and 79.2% in Turkey [n=5,799; *S.*Reading(1,041, 18.0%), *S.*Hadar(790, 13.6%), *S.* Schwarzengrund(449, 7.7%)]. Such variation resulted in low *Se* for Beef[SoC= 33.9(32.6-35.4%); SoC+StW= 28.1(27.1-29.1%)], Pork[72.6(71.0%-74.1%);36.1(35.0%-37.2%)], Chicken[66.5(65.8-67.2%); 66.0(65.3-66.6%)], Turkey[49.7(47.6-51.8%); 28.5(27.2-29.8%)], and all meats[60.9(60.3-61.5%);48.8%(48.3%-49.3%)]. FNs primarily affected *S*. Montevideo (beef), *S*. Anatum (Pork), *S*. Enteriditis (chicken), and *S*. Reading (turkey). *Sp* ranged from 90.6(89.9-91.3%) for SoC, to 98.7(98.3%-99.0%) for SoC+StW, both on beef isolates. A 300 beef combos/day facility(10% testing, 3% *Salmonella* prev.) yielded 89(72-109) FNs out of 125(103-147) combos contaminated with virulent serovars, yearly.

Significance: Serovar diversity in meats resulted in poor *Se* and high number of FNs for currently proposed HPS virulence targets.

P1-098: PLASMID DIVERSITY IN SALMONELLA KENTUCKY ST152 ISOLATED FROM LIVE POULTRY ENVIRONMENTS ON OREGON'S AGROTOURISM FARMS

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Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Agrotourism is a growing industry in several U.S. states, including Oregon. These operations often include activities allowing interactions between visitors & animals.

Purpose: To evaluate zoonotic illness risks associated with agrotourism by estimating the pathogen burden and spread within farms.

Methods: Environmental samples (n = 25–36) from agrotourism operations with live poultry (n = 10) were analyzed for *Salmonella* using enrichment and isolation procedures. All isolates were whole genome sequenced (WGS) using Illumina, with selected isolates also sequenced by MinION. Plasmids were identified using a hybrid assembly approach together with plasmid marker screening of short-read assemblies. Incompatibility groups were assigned by PubMLST.

Results: *Salmonella* was not detected on five farms, detected in a single sample on four farms, and detected in 23 of 30 of samples (77%) from one farm. The highest burden of

Salmonella was from the only farm that raise broilers for slaughter (<1000/yr). Isolates from this farm (n = 42) were predominantly *S.* Kentucky ST152 (90%) with the balance being *S.* Alachua ST1298. Eight unique plasmids were identified across the *S.* Kentucky strain set. Three mobilizable plasmids were identified, including a conserved 3.5-kb plasmid (38/38) and a rare 6.6-kb plasmid (1/38). A mobilizable 47-kb plasmid thought to be conserved in *S.* Kentucky was confirmed in all strains. Four conjugative plasmids were identified with the most common being a 68.9-kb IncFII plasmid (32/38) which carried the aerobactin gene cluster. The other conjugative plasmids were infrequent: 83-kb IncI/FII plasmid (4/38), 68.4-kb IncFII (1/38), and 42-kb IncP (1/38). None of the plasmids identified carried genes associated with antibiotic resistance.

Significance: Plasmid characterization of closely related *S.* Kentucky strains provided a unique perspective on the genomic diversity and provide context for mobile gene acquisition and spread within a single farm.

P1-099: LINEAGE STABILITY OVERRIDES RESERVOIR ADAPTATION IN THE PLASMIDOME OF SALMONELLA ENTERITIDIS FROM LOW-MOISTURE FOODS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Low-moisture foods (LMF), including nuts, flour, and spices, are increasingly implicated in outbreaks of *Salmonella enterica* serovar Enteritidis.

Purpose: It remains unclear whether LMF-associated isolates exhibit reservoir-specific plasmidome signatures after accounting for lineage structure. I Pathogen Detection SNP clusters.

Methods: We analyzed 2,897 non-clinical Enteritidis genomes from NCBI Pathogen Detection, including 45 LMF isolates and 2,852 controls spanning 51 SNP clusters. To minimize population-structure confounding, primary tests were restricted to mixed clusters containing both reservoirs. Replicons were identified with PlasmidFinder, plasmid content and mobility were assessed with MOB-suite, and reservoir associations were evaluated using Fisher's exact tests and Cochran-Mantel-Haenszel stratified analyses with false-discovery correction.

Results: Core virulence-associated replicons, including IncFIB(S) and IncFII(S), were near-

ubiquitous in both groups, and no accessory replicon family showed significant LMF enrichment after correction. Plasmid burden and predicted mobility profiles were also similar between reservoirs. However, rare LMF-specific accessory elements were detected in a small subset of isolates, including an IncI2 plasmid carrying *proQ* and a compact resistance module containing TEM-family beta-lactamase and ant(3'')-Ia.

Significance: These findings indicate that lineage stability explains most plasmidome patterns across reservoirs, while low-frequency accessory plasmids may still contribute niche-relevant traits. This lineage-aware framework provides a baseline for testing adaptation hypotheses in dry-food associated *Salmonella*.

Award Application: Developing Scientist Award

P1-100: CHARACTERIZING THE CELL SURFACE-ASSOCIATED PROTEINS OF SALMONELLA TYPHIMURIUM STRAIN SL1344 USING A BIOTINYLATION-BASED PROTEIN EXTRACTION METHOD: A PROOF-OF-CONCEPT STUDY

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Salmonella*'s success as a pathogen is partly due to its use of cell surface-associated proteins (CSAPs; e.g., fimbriae, flagella) to mediate host interactions, making their isolation essential for understanding pathogenesis and improving detection and control strategies. Isolating CSAPs is challenged by their chemical heterogeneity, low abundance, and the lack of a universal isolation method.

Purpose: In this proof-of-concept study, we investigated the cell surface biotinylation method for extracting CSAPs of *Salmonella enterica* serovar Typhimurium strain SL1344.

Methods: CSAPs from the SL1344 proteome (*n*=4,712 protein sequences) were identified *in silico* using PSORTdb4.0, SOSUIGramN, and BUSCA, and their expression was experimentally assessed by extracting biotinylated proteins (BPE) from early stationary-phase cells at 37°C

using the Pierce Cell Surface Protein Biotinylation Kit. Total proteome extracts (TPE) generated by sonication served as controls. These protein extracts were analyzed by SDS-PAGE and LC-MS/MS, with cell surface biotinylation efficiency confirmed by immunofluorescence staining. All experiments were triplicated.

Results: SDS-PAGE analysis of the BPE confirmed successful recovery of multiple proteins, and immunofluorescence imaging validated efficient biotinylation (~26X higher intensity in biotinylated samples). LC-MS/MS identified 632 and 2,779 proteins in the BPE and TPE, respectively, with 8 and 2,155 proteins uniquely identified by each method. In comparison with *in silico* predicted CSAPs (n=156), 60 were detected by at least one method, of which 24 were detected only in the TPE, suggesting greater sensitivity of this method (Fisher's exact test, P<0.05). However, $\log_2$ fold-change analysis showed more CSAPs enriched in the BPE (n=13) than in the TPE (n=3).

Significance: While BPE results in lower CSAP diversity, it selectively enriches for CSAPs that can be useful for downstream research in biocontrol and novel detection methods.

Award Application: Developing Scientist Award

P1-101: TRANSCRIPTIONAL PROFILE OF CRONOBACTER SAKAZAKII TO GROWTH PHASE AND NUTRIENT AVAILABILITY

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: *Cronobacter sakazakii,* an opportunistic foodborne pathogen that affects neonates and infants, has demonstrated persistence under conditions that restrict growth and metabolic activity. Factors such as nutrient availability and bacterial growth phase are key determinants of physiology and environmental stress tolerance, yet remain underexplored for this microorganism.

Purpose: Characterize growth phase transcriptomic responses of *Cronobacter sakazakii* across media with differing nutrient content and strains.

Methods: Growth curves for three *C. sakazakii* strains (ATCC 12868 and two clinical isolates) were generated in tryptic soy broth (TSB) (30 g/L) and low-concentration TSB (0.3 g/L). Cells were sampled at log (3h) and stationary phase (24h), and total RNA extraction was performed using TriReagent. RNA quality and concentration were assessed prior to library preparation. Ribosomal RNA was depleted, and RNA-seq libraries were constructed and sequenced on an Illumina NovaSeq platform. Raw sequencing reads were mapped with STAR 2.7.11, and differential gene expression analysis was performed with DESeq2.

Results: Transcriptional analysis revealed distinct growth-phase-dependent gene expression profiles among differing nutrient conditions and strains. Log-phase cells had higher transcript levels of genes involved in translation, energy metabolism, and cell division, whereas stationary-phase cells showed increased expression of genes associated with stress response and nutrient acquisition pathways ($\log_2$FC 1; FDR < 0.05). There were also transcriptional differences among strains under the same growth conditions ($\log_2$FC 1; FDR <0.05).

Significance: Transcriptomic profiling provides insight into bacterial physiological states that may support persistence and survivability in low-moisture food-processing environments and informs mitigation strategies of *C. sakazakii* in food systems.

Award Application: Developing Scientist Award

P1-102: SURVIVING THE ELEMENTS: HEAVY METAL TOLERANCE WITHIN REPEXH02 E. COLI O157:H7

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Recent analysis of genomic and epidemiological data has shown that a specific subtype of *E. coli* O157:H7 has been associated with illnesses since 2016. The CDC has designated this subtype (REPEXH02) as one that is reoccurring, emerging, or persistent. Available data appear to indicate the potential for environmental reservoirs of this subtype, as it has so far only been found in a limited geographic area. Comparative genomics has identified genetic differences among isolates within the REPEXH02 group, including the presence of a mutation in the arsenic tolerance operon repressor.

Purpose: To quantify arsenic tolerance among REPEXH02 isolates and link tolerance to genomic differences.

Methods: A set of 31 REPEXH02 isolates were grown in LB broth and exposed to sodium arsenate at concentrations ranging from 13.3 PPM to .0259 PPM. A set of 5 unrelated REPEXH01 strains were used for comparison. Minimum inhibitory concentration (MIC) was determined based on the minimum concentration of arsenate that resulted in OD600 < 0.1 after 24 h at 37C. To identify genetic differences among the isolates, the core genome was determined using Roary (v3.13.0) and the core genome phylogeny was constructed using

RAxML-NG (v1.2.0).

Results: MICs for arsenate varied among strains, with the REPEXH01 isolates having the lowest at 1.7 ppm. REPEXH02 isolates had MICs ranging from 3.3 to 6.6 ppm, and those with the highest MIC were environmental isolates that contained the arsR nonsense mutation. The presence of the arsR mutation varied among REPEXH02 isolates with MIC of 3.3 ppm, indicating that other genomic differences may contribute to arsenic tolerance.

Significance: REPEXH02 isolates do have higher tolerance to arsenic compared to *E. coli* O157:H7 from other genetic subtypes. This may contribute to persistence of REPEXH02 in the environment, specifically in areas with higher levels of arsenic.

P1-103: EXPRESSION OF VIRULENCE GENES IN LISTERIA MONOCYTOGENES SHIFTS WITH SUB-LETHAL ELECTRON BEAM EXPOSURE

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Electron beam (eBeam) treatment is used in the food industry for shelf-life extension and is limited to doses of up to 1 kGy. Products that have received phytosanitary doses may harbor pathogens that have received a sub-lethal ionizing treatment dose. Sub-lethally exposed *Listeria monocytogenes* may undergo changes related to stress response and virulence.

Purpose: To determine whether sub-lethal X-ray and eBeam exposure changes the expression of genes related to virulence and stress response in *Listeria monocytogenes*.

Methods: Three strains of *L. monocytogenes* were grown to 10^8 CFU and suspended in PBS buffer. The cultures were exposed to a sub-lethal eBeam dose of 1 kGy. Real-time quantitative PCR was used to evaluate the relative expression of genes related to virulence (*hly*, *plcA*, and *iap*) and a gene related to stress response (*sigB*). The control sample (no treatment) and treated sample (1 kGy eBeam) were evaluated using SYBR® Green methods. The housekeeping gene 16S was used to calculate relative gene expression.

Results: Relative gene expression was calculated using the □□Ct method, using the Ct values

from the control and treated samples, along with the target and housekeeping genes. Relative expression analysis revealed significant changes in genes associated with stress response and virulence following sub-lethal ionizing treatment doses. Immediately after exposure, all four target genes were significantly overexpressed in all three strains of *L. monocytogenes.* Significant differences were also observed among the three *Listeria monocytogenes* strains selected for the study.

Significance: Elucidating molecular response to sub-lethal X-ray and eBeam doses will help us understand the stress response and behavior of *Listeria monocytogenes*.

Award Application: Developing Scientist Award

P1-104: HOW RIBOSOME ENGINEERING ENHANCES THE PRODUCTION OF PAENIBACILLIN, A PROMISING ANTIMICROBIAL ADDITIVE: INSIGHTS VIA COMPARATIVE GENOMICS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Paenibacillin is a natural antimicrobial peptide produced by *Paenibacillus polymyxa* OSY-DF (OSY-DF), but low yield in the wild-type strain limits its industrial applications.

Purpose: A prior spontaneous mutant selected through ribosome engineering, *P. polymyxa* OSY-EC (OSY-EC), exhibited higher titers of paenibacillin and restricted growth compared to the wild-type strain, but the genetic bases for these differences have not been fully deciphered.

Methods: The mutant OSY-EC was isolated after sequential gentamicin (2.5 μg/ml) and rifampicin (5 μg/ml) treatment of the wild-type OSY-DF. Genomes were annotated via Prokka 1.15.6 and NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Pangenome analysis (Panaroo 1.5.2) and whole-genome alignment (MUMmer 4.0.1) identified gene presence-absence variations, structural variants (SVs), and single nucleotide polymorphisms (SNPs). *Kyoto Encyclopedia of Genes and Genomes* (KEGG) enrichment analysis was used to determine functional implications of identified mutations.

Results: Pangenome analysis revealed an identical gene set of 5,010 genes in OSY-DF and OSY-EC. One SV was identified in OSY-EC, which leads to a 99-amino acid insertion in an S-layer homology domain protein. A total of 572 SNPs were identified across 44 genomic loci (rRNA/tRNA hubs and protein-coding regions), with dense clusters in 16S rRNA (9), 23S rRNA (13), and tRNAs (9 Ala, 2 Lys, 2 Cys, 2 Leu). Mutated protein-coding genes were enriched in three pathways: metabolism of terpenoids and polyketides (p=7.7×10^{-9}), nitrogen cycle (p=0.027), and nitrogen metabolism (p=0.032). In summary, ribosome engineering systematically reprograms the cell by remodeling translational apparatus and biosynthetic pathways. These findings may be responsible for higher yields of paenibacillin and limited growth of OSY-EC.

Significance: This study provides a blueprint for applying ribosome engineering to enhance natural antimicrobials production.

Award Application: Developing Scientist Award

P1-105: GENOMIC AND PHENOTYPIC CHARACTERIZATION OF NOVEL LACTIC ACID BACTERIA FOR CONSORTIUM-BASED KIMCHI FERMENTATION

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Kimchi is a traditional Korean food with high nutritional and microbiological value, produced through native fermentation; however, controlled fermentation of kimchi improves consistency and sensory quality. This study aims to establish a novel microbial consortium for the controlled fermentation of kimchi and to compare the genomic and phenotypic characteristics of lactic acid bacteria (LAB) during fermentation using native and controlled methods.

Methods: AVITI shotgun metagenomic sequencing was performed to profile the microbial communities of 5 naturally fermented and commercial kimchi and sauerkraut products. In parallel, 9 LAB isolates were recovered, and whole-genome shotgun sequencing was performed to determine their taxonomic profiles and functional characteristics. A consortium comprising the 9 isolates was used to produce lab-made kimchi, and comparative metagenomic and phenotypic analyses were conducted throughout the fermentation process to track bacterial growth, pH, lactic acid, and sugar consumption under both controlled and native fermentation conditions.

Results: Metagenomics of commercial kimchi revealed a high abundance of LAB and a high number of carbohydrate transport and metabolism genes (~200), consistent with the lab-

made consortium. Additionally, metabolic pathway mapping of the consortium suggested synergistic cross-feeding interactions among the different LAB. During kimchi fermentation, bacterial populations reached ~8 log CFU/g in controlled kimchi vs ~2 logCFU/g in natively fermented kimchi. Moreover, controlled kimchi showed a faster drop in pH, higher lactic acid concentrations (12-fold increase), and faster sugar consumption than natively fermented kimchi.

Significance: This work highlights the importance of rationally combining LAB to synergize beneficial outcomes during kimchi fermentation. It also helps develop novel starter cultures, thereby advancing controlled fermentation to improve consistency and functionality.

Award Application: Developing Scientist Award

P1-106: NANOMATERIAL INTERACTIONS WITH AGRI-FOOD MICROBIOME: IMPACTS ON MICROBIAL COMMUNITIES AND PATHOGEN DYNAMICS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: The agri-food microbiome plays a vital role in nutrient cycling, nitrogen fixation, and overall soil health, but its integrity is increasingly challenged by emerging environmental stressors such as nanomaterials (NMs). The widespread use of NMs in agriculture, in addition to their mobility and persistence in soil, raises concerns about potential unintended impacts on microbial communities and pathogen dynamics.

Purpose: This study investigates how commonly used metallic NMs influence microbial community composition and persistence of pathogens in agri-food environments.

Methods: Seven metallic NMs commonly used in agrochemicals, i.e., Ag, CuO, CeO_2, Fe_2O_3, MgO, TiO_2, and ZnO were applied to soil collected from a site classified under the Apopka soil series. Eight replicate pots per treatment, including an untreated control, were maintained under greenhouse conditions for 12 weeks with periodic irrigation to simulate rainfall. Soil samples were collected cross sectionally at two depths, at time points: zero, 4 weeks, 8 weeks, and 12 weeks. Aerobic plate counts (APC) were used to access bacterial responses to NM exposure and migration over time. Samples will undergo marker gene, multi-omics, and

machine-learning analyses to evaluate the broader effects on native microflora and foodborne pathogens.

Results: Silver (Ag) NMs caused a pronounced decline in bacterial abundance at both depths, with significant reductions in the middle ($p < 9.31 \times 10^{-10}$) and bottom ($p < 0.000128$) layers relative to controls. Within the Ag treatment, no significant temporal variation in bacterial counts was observed at either depth (middle: $p = 0.39$; bottom: $p = 0.508$; 95% confidence intervals).

Significance: These findings highlight the potential for Ag NMs to alter microbial populations and influence pathogen persistence, providing insights into their environmental safety, food security implications, and responsible end-of-life management.

P1-107: MICROBIOTA MODULATION IN CHICKENS REDUCES SALMONELLA COLONIZATION AND PROMOTES BENEFICIAL HOST CECAL GENE EXPRESSION

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Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Early-life exposure of chicks to commensal microbiota can reduce *Salmonella* colonization through competitive exclusion, but undefined microbial products raise concerns for consistency and regulatory approval.

Purpose: This study compared the anti-*Salmonella* effects of mature-bird cecal contents (CCG) and a defined 15-species bacterial consortium (DCG) to a sterile control (CON) and examined associated host transcriptional responses.

Methods: One-day-old White Leghorn chicks were orally gavaged with CCG, DCG, or CON. After one week, 12 birds were sampled prior to challenge, while remaining birds were orally inoculated with *Salmonella* enterica serovar Heidelberg (SH2813) and sampled (12 birds per group per timepoint) at 3-, 14-, and 28-days post-inoculation (DPI). Cecal contents were used to quantify *Salmonella* and characterize microbiota composition, and cecal tissues were analyzed by RNA sequencing.

Results: *Salmonella* was below 10 CFU/g at all timepoints in CCG birds, while DCG birds exhibited a 2-$\log_{10}$ reduction compared to CON at 28 DPI (adjusted $p < 0.05$). CCG microbiota displayed greater diversity across all timepoints, whereas DCG communities showed transient reductions in diversity that partially recovered by 28 DPI. Both treatments altered host gene expression, including genes involved in intestinal transport, barrier function, and immune regulation, with CCG showing broader immune-related responses at later timepoints and DCG affecting transporter-related pathways prior to challenge.

Significance: These results demonstrate that both complex and defined microbial communities can reduce the duration and density of *Salmonella* colonization, while eliciting distinct host responses. The findings support development of targeted, microbiota-based strategies to improve poultry health and food safety.

P1-108: METAGENOMICS-DRIVEN ISOLATION OF SALMONELLA IN FOOD

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Polycontamination of food, defined as the presence of multiple hazardous microbes in food, can confound a food safety outbreak investigation. A metagenomics-informed recovery of a microbial contaminant in food could allow the targeted isolation of a pathogen to match the cause a foodborne clinical case.

Methods: A bacterial suspension of three *Salmonella* serovars, namely Enteritidis, Typhimurium and Heidelberg (approximately 200 colony forming units per serovar) was applied to chicken breast (50 g). Spiked chicken was kept at 4°C for 72 h, placed in 450 ml of Buffered Peptone Water (BPW) and subjected to stomaching for 1 min at 125 rpm followed by an overnight incubation at 37°C. Recovered BPW was split equally into two for (a) metagenomic sequencing and (b) isolation of colonies using a standard *Salmonella* recovery protocol. DNA extract from contaminated BPW was subjected to genomic amplification followed by nanopore sequencing for 24 h (GridION, Oxford Nanopore Technologies). Raw sequence reads were analyzed on the CZID bioinformatic platform (https://czid.org). For *Salmonella* recovery, samples were cultured in RSV and TBS selective media before being plated on Bis and XLT-4 agar plates. Ten colonies were picked from each plate and analyzed using a multiplex PCR able to identify the three serovars.

Results: Bioinformatic analysis revealed the presence of multiple bacteria in the chicken samples: *Salmonella*, *Hafnia*, *Escherichia coli*, *Morganella*, *Aeromonas*, *Pseudomonas*, *Serratia* and *Proteus*. Sequences of the three *Salmonella* serovars, namely Enteritidis, Typhimurium, and Heidelberg were detected (breadth coverage > 10%; depth coverage 1x). Colonies of all three serovars were recovered as confirmed by a multiplex PCR assay ($mPCR_{SE+ST+SH+STh+SInf}$).

Significance: Metagenomic analysis reliably demonstrated the presence of multiple bacterial contaminants in food allowing prompt mitigation efforts to protect consumers from foodborne illnesses.

Award Application: Developing Scientist Award

P1-109: COMPREHENSIVE SHOTGUN METAGENOMIC ANALYSIS OF BISON CARCASSES PRE- AND POST-ANTIMICROBIAL TREATMENT AND CORRESPONDING FECAL MICROBIOMES

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Demand for lean, high-protein bison meat is increasing, yet data on bison carcass microbial ecology and antimicrobial interventions remain limited. Shotgun metagenomic sequencing allows culture-independent analysis of microbial communities and functions.

Purpose: To characterize the microbial composition and functional profiles of bison carcasses and fecal samples using shotgun metagenomic sequencing.

Methodology: Bison carcass samples, pre- and post-antimicrobial treatment (n=6), and fecal samples (n=3) and experimental replicates (n=3) were collected from bison at a commercial processing facility. The antimicrobial treatment involved steam and lactic acid spray (2.5-3%). Genomic DNA was extracted from carcass wash suspension and fecal samples. Shotgun metagenomic sequencing was performed using Illumina NovaSeq 6000 platform. Raw sequence quality was assessed using FastQC and clean reads were assembled using

MEGAHIT. Antibiotic profiling was conducted by aligning sequences to the Comprehensive Antibiotic Resistance Database (CARD).

Results: Shotgun metagenomic analysis revealed distinct microbial communities in carcass and fecal samples. *Lactiplantibacillus plantarum* was the most abundant species in the bison carcass microbiome, whereas uncultured *Methanobrevibacter* species dominated the fecal microbiome. Principal Component Analysis showed that carcass microbiomes clustered closely regardless of antimicrobial treatment, while fecal microbiomes exhibited high diversity and distinct community composition. Antibiotic resistance genes (ARGs) were not detected in carcass samples; however, 21 ARGs were identified in the fecal microbiomes, with vancomycin resistance gene cluster being dominant.

Significance: This culture-independent shotgun metagenomic approach enables comprehensive assessment of microbial communities across different processing stages and sample types, providing valuable insights into the microbial ecology of processed bison.

Award Application: Developing Scientist Award

P1-110: EFFECTS OF ADAPTIVE MULTI-PADDOCK GRAZING ON SOIL MICROBIOME COMPOSITION AND FUNCTION IN PECAN ORCHARDS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Adaptive multi-paddock (AMP) grazing, a major regenerative agriculture practice, has received increasing attention in U.S. tree nut production due to its potential benefits for productivity, soil health, and environmental sustainability. Soil microbiomes may play a significant role in these potential benefits.

Purpose: The study aimed to evaluate the effects of conventional and AMP grazing on soil microbiome in pecan orchards.

Methods: A total of twenty soil samples (eight from conventional and twelve from AMP grazed orchards) were collected. Genomic DNA was extracted using PowerSoil Pro Kit. Amplicon sequencing (bacterial 16S rRNA gene and the fungal ITS region) was performed, along with shotgun metagenomic sequencing, using Illumina NovaSeq 6000 platform. Amplicon sequences were taxonomically classified using the Silva database for bacteria and the UNITE database for fungi. Metagenome clean reads were assembled using MEGAHIT and functional annotation was conducted using the KEGG database.

Results: Actinobacteriota was identified as the most dominant phylum in both conventional and AMP grazed soils, followed by Proteobacteria, and Acidobacteriota. Alpha diversity analyses revealed higher bacterial and total microbiome richness under AMP grazing, while fungal richness showed no consistent differences between grazing systems. Non-metric Multidimensional Scaling (NMDS) analyses indicated reduced heterogeneity and tighter clustering in bacterial and total microbiome communities among soil samples under AMP grazing compared to conventional grazing. In contrast, fungal communities remained highly diverse across both grazing systems. Functional annotation demonstrated greater metabolic potential in AMP-grazed soils.

Significance: These findings provide comparative evidence that AMP grazing alters soil microbiome composition and functional potential in pecan orchards, enhancing our understanding of regenerative grazing practice in perennial agroecosystems.

Award Application: Developing Scientist Award

P1-111: METAGENOMIC COMPARISON OF SOIL ANTIBIOTIC RESISTOMES UNDER CONVENTIONAL AND ADAPTIVE MULTI-PADDOCK GRAZING IN PECAN ORCHARDS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Antibiotic resistance is widely recognized as a critical One Health challenge, with soil serving as a major reservoir of antibiotic resistance genes (ARGs). Adaptive multi-paddock (AMP) grazing, a core regenerative agriculture practice, has been shown to improve soil health and ecosystem functioning. However, its influence on the diversity and composition of soil antibiotic resistomes remains poorly understood.

Purpose: This study aimed to compare the distribution and composition of soil antibiotic resistomes in pecan orchards managed under conventional and AMP grazing systems.

Methods: A total of twenty soil samples were collected, including eight from conventionally grazed orchards and twelve AMP-grazed orchards. Soil genomic DNA was extracted using DNeasy PowerSoil Pro Kit. Shotgun metagenomics sequencing was performed on NovaSeq 6000 platform. Metagenome clean reads were assembled using MEGAHIT v1.2.9 with default parameters. Antibiotic resistome profiling was conducted using the Comprehensive Antibiotic Resistance Database (CARD)

Results: AMP-grazed soils exhibited a greater number of detected ARGs compared to conventionally grazed soils. At the sample level, conventionally grazed soils contained 14–17 ARGs per sample, whereas AMP-grazed soils contained 17–19 ARGs per sample. Vancomycin resistance gene clusters (*vanA*, *vanB*, *vanG*, *vanL*, *vanM* and *vanO*) were the dominant ARGs in soils from both grazing systems, followed by oxytetracycline (*otrA*), and and streptomycin (*rpsL*). Several low-abundance resistance genes, including *HelR*, *qacG*, *FosI*, and *rsmA*, were detected ($p>0.05$) exclusively in conventionally grazed soils, whereas *murA* ($p>0.05$), *rox* ($p<0.05$) and *tet(45)* ($p>0.05$) were detected only in AMP-grazed soils.

Significance: These findings demonstrate that grazing management practices are associated with variation in the distribution and composition of soil antibiotic resistomes in pecan orchard agroecosystems.

Award Application: Developing Scientist Award

P1-112: ALTERED GUT MICROBIOME STRUCTURE ASSOCIATED WITH SALMONELLA ENTERICA COLONIZATION IN COWS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Interactions between gut microbial communities and enteric pathogens play a critical role in animal health and the emergence of zoonotic risks in dairy production systems.

Purpose: This study aimed to investigate associations between cow gut microbiome, host health status, and the genomic characteristics of colonizing *Salmonella enterica*.

Methods: Fecal samples were collected from 155 dairy cows and classified based on veterinary diagnoses recorded on the day of sampling and three days later. Cows that remained clinically healthy on both days were classified as healthy (n = 33), while cows diagnosed with metabolic or infectious conditions (including diarrhea, mastitis, metritis, or ketosis) or referred for hospital evaluation on either day were classified as sick. *Salmonella* was isolated from fecal samples. Disease-associated microbiome profiles were characterized using 16S rRNA gene sequencing, and whole-genome sequencing was performed on *Salmonella* isolates to assess serovar distribution, virulence factors, and antimicrobial-resistance genes.

Results: A total of 84 *S. enterica* isolates representing ten serovars were identified, with Montevideo, Senftenberg, Anatum, and Agona being most prevalent. Metritis cases were exclusively associated with Montevideo isolates, whereas mastitis cases were linked to multiple serovars. Distinct virulence and antimicrobial-resistance profiles were identified across serovars and host health conditions, including variation in fimbrial adhesion-associated genes. Notably, Anatum and unclassified serovars were more frequently recovered from healthy cows and carried fewer antimicrobial resistance genes.

Significance: These findings suggest that differences in gut microbiome may modulate *Salmonella* population structure and genomic traits in dairy cows. Understanding these microbial and pathogen interactions improves insight into disease risk and zoonotic potential within dairy production systems.

P1-113: METAGENOMIC ANALYSIS REVEALS BSAAO-DRIVEN SOIL MICROBIOME SHIFTS RESULTING IN EXTENDED ESCHERICHIA COLI PERSISTENCE

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Biological soil amendments of animal origin (BSAAOs) are widely used to improve soil fertility but may promote pathogen persistence.

Purpose: This study examined how BSAAO treatments influence soil microbiota and the persistence of a surrogate pathogen, *Escherichia coli*.

Methods: A 140-day longitudinal metagenomic study was conducted in agricultural soils from Florida and Georgia under four treatments: uninoculated soil, unamended control, composted poultry litter, and heat-treated poultry pellets. Soil microbial communities were

profiled using 16S rRNA gene amplicon sequencing and shotgun metagenomics. While 16S sequencing captured overall community structure, shotgun metagenomics enabled species-level taxonomic resolution, functional pathway and resistome characterization, and inference of microbial interaction networks. Machine learning integrated metagenomic and environmental variables to identify microbial shifts and predictors of pathogen persistence ($p < 0.05$).

Results: Soil microbiome diversity and composition were strongly influenced by geographic location and BSAAO treatment. Shotgun metagenomics revealed treatment-specific restructuring of microbial interaction networks and functional capacities not fully resolved by 16S-based analyses. BSAAO application significantly altered microbial composition and function, coinciding with prolonged *E. coli* survival. Environmental factors, particularly soil moisture, further modulated microbial dynamics and pathogen persistence. Species-level analyses identified multiple *Bradyrhizobium* species associated with reduced *E. coli* survival, whereas *Anaeromyxobacter oryzae* was linked to enhanced persistence. Functional profiling showed enrichment of pathways such as hexitol fermentation in BSAAO-amended soils.

Significance: These findings provide new insight into soil microbial interactions, highlighting microbiome shifts associated with BSAAO application and extended pathogen persistence.

P1-114: IMPACT OF FRUIT ADDITION ON MICROBIAL COMMUNITIES AND METABOLITE PROFILES OF GINGER BEER, A TRADITIONAL BEVERAGE DERIVED FROM GINGER ROOTS

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Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Ginger beer is a fermented beverage produced through the activation of ginger autochthonous microbial communities. Incorporation of fruits into ginger beer may support the development of functional plant-based beverages. Mangaba (*Hancornia speciosa*) and cajarana (*Spondias dulcis*) are underexploited Brazilian fruits; impacts of these fruits on ginger beer remain unknown.

Purpose: To assess the effects of incorporating mangaba and cajarana on microbial communities and phenolics in ginger beer.

Methods: Fruit-enriched ginger beers were produced with ginger (4% w/v), sugar (15% w/v),

lemon juice (1% v/v), cajarana (15% w/v) or mangaba pulp (15% w/v), ginger bug starter (25% v/v), and sterile water. Fermentation was conducted at 25°C for 7 days. Ginger beer without fruit was used as a control. Phenolics were quantified using HPLC/DAD. Bacterial and fungal communities were analyzed by sequencing the 16S rRNA and ITS regions, respectively, on an Illumina MiSeq platform. Statistics considered $p<0.05$

Results: Fruit incorporation did not alter Chao1, Shannon, or InvSimpson indices. Control exhibited distinct bacterial and fungal profiles compared with fruit ginger beers, which did not differ significantly from each other. The core microbial community across all formulations was dominated by *Enterobacter*, *Pectobacterium*, and *Pseudomonas*. *Candida* characterized the control, *Hanseniaspora* distinguished cajarana ginger beer, and *Pichia* the mangaba ginger beer. Fruits increased phenolics such as naringin, quercetin, rutin, trans-resveratrol, and epigallocatechin gallate. Mangaba ginger beer showed the highest concentrations of chlorogenic acid, rutin, naringin, and trans-resveratrol.

Significance: Findings indicate that fruit addition preserves microbial community structure while enhancing phenolic content in ginger beer, particularly with mangaba. These results support the development of plant-based fermented beverages using underexploited fruits.

P1-115: DEFINING MICROBIAL COMMUNITIES AND PREDICTING SALMONELLA SEROTYPES IN GROUND MELON SEEDS (EGUSI) USING SHOTGUN METAGENOMICS

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Shotgun metagenomics is an approach that can be used to screen foods for pathogenic bacteria as well as detecting the presence of various *Salmonella* serotypes.

Purpose: This study used shotgun metagenomics to examine the complex microbiota of ground melon seeds and the presence of *Salmonella* serotypes in both the universal pre-enrichment broths (UPB) as well as in the selective enrichment broths (SX2).

Methods: One sample of ground melon seeds consisting of 30 subunits was analyzed by creating 6 composites. Each composite was diluted 1:10 in UPB and incubated at 35°C. After 24 hours. 0.1mL UPB was added to SX2 broth and incubated at 42°C for 24 hours. 1mL aliquots were taken from enriched UPB and SX2 broths for DNA extractions using the Zymobiomics™ DNA Miniprep kit. Shotgun metagenomics was performed on DNA extractions using the Illumina DNA Prep Kit for library preparation. Libraries were sequenced on the Illumina MiSeq using V3 chemistry. FASTQ data files were transferred to GalaxyTrakr for analysis. Species level taxonomy were described using metaphlan and the predicted *Salmonella* serotypes were analyzed using bettercallsal.

Results: Shotgun sequencing identified many different taxa from UPB and SX2 broths. The most abundant bacterial species in both enrichments was found to be *Klebsiella pneumoniae* ranging from 27.5-56.6% in UPB and 26-65.2% in SX2 broth. *Cronobacter sakazakii* was the second most common ranging from 5.1-10% in UPB and 5.5-15.2% in SX2. *Salmonella enterica* ranged from 0-3.3% in UPB and 4.5-8.5% in SX2. Four different *Salmonella* serotypes were predicted in the UPB broth and an additional eight in SX2 broths.

Significance: This study demonstrates that shotgun metagenomic sequencing can be used to provide useful information about the microbiome composition and diversity within pre-enrichment and selective enrichment broths used in food analysis. *Salmonella* serotypes can be predicted using the bioinformatic tool bettercallsal.

P1-116: ASSESSING THE EFFICACY OF POST-HARVEST SANITIZATION METHODS AND REFRIGERATED STORAGE ON ROMAINE LETTUCE MICROBIOME USING 16S RRNA SEQUENCING

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Presentation type/Format: Poster

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Many leafy greens undergo a sanitization wash post-harvest to reduce microbial loads of spoilage organisms and foodborne pathogens. While the efficacy of chlorinated wash is widely accepted, the specific impacts of this wash on bacterial community have not been fully explored.

Purpose: Evaluate the shifts in bacterial communities including the abundance of spoilage organisms on romaine lettuce following a chlorine wash and after one week of refrigerated storage.

Methods: Five samples of field-picked whole romaine heads, romaine hearts and post-chlorinated water (200 ppm) wash processed chopped romaine samples were collected from Yuma, AZ. Leaf samples (25 g) were pummeled using a stomacher on high speed in a 1:10 dilution of phosphate buffered saline. The supernatant was centrifuged to pelletize microbial cells and prepared for 16S rRNA sequencing. Sequence data were processed and analyzed

using R (RStudio).

Results: There was a significant reduction (p<0.05) in amplicon sequence variants richness and evenness in washed (chopped) romaine lettuce as compared to the unwashed (heart) and outer leaf (whole romaine head). In addition, beta diversity showed a significant shift (p.adj<0.05) in community composition for washed romaine samples after one week storage. Each sample type exhibited distinct microbial communities (p.adj<0.05). However, only washed romaine showed a significant change (p.adj<0.05) in composition from Day 0 to Day 7. ANCOM-BC analysis associated *Actinobacteriota* and *Proteobacteria* with significant increase (p.adj<0.05) in phylum-level shifts relative to Day 0 washed romaine. Genus *Pseudomonas* exhibited significant increased abundance for all sample types.

Significance: Overall, results indicate that bacterial community differs between unwashed romaine hearts before and after storage, with genus *Pseudomonas* showing a significant increase in abundance between Day 0 and Day 7 washed romaine, which could alter the shelf life of the product.

P1-117: MICROBIOTA-COMPATIBLE INDUSTRIAL APPLICATION OF A LISTERIA-TARGETING BACTERIOPHAGE IN BABY SPINACH

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Bacteriophages are increasingly proposed as targeted interventions to control foodborne pathogens in fresh produce. However, their impact on the native microbiota of leafy greens under industrial processing conditions remains poorly understood

Purpose: This study evaluated the effect of a *Listeria*-specific bacteriophage cocktail applied at industrial scale during commercial processing of baby spinach on the native bacterial microbiota during storage.

Methods: Twenty independent industrial processing trials were conducted over one year in a commercial baby spinach facility. A *Listeria*-targeting bacteriophage cocktail was applied

during processing. Bacterial communities were analyzed using 16S rRNA gene sequencing immediately after processing (day 0) and after 10 days of storage under commercial (4°C followed by 7°C) and abusive (10°C) temperature conditions. Microbial diversity and community composition were compared between treated and untreated samples.

Results: Freshly processed spinach showed diverse bacterial communities dominated by Proteobacteria and Firmicutes, with *Pseudomonas*, *Bacillus*, *Exiguobacterium*, *Pantoea*, and *Acinetobacter* as core genera. Phage application did not significantly affect microbial richness, diversity, or overall community structure. In contrast, storage time and temperature strongly influenced microbiota dynamics. After 10 days, samples showed reduced diversity and increased relative abundance of *Pseudomonas* and *Flavobacterium*, with decreases in *Bacillus* and *Exiguobacterium*. Community clustering was driven mainly by storage conditions rather than phage treatment.

Significance: Industrial application of a *Listeria*-targeting bacteriophage cocktail did not disrupt the native microbiota of baby spinach, supporting its use as a microbiota-compatible food safety intervention.

P1-118: PRECISION HARPOON OR WIDER NET? ASSESSING TARGETED METAGENOMIC ENRICHMENT FOR ROBUST SPECIES-LEVEL IDENTIFICATION OF ARTHROPOD CONTAMINANTS

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Presentation type/Format: Technical

Category: Molecular Analytics, Genomics and Microbiome

Abstract:

Introduction: Traditional microscopy for identifying arthropod contaminants in food is labor-intensive and often fails to provide species-level resolution for highly fragmented specimens.

Purpose: To evaluate whether expanding a custom mitochondrial DNA hybridization capture panel improves the detection and resolution of regulatory-relevant arthropod pests in a food background.

Methods: Eight mock communities containing 23 insect taxa (7 orders) were prepared with a wheat flour genomic DNA background (food mass 4X total insect mass). Metagenomic libraries were prepared using the KAPA HyperPlus kit with dual-indexed adapters. We compared shotgun sequencing against targeted capture using two arthropod biotinylated

RNA bait panels: v1 (1,988 species, 280K probes) and v2 (5,522 species, 480K probes). Taxonomic assignment was performed via the mitoK-mer pipeline, using a hierarchical structure to classify reads based on diagnostic k-mer matches.

Results: Targeted capture shifted all libraries from background-heavy profiles to insect-dominated ones, with the 23 target species increasing from an average of 66.9% of reads in shotgun libraries to 98.6% in both bait versions. Both panels achieved a 24-fold reduction in non-target signal, effectively suppressing the wheat flour background. While bulk enrichment was equivalent, the v2 panel increased mitogenome redundancy and filled crucial representation gaps for stored products and nuisance pest species, improving the workflow’s robustness for a variety of surveillance scenarios. All 23 targets were correctly identified to the species level, including closely related stored-product beetles.

Significance: While v1 serves as a precision harpoon for known targets, v2’s wider net and finer mesh are prioritized for real-world surveillance where contaminant identity is unknown. This increased robustness and taxonomic breadth offset the higher cost, offering a scalable framework for evidence-based decisions.

P1-119: EFFECT OF PHOSPHINE TREATMENT AND INTERSTITIAL CARBON DIOXIDE ON DEOXYNIVALENOL PRODUCTION IN WHEAT UNDER VARYING STORAGE CONDITIONS

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Presentation type/Format: Technical

Category: Food Toxicology

Abstract:

Introduction: Deoxynivalenol (DON) is a toxic *Fusarium* metabolite that can accumulate in cereals, causing economic losses and health risks. The role of interstitial CO_2 in DON production remains poorly understood, and the risk of DON accumulation after phosphine treatment remains unexplored.

Purpose: To determine how interstitial CO_2, grain moisture content, airtight duration, and *Fusarium* treatments affect DON production in phosphine-treated wheat.

Methods: Wheat (45 kg) was fumigated with phosphine (2 g/m^3), stored for 3 months, and then conditioned to 15, 17, 20, or 23% moisture content. Four *Fusarium* treatments were applied: naturally contaminated, *Fusarium*-inoculated, disinfected, or disinfected + *Fusarium*-inoculated, and samples were stored under airtight conditions for 1, 3, 6, or 12 days. A factorial design (4×4×4) with triplicates (n = 192; non-airtight controls n = 48) was used to evaluate the effects of different storage conditions (moisture content, airtight duration, and

Fusarium treatment) on CO_2 and DON production. Statistical analysis was performed using ANOVA and Tukey HSD. Interstitial CO_2 was measured by GC, DON concentrations were measured by HPLC every 12th day, and correlations between interstitial CO_2 and log_{10}-transformed DON were determined.

Results: Moisture content was the primary determinant of both interstitial CO_2 and DON levels. *Fusarium*-inoculated wheat consistently produced the highest DON concentrations. Interstitial CO_2 was strongly correlated with DON ($r = 0.87$, $p<0.001$), and samples with interstitial CO_2 <20% remained below DON safety thresholds. Germination was not adversely affected immediately after phosphine treatment but declined slightly with storage time.

Conclusion: Phosphine treatment does not completely prevent fungal growth in stored wheat. Interstitial CO_2 is a reliable indicator of DON risk in stored wheat, and maintaining grains' interstitial CO_2 <20% provides a practical target to minimize DON risk during storage.

Award Application: Developing Scientist Award

P1-120: BENCHMARKING THE INVISIBLE: A TIERED FRAMEWORK FOR DERIVING BENCHMARK DOSE FROM FRAGMENTED TOXICOLOGICAL DATA ON NANO- AND MICROPLASTICS

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Presentation type/Format: Technical

Category: Food Toxicology

Abstract:

Introduction: The widespread presence of nano- and microplastics (NMPs) has raised increasing concern regarding their potential neurotoxicity, yet quantitative dose–response

benchmarks suitable for human health risk assessment remain scarce.

Purpose: The objective of this study was to derive neurotoxicity focused benchmark dose (BMD) intervals from terrestrial mammalian oral exposure studies using a structured evidence synthesis and Bayesian modeling framework.

Methods: A two-phase approach was applied, beginning with a PRISMA-guided systematic review across seven databases, yielding 22 eligible terrestrial mammalian studies from 41,784 records; eligibility was informed by structured quality assurance/quality control (QA/QC) and risk-of-bias (RoB) assessments. A total of 134 neurotoxicity endpoints were modeled using Bayesian dose–response analysis with a 10% benchmark response, MCMC fitting (30,000 iterations, 65% burn-in), and posterior probability–weighted model averaging. Endpoints were screened using predefined interval-stability criteria (BMD/BMDL < 20; BMDU/BMDL < 50; BMD/lowest dose < 10) and prioritized by data density and exposure duration.

Results: Of 134 modeled neurotoxicity endpoints, 79 (59%) met interval-quality criteria, and 26 were prioritized as robust points of departure. Chronic polystyrene nano- and microplastics (PS-NMPs) yielded the most sensitive benchmarks, with prioritized BMDs of ~10^{-4}–10^{-3} mg·kg^{-1}·day^{-1} and BMDLs as low as 1.61×10^{-4} mg·kg^{-1}·day^{-1}. These low-dose benchmarks were associated with cognitive impairment, synaptic loss, neuroinflammation, and mitochondrial dysfunction. Other polymers (PP, PET, LDPE, PLA) showed higher and more uncertain BMD intervals due to limited and short-term data.

Significance: Neurotoxicity emerges as a sensitive and integrative endpoint for NMP hazard characterization, with BMD intervals providing an uncertainty-aware basis for quantitative risk assessment and regulatory prioritization.

Award Application: Developing Scientist Award

P1-121: ASSESSING BIOLOGICAL ENDPOINTS OF MICRO- AND NANOPLASTIC EXPOSURE RELEVANT TO FOOD SAFETY

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Presentation type/Format: Technical

Category: Food Toxicology

Abstract:

Introduction: Micro- and nanoplastic (MNP) are detected across the food chain with reported toxicological effects spanning all levels of biological organization. The full extent of MNP effects on human health remain unclear. The inherent heterogeneity of MNPs, combined with the high diversity of species, model systems and exposure scenarios used, complicates extrapolation of results, hampers cross-study comparisons and comprehensive risk assessment.

Purpose: This study aims to provide insights into MNP toxicity and facilitate identification of the most common molecular and cellular "hotspots" as a function of particle characteristics and species specificity.

Methods: Relevant publications on MNP effects were retrieved from six databases, using pre-defined Boolean search strings. The publications were screened for eligibility and quality based on pre-determined criteria. Following a risk-of-bias assessment and selection of publications, critical data elements, including experimental model characteristics, target organ information, detailed specifications of the intervention, were extracted in a systematic manner.

Results: The review will quantitatively synthesize reported toxicity endpoints associated with microplastic exposure, including oxidative stress, inflammatory responses, membrane integrity disruption, and bioenergetic alterations. These endpoints will be categorized and mapped onto upstream molecular mechanisms and broader cellular pathways, such as apoptosis, autophagy, and immune activation. Additionally, findings will be integrated with existing data on microplastic abundance and heterogeneity in food sources to contextualize observed biological effects.

Significance: The resulting integrated dataset of molecular and cellular hotspots will serve as a foundation for future predictive hazard characterization and quantitative risk assessment

P1-122: MICROPLASTICS SUPPORT COLONIZATION AND ALTER ANTIMICROBIAL SUSCEPTIBILITY OF CAMPYLOBACTER JEJUNI

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Microplastics (MPs) are increasingly recognized as environmental reservoirs for microorganisms, yet their role in the ecology and antimicrobial behavior of *Campylobacter jejuni* remains poorly understood.

Purpose: This study examined the ability of *C. jejuni* to colonize MPs and evaluated the impact of MP-associated biofilms on antimicrobial susceptibility.

Methods: Five *C. jejuni* isolates from poultry, water, and human sources, together with reference strain NCTC 11168, were assessed for biofilm formation on MPs over 24–72 h using culture-based methods. Antimicrobial susceptibility of cells detached from MP biofilms was determined by Etest, and whole-genome sequencing was used to identify adherence, biofilm, and antimicrobial resistance determinants. Experiments were performed in triplicate and repeated in three independent runs.

Results: All strains rapidly colonized MPs within 24 h, with attachment levels ranging from 1.09 to 5.78 log CFU/MP. NCTC 11168 showed the strongest biofilm formation. The presence of *peb3* and specific gene combinations (*capA, capB, cj1725,porA*) was associated with

enhanced attachment. Antimicrobial resistance genotypes matched phenotypes; only one isolate (ST-6209/CC464) was resistant to ciprofloxacin, nalidixic acid, and tetracycline. Cells detached from MP biofilms showed increased antimicrobial susceptibility, with up to a 4.6 $\log_2$-fold reduction in MICs compared with planktonic cells.

Significance: MPs support *C. jejuni* colonization and persistence while altering antimicrobial susceptibility, highlighting their potential role as ecological modifiers relevant to food safety.

P1-123: RISK INTERPRETATION OF ARSENIC, LEAD, CADMIUM, AND MERCURY REPORTED IN U.S. BABY FOODS

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Presentation type/Format: Technical

Category: Food Toxicology

Abstract:

Introduction: Recent U.S. state regulations require that baby food manufacturers test their products for arsenic, lead, cadmium, and mercury, and publish these results on their websites. Although caregivers have access to these data, they likely lack the training necessary to interpret whether the reported concentrations are sufficient to pose a health risk if these products are consumed.

Purpose: The aim of this research is to use a standard health risk assessment approach to interpret these reported metals concentrations.

Methods: We reviewed manufacturers' websites (n=9), extracted reported testing results, and applied standard risk assessment methods to explore the potential for health risk following routine consumption, considering a range of product types and intakes per day. Estimated exposures were compared to health-based guidance values published by the US-FDA, U.S. State of California, US EPA, and EFSA.

Results: Of the 1,362 samples, some baby food products contained metals at concentrations below health benchmarks, indicating no anticipated risk even following daily consumption of 5 items. However, there were also baby food products that, under certain intake assumptions, resulted in heavy metal doses exceeding health benchmarks, including from

some products reported as having metals concentrations “below the limit of detection” (legally defined as up to 6 ppb).

Significance: Our study highlights the need for clearer risk communication as reporting metals concentrations alone is not sufficient for risk-based decision making. In some instances, an apparent health risk may reflect generous intake assumptions rather than unacceptable metals concentrations in the foods. To better inform decision making, caregivers should also be made aware of non-zero exposures from feasible alternatives. More robust market share and expected intake data are needed to make informed inferences about the likelihood of exposure and potential for population health risk.

P1-124: PRESSURE SENSITIVITY OF O157 AND NON-O157 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI AUGMENTED BY EUGENOL, ALLICIN, AND MILD HEAT

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Various O157 and non-O157 serogroups of Shiga toxin-producing *Escherichia coli* could cause rare but important health complications such as Hemolytic Uremic Syndrome. Application of high-pressure processing can be an important intervention to prevent, eliminate, and reduce the public health burden of this pathogen in the food chain.

Purpose: Our study investigated the impact of high-pressure processing with the addition of two bioactive compounds (Eugenol and Allicin) for the reduction of *Escherichia coli* O157: H7 and six non-O157 serogroups (O26, O45, O103, O111, O121, and O145).

Methods: Elevated hydrostatic pressures of 325 and 650 MPa were used at 4.4 and 60.0°C for inactivation of the pathogen. Studies were randomized block designs with two biologically independent replications (i.e., as blocks), each consisting of three replications. Temperature values of the treatments were precisely controlled by a circulating water bath connected to a stainless-steel jacket surrounding the high-pressure processing chamber. Results were

statistically analyzed using Tukey-adjusted ANOVA at the type I error level of 5%. Additionally linear and non-linear inactivation indices were calculated.

Results: Both antimicrobial compounds at 3% concentration were able to augment ($P < 0.05$) the efficacy of the 325 and 650 MPa treatments. Application of mild heat was additionally effective to enhance ($P < 0.05$) the decontamination efficacy of the treatment in the context of thermally assisted high-pressure processing. Similar trends were obtained after calculation of D-value (linear-regression based) and K_{max} (non-linear models).

Significance: Our results, thus, indicate that industrial application of high-pressure processing could be enhanced by benefiting from synergism with mild heat and/or novel bioactive compounds.

Award Application: Developing Scientist Award

P1-125: STRESS-INDUCED FORMATION OF CAMPYLOBACTER JEJUNI PERSISTERS IN AGRI-FOOD ENVIRONMENTS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Many foodborne pathogens, including *Campylobacter jejuni*, can enter a persister state in response to environmental stressors that promotes survival under harsh conditions.

Purpose: The objective of this study was to evaluate the impact of simulated agri-food systems and real food environments on persister formation in different *C. jejuni* strains.

Methods: *C. jejuni* persisters were induced in broth under simulated agri-food stress conditions (aerobic, osmotic, or cold stress) and compared with unstressed cultures. Biphasic time-kill curves for ciprofloxacin and ampicillin were created by plating on agar every hour and best-fit curves of the second phase were used to calculate initial persister fractions. Afterwards, persister formation was evaluated in milk and chicken juice subjected to the same stress conditions to assess the impact of food matrices on persister formation.

Results: All killing curves were plotted using two technical replicates from at least 2 biological replicates. Substantial strain-dependent variability was observed under unstressed conditions, with persister fractions ranging from over 10% to under 0.5% depending on the strain and antibiotic tested. Stress exposure generally increased persister fractions with strain F38011 under aerobic stress challenged with ciprofloxacin yielding 31.3% persisters

and strain 81–116 under osmotic stress challenged with ampicillin yielding 24.8% persisters. These findings suggest an interaction between antibiotic mechanism of action and persister induction conditions. In real food matrices, time-killing curves often lacked clearly defined biphasic kinetics but those observed generally yielded higher persister fractions than those observed in broth.

Significance: These findings demonstrate that agri-food environments can promote *C. jejuni* persister formation, potentially enhancing survival in food systems and persistence during transmission to humans.

Award Application: Developing Scientist Award

P1-126: PHYSIOLOGICAL CHARACTERIZATION OF VIABLE BUT NON-CULTURABLE SALMONELLA BY THE EXPOSURE OF SODIUM HYPOCHLORITE

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Salmonella* in fresh produce can still survive due to the ability to enter a viable but non-culturable (VBNC) state by the exposure of washing water containing chlorine.

Purpose: This study aimed to characterize the physiological properties of VBNC *Salmonella* induced by various concentrations of sodium hypochlorite.

Methods: The VBNC *S.* Enteritidis (SE) and *S.* Typhimurium (ST) was induced by the exposure to various concentrations of sodium hypochlorite. The culturability and viability of SE and ST were confirmed using TSA plate method and PMA-qPCR, respectively. The VBNC SE and ST were assessed using LIVE/DEAD BacLight kit. Their morphological changes, metabolic activity, and enzymatic profiles were analyzed by TEM, ATP luminescence (BacTiter-Glo assay), and API ZYM, respectively. Their resuscitation efficacy was evaluated in TSB with or without sodium pyruvate.

Results: VBNC induction in SE and ST was confirmed at 30 ppm sodium hypochlorite without

colony formation whereas viability of VBNC SE and ST were determined to be 8.4% and 5.3%, respectively. The cell size of SE and ST were reduced up to 47% and 51% and their ATP level was maintained 23% and 21%, respectively. Compared with the vegetative state, VBNC *Salmonella* exhibited reduced activity for most enzymes, with complete loss of α-galactosidase and α-glucosidase whereas acid phosphatase and naphthol-AS-BI-phosphohydrolase activities were sustained. The sodium pyruvate-meditated resuscitation was found to be the most efficient strategy to recover VBNC *Salmonella*.

Significance: This study elucidates the distinct physiological characteristics of chlorine-induced VBNC *Salmonella* and demonstrates their resuscitation potential, suggesting the development of culture-based detection strategies for VBNC *Salmonella* in food.

P1-127: HIGH-THROUGHPUT KINETIC PHENOTYPING OF MICROBES VIA AUTOMATED 3D DIGITAL CULTURING

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Conventional microbial culturing and phenotyping methods are inherently slow, manual, and labor-intensive processes that rely on subjective endpoint visual inspections, struggling to provide timely and reproducible results.

Purpose: This study demonstrates a novel method enabling parallel culturing and time-lapse imaging of 96 microbial samples.

Methods: A panel of eight *Salmonella* and eight non-*Salmonella* competitor strains was evaluated using a 3D sandwiched culturing format in 96-well plates containing selective XLT4 agar. Strains were inoculated at 5 log CFU, automatically serially diluted across six wells, and imaged hourly at 37°C for real-time kinetic analysis.

Results: Time-lapse imaging clearly captured a signature *Salmonella* metabolic profile: sequential xylose fermentation (pH drop) followed by lysine decarboxylation (pH rise), visible as a "hump" in the media hue plot that peaked around 6 hours for high-titer wells (5 log CFUs). Non-*Salmonella* strains, conversely, lacked this characteristic signature; their metabolic activities were either entirely inhibited (*Proteus mirabilis*, *E. coli*), remained acidic (*Citrobacter*

freundii, *Klebsiella* species), or showed delayed decarboxylation (*P. aeruginosa*, *Shigella sonnei).* This objective analysis proved crucial for accurate differentiation, allowing clear distinction between *S.* Choleraesuis (a H_2S- strain difficult to distinguish by endpoint inspection) and *P. aeruginosa*. Furthermore, dynamic monitoring of colony morphology revealed a critical timing difference: *Salmonella* colonies developed definitive black centers in 8 to 12 hours, significantly faster than *Citrobacter freundii* (>16 hours).

Significance: The digital culturing and phenotyping method provides a novel, objective, and high-throughput solution that significantly enhances the accuracy and speed of phenotypic research on microbes, accelerating critical food safety surveillance and public health interventions.

P1-128: TEMPERATURE EFFECTS ON THE PERSISTENCE OF AICHI VIRUS IN A HYDROPONIC NUTRIENT SOLUTION

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: With the growing demand for fresh produce and less cultivatable land, controlled environmental agriculture (CEA) using hydroponic systems is used. However, produce safety in CEA needs to be maintained to prevent outbreaks and recalls.

Purpose: This research aimed to determine the ability of Aichi virus (AiV) to persist in hydroponic nutrient solutions at varied temperatures (4, 15, 20, and 25℃) encountered during produce cultivation, transport and storage.

Methods: Commercial hydroponic solutions (1.8 mL, pH 5.8 or pH 7) in 2-mL tubes were mixed with 200-µL of AiV (~6.5 log PFU/mL) and stored for 21 days at 4, 15, 20, or 25℃. Infectivity of recovered AiV was determined using standard plaque assays. All experiments were replicated thrice. Data were statistically analyzed for differences over time and temperature, using ANOVA with Tukey's adjustments (JMP v17).

Results: AiV was found to retain infectivity after storage at all tested temperatures for 21 days, with highest persistence at 4°C in hydroponic nutrient solution at pH 5.8. The D-values were 323.94 ± 8.58 (4°C), 25.34±4.27 (15°C), 52.46±14.44 (25℃), and 15.38±1.61 (20°C) days. AiV showed an increased reduction of 1.51±0.27 log PFU at 20°C after 21 days at pH 5.8. The lower temperatures favored the persistence of AiV over higher temperatures at neutral pH 7.

AiV showed exceptional stability at all the tested temperatures (reductions ranged from 0.3 log to 1.51 log PFU) over the 21 day period.

Significance: The results provide the baseline information needed to determine the risk associated with cross-contamination of AiV in hydroponic nutrient solutions during harvest and post-harvest of produce. Thus, appropriate preventive/control strategies should be implemented to prevent viral contamination.

Award Application: Developing Scientist Award

P1-129: CHARACTERIZING THE EFFECTS OF SHORT-CHAIN FATTY ACIDS ON CAMPYLOBACTER JEJUNI VIRULENCE

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Campylobacter jejuni* is a leading cause of bacterial gastroenteritis worldwide, with diverse clinical presentations, including bloody/inflammatory or watery diarrhea. We investigated the effects of short-chain fatty acids (SCFAs) on the virulence and physiology of *C. jejuni* strains associated with different clinical diarrheal manifestations.

Purpose: SCFAs are key components of the intestinal tract; therefore, understanding their impact on *C. jejuni* pathogenicity is essential for elucidating host-pathogen interactions and potential therapeutic options.

Methods: Ten *C. jejuni* strains (five associated with watery diarrhea and five with bloody/inflammatory diarrhea) were used. Growth curve, invasion, and attachment assays with Caco-2 intestinal epithelial cells were conducted for all strains treated with no SCFAs, 0.03 mM, or 3 mM sodium butyrate, sodium acetate, or sodium propionate to assess the effects of different SCFAs and strain variation. All assays were performed in triplicate for each strain and SCFA concentration.

Results: SCFAs affected *C. jejuni* growth, attachment, and invasion at the group, diarrheal manifestation, and individual strain levels. Pairwise *t*-tests compared untreated controls with SCFA-treated groups. Generation time assays demonstrated that 0.03 mM acetate caused a 2.37-hour delay in growth ($p = 0.004$) and propionate caused a 2.68-hour delay in growth ($p <$

0.001). 0.03 mM butyrate caused a 0.3-hour slower growth in watery diarrheal strains compared to bloody diarrheal strains (p = 0.01). 0.03 mM propionate reduced attachment by 0.69% (p = 0.03) and 3 mM reduced attachment by 0.8% (p = 0.005). SCFAs did not appear to significantly affect the invasion of Caco-2 cells. Our data indicate that the SCFAs affect overall treated groups, differing diarrheal groups, and individual strains.

Significance: Understanding the effects of SCFAs on *C. jejuni* virulence provides insight into how gut microbiome diversity influences disease severity.

P1-130: DESIGNING DECISION-RELEVANT CLOSTRIDIUM BOTULINUM CHALLENGE TESTS FOR CHILLED FOODS: WHEN TEMPERATURE ABUSE SHIFTS FROM COUNTS TO TOXIN

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: *Clostridium botulinum* challenge testing for refrigerated ready-to-eat foods is technically demanding and requires correct upfront framing to generate decision-relevant evidence for industry.

Purpose: To demonstrate a proof-of-concept workflow for *C. botulinum* challenge testing aligned with industrial questions, and to illustrate how early choices (process lethality, strain selection, storage scenarios, endpoints) shape conclusions.

Methods: A chilled multi-component ready-to-eat product was inoculated with a cocktail representing proteolytic (Group I/type A) and non-proteolytic psychrotrophic (Group II/type E) *C. botulinum*, targeting ~10^3 spores/g. A mild pasteurization step was applied; the delivered thermal lethality (VP range 1076-1310) was verified and contrasted with the nominal target. 3

independant replicates were performed (i) 1/3 of the storage period at 4°C followed by 2/3 at 8°C, then an additional 7 days at 8°C, with a cold-chain break on day 21 (2 h at 20°C); and (ii) 1/3 of the storage period at 4°C followed by 2/3 at 20°C, then an additional 7 days at 20°C. At multiple timepoints up to the end of shelf life, samples were analyzed for *C. botulinum* by MPN, and frozen subsamples were used for botulinum neurotoxin testing at selected timepoints by lethality test.

Results: In Scenario 1, a positive growth potential was estimated only at day 49 (Δ=2.86 $\log_{10}$ CFU/g based on measured MPN values), with no neurotoxin detected while *C. botulinum* type A was evidenced. In Scenario 2 (20°C misuse), measured levels exceeded 5 $\log_{10}$ CFU/g by day 24, with a growth potential of 4.50 $\log_{10}$ CFU/g, and botulinum neurotoxin type E was detected.

Significance: This proof of concept shows that *C. botulinum* challenge testing is feasible for industry, but must start from verified process delivery and a justified study design (strain set, realistic misuse scenarios, and endpoints) to support actionable process and shelf-life decisions.

P1-131: GAP ANALYSIS ON CRONOBACTER SPP. IN FOODS INTENDED FOR INFANTS

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Presentation type/Format: Technical

Category: General Microbiology

Abstract:

Introduction: Food safety incidents linked to infant formula and various microbial pathogens have made the news recently. Infants are particularly vulnerable to infection, including *Cronobacter* spp. which can cause severe and life-threatening illness. Outbreaks of *Cronobacter* spp. illness have been mainly associated with contaminated powdered infant formula (PIF). *Cronobacter* spp. have also been detected in infant cereals; a food-pathogen combination that remains poorly understood.

Purpose: To summarize the current body of knowledge on *Cronobacter* spp. as a foodborne pathogen, focusing on PIF and infant cereals, to identify knowledge gaps and explore opportunities for food safety improvements.

Methods: Data were gathered and analyzed from the scientific literature, databases, government and private sources.

Results: PIF remains the primary transmission vehicle for *Cronobacter* spp. in infants and is appropriately targeted for food safety measures. Internationally, recommendations for the

preparation and handling of PIF vary: some jurisdictions apply uniform guidance, while others distinguish between at-risk and healthy infants; water temperature guidelines for reconstitution also differ. *Cronobacter* spp. have been detected in infant cereals, however no related cases or outbreaks have been reported. Authoritative data indicate that infant cereals, often introduced before the recommended age of six months, are among the first complementary foods offered to infants. Knowledge gaps persist regarding reportability, susceptibility, dose-response, and handling practices.

Significance: Greater international alignment could promote consistent public messaging and enhance protection against *Cronobacter* spp. infections. While knowledge gaps around infant cereals hinder targeted food safety improvement actions, reinforcing the appropriate age of introduction of solids may help reduce *Cronobacter* spp. infections.

P1-132: COMPARATIVE ANALYSIS OF MICROBIAL ACTIVITY IN YELLOW MEALWORM (TENEBRIO MOLITOR) LARVAE BATCHES ACROSS VARIOUS TIME POINTS AND ORIGINS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Edible insects such as *Tenebrio molitor* (yellow mealworm) larvae are increasingly promoted as sustainable protein sources; however, microbial profiles may vary by geographic region due to differences in climate, environment, and rearing practices, potentially affecting food safety under standardized processing.

Purpose: This study evaluated batch-to-batch microbial variation in U.S.-sourced fresh mealworm larvae and compared these values with published data from Europe and South America to assess food safety implications.

Methods: Live mealworm larvae were obtained from a single U.S. supplier across three

seasonal production batches (June 2024, September 2024, February 2025). Larvae were separated from frass, euthanized by freezing, homogenized, and analyzed without further processing. Six technical replicates per batch (2 g each) were serially diluted and plated to enumerate aerobic bacteria (35°C, 48 h), total anaerobes (35°C, 48 h, anaerobic), psychrotrophic bacteria (20°C, 72 h), and yeast and mold (22.5°C, 5 d). Data were analyzed using one-way ANOVA with appropriate post hoc tests; batch means were compared with literature values (α = 0.05).

Results: Significant batch-to-batch variation was found in microbial groups, with aerobic counts between 7.1 to 7.9 log CFU/g, notably higher in the September batch ($p<0.0001$). Anaerobic and psychrotrophic counts showed differences ranging from 7.61 to 8.08 CFU/g ($p=0.002$). Yeast and mold counts varied from 3.5 to 5.1 log CFU/g, with one batch being significantly lower ($p<0.0001$). While aerobic and yeast/mold counts matched literature ranges, anaerobic and psychrotrophic counts did not.

Significance: Geographic origin and seasonal production influence microbial loads in fresh mealworms, indicating that insects from different regions may respond differently to identical processing. Standardized microbiological benchmarks are needed to support risk assessment and safe commercialization of edible insects.

Award Application: Developing Scientist Award

P1-133: THE SILENCING OF RECA GENE BY ZIF-8/ASO/RECA-MRNA REDUCED THE ADHESION CAPACITY OF ENTEROHEMORRHAGIC, ENTEROTOXIGENIC, AND ENTEROAGGREGATIVE ESCHERICHIA COLI TO CACO-2 CELLS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Diarrheagenic *Escherichia coli* pathotypes, a major public health challenge, cause foodborne illnesses. Their high variability in virulence factors and antibiotic resistance complicates antibiotic use during infection.

Purpose: To determine the impact of ZIF-8/ASO/*recA*-mRNA on the adhesion capacity of diarrheagenic *E. coli* pathotypes to Caco-2 cells.

Methods: *E. coli* K-12, EHEC, EAEC, and ETEC strains were pre-exposed to 16, 32, 64, and 128 nM/mL of ZIF-8/ASO/*recA*-mRNA for 3, 6, and 24 h. Then, adhesion capability and adhesion patterns of treated *E. coli* to Caco-2 cells was determined for 3 h using plate count and fluorescence staining assays respectively. The cytotoxicity of ZIF-8/ASO/*recA*-mRNA on Caco-2 cells was determined by the MTT assay. Statistical analyses were performed using an analysis of variance and Duncan's multiple range test; statistical significance was set at $p \leq 0.05$. Three

independent biological samples were analyzed, each with three technical replicates.

Results: Adding ZIF-8/ASO/*recA*-mRNA to Caco-2 cells maintained cell viability at 95.5±6%. Most concentrations of compound reduced *E. coli* adhesion to Caco-2 cells compared to the control ($p < 0.05$; control normalized to 100%). A dose-dependent effect was observed in most pathotypes. The lowest adhesion levels were at 128 nM/mL, with adherent cells of 67±2% ($\bar{x}$ = 4.3±0.1 $\log_{10}$ CFU) for *E. coli* K-12, 78.6±0.2% ($\bar{x}$ = 4.4±0.1 $\log_{10}$ CFU) for EAEC, 68.2±2.8% ($\bar{x}$ = 4.4±0.1 $\log_{10}$ CFU) for ETEC, and 42.5±6.3% ($\bar{x}$ = 2.6±0.6 $\log_{10}$ CFU) for EHEC. EHEC was the most affected pathotype. Pre-exposure to 128 nM/mL also affected the adhesion patterns in all pathotypes.

Significance: These findings present a potential avenue for the development of non-antibiotic therapies targeting *recA* for the control of pathogenic strains, representing a promising strategy for public health and the food industry.

Award Application: Developing Scientist Award

P1-134: HORIZONTAL TRANSFER OF QACS RESISTANCE GENES BETWEEN LISTERIA INNOCUA AND LISTERIA MONOCYTOGENES STRAINS ISOLATED FROM A FROZEN VEGETABLE PRODUCTION ENVIRONMENT

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: The exchange of disinfectant resistance genes can occur between non-pathogenic *Listeria* spp. and *L. monocytogenes*, potentially contributing to the persistence of this pathogen in food production environments.

Purpose: The objective of this study was to determine the presence of quaternary ammonium compound (QACs) resistance genes in *Listeria* spp. strains and to evaluate the effect of temperature and substrate on interspecies gene transfer.

Methods: A total of 23 *L. monocytogenes* strains, four *L. innocua* and 37 *Listeria* spp. isolates recovered from surfaces in a frozen vegetable processing facility were used. The presence of the *bcr*ABC gene was assessed by PCR. Two *L. innocua* strains carrying the *bcr*ABC gene and two *L. monocytogenes* strains lacking it were selected to prepare mixed cultures; four co-

cultures were prepared, inoculated into tryptic soy broth and spinach extract, and incubated at 4°C, 25°C, and 36°C for 10 days. Phenotypic resistance to QACs was evaluated in both native and recipient *L. monocytogenes* strains using well-diffusion assays and Student's *t*-test

Results: The *bcr*ABC gene was detected in 73.9% (17/23) of *L. monocytogenes* isolates and in 50% (2/4) of *L. innocua* strains, while it was not found in the remaining *Listeria* spp. species. Horizontal transfer of the *bcr*ABC gene was observed in 7.9% (17/216) of reactions across both substrates ($p > 0.05$), with higher frequencies at 25°C (4.6 %) and 36°C (2.8 %). An increased resistance to QACs at concentrations of 50, 500, and 1000 ppm in recipient strains compared to native ones were observed.

Significance: Our findings suggest that horizontal gene transfer between non-pathogenic *Listeria* species and *L. monocytogenes* may play a relevant role in the persistence of disinfectant-resistant strains in food processing environments.

Award Application: Developing Scientist Award

P1-135: IMPACT OF LISTERIA MONOCYTOGENES ON THE EXTRACELLULAR POLYMERIC SUBSTANCE (EPS) COMPOSITION IN BIOFILMS WITH PSEUDOMONAS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Listeria monocytogenes* is a foodborne pathogen that persists in food processing environments and poses a significant food safety risk. *L. monocytogenes* grows better in biofilms with *Pseudomonas* compared to mono-species biofilms. However, it is unknown whether the presence of *L. monocytogenes* impacts the composition of extracellular polymeric substances (EPS) in a biofilm with *Pseudomonas.*

Purpose: We investigated whether the total protein and carbohydrate content of the biofilm EPS is altered when *Pseudomonas* is grown with *L. monocytogenes* in a multispecies biofilm.

Methods: Six strains of *Pseudomonas* species and two strains of *L. monocytogenes* were grown in mono and dual-species biofilms over three days at 12°C with two biological replicates per biofilm. Biofilms were harvested to quantify *L. monocytogenes* and *Pseudomonas* by plating on selective agars. The protein and carbohydrate content of the cell-free biofilm EPS was quantified using the Pierce BCA and the phenol sulfuric acid assay, respectively. Data were analyzed using paired *t*-tests with Bonferroni correction.

Results: *Pseudomonas* and *L. monocytogenes* grew to an average of 6.41 $\log_{10}$ and 4.25 $\log_{10}$

CFU/biofilm sample in dual-species biofilms, respectively. Total protein concentration in EPS from *Pseudomonas* mono-species biofilms did not significantly differ from corresponding dual-species biofilms grown with *L. monocytogenes* (3.75 µg/mL) (p>0.05). Similarly, total carbohydrate concentration of EPS in *Pseudomonas* mono-species biofilms (73.11 µg/mL) did not differ significantly (p>0.05) from *Pseudomonas–L. monocytogenes* dual species biofilms (84.05 µg/mL).

Significance: Although *L. monocytogenes* consistently survived in biofilms with *Pseudomonas,* it did not significantly impact the total protein and carbohydrate concentrations in the EPS. Therefore, further investigation of specific interactions enabling the survival of *L. monocytogenes* in biofilms with *Pseudomonas* is needed.

Award Application: Developing Scientist Award

P1-136: BIOCIDE RESISTANCE IN BACKGROUND MICROORGANISMS ASSOCIATED WITH FOOD PROCESSING ENVIRONMENTS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Biocide resistance in food environmental microbiomes is a growing concern, as repeated sanitizer exposure may enable survival and persistence of pathogenic microorganisms.

Purpose: To determine phenotypic biocide resistance among food environmental microorganisms.

Methods: *Citrobacter rodentium* (ATCC 51459), *Enterococcus faecium*, *Escherichia coli* (ATCC 25922), *E. coli* O121, O157:H7, O26, and O91, *Klebsiella aerogenes* (ATCC 13048), *Lactobacillus acidophilus*, *Lactococcus lactis*, *Listeria innocua* (ATCC 33090), *L. ivanovii* (ATCC 19119), *L. monocytogenes* (ATCC 19115), *L. seeligeri* (ATCC 35967), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* serovars Anatum, Braenderup, Enteritidis, Heidelberg, and Typhimurium, and *Staphylococcus aureus* (ATCC 25923) were evaluated using a broth dilution assay (10^5 CFU/mL) to determine MICs of KC-631 (acid QAC), KC-610 (PAA), and KC-644 (neutral QAC) after 24 h at 35°C.

Results: MICs (ppm) for KC-631, KC-610, and KC-644, respectively, were: *C. rodentium* (2.34, 7.81, 3.13), *E. faecium* (ND, 15.63, 0.39), *E. coli* (2.34, 15.63, 3.13), *E. coli* O121 (4.69, 15.63, 3.13), *E. coli* O157:H7, O26, and O91 (2.34, 15.63, 3.13), *K. aerogenes* (2.34, 15.63, 3.13), *L.*

acidophilus (ND, ND, 0.39), *L. lactis* (ND, ND, 0.39), *L. innocua* (0.59, 15.63, 0.39), *L. ivanovii* (ND, 15.63, 0.39), *L. monocytogenes* (ND, 15.63, 0.39), *L. seeligeri* (ND, 15.63, ND), *P. aeruginosa* (9.38, 15.63, 12.5), *Salmonella* Anatum, Braenderup, Enteritidis, and Typhimurium (4.69, 15.63, 6.25), *Salmonella* Heidelberg (4.69, 7.81, 6.25), and *S. aureus* (0.59, 7.81, 0.39); ND = not determined. Two independent biological replicates yielded consistent MIC values with no observed differences. Statistical analysis was not conducted, as MIC endpoints are interpreted by replicate agreement.

Significance: These results show organism- and biocide-specific susceptibility differences, emphasizing effective sanitizer application to prevent sublethal exposure and microbial persistence.

Award Application: Developing Scientist Award

P1-137: BIOFILM FORMATION BY WILD-TYPE AND PRESSURE-STRESSED ESCHERICHIA COLI O157:H7 AND SALMONELLA ENTERICA SEROVARS ON STAINLESS STEEL IN MILK AT 4°C AND 37°C

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Biofilms formed by foodborne pathogens on food-contact surfaces pose significant public health risks in dairy processing. Limited information exists on how pressure-induced stress adaptation affects biofilm formation capacity in milk environments.

Purpose: To investigate biofilm formation by wild-type and pressure-stressed *Escherichia coli* O157:H7 and *Salmonella enterica* serovars on stainless steel surfaces in milk for twenty-eight days at refrigeration and physiological temperatures.

Methods: A four-strain cocktail of each pathogen (wild-type and pressure-stressed at 103 MPa, 25°C for 15 min) was inoculated in sterile milk and cultured on stainless steel coupons at 4°C and 37°C. A randomized block design with two biologically independent repetitions was employed. Biofilm cell counts were log-transformed and analyzed using Tukey-adjusted ANOVA.

Results: Approximately 2-log reductions in biofilm counts were observed for both wild-type and pressure-stressed phenotypes compared to controls ($p < 0.05$). No significant differences in biofilm formation were detected between wild-type and pressure-stressed phenotypes ($p > 0.05$). Viable cells remained above detection limits on steel coupons for twenty-eight days at both temperatures.

Significance: The persistence of biofilm-forming capacity following pressure stress demonstrates the adaptability of these pathogens in dairy environments, underscoring the need for comprehensive control strategies beyond traditional chemical interventions in food processing facilities.

P1-138: VALIDATION OF A RAPID METHOD TO DETECT YEAST AND MOLD IN A BROAD RANGE OF FOODS FOLLOWING CHINA GB 4789.45

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Rapid methods to detect yeast and mold allow quick response and support efficient quality control in food industries. However, few have been validated under GB 4789.45-2023 General Rules for Validation of Microbiological Test Methods, the Chinese standard for validating alternative microbiological methods.

Purpose: This study aims to validate the performance of a rapid alternative method for detecting yeast and mold in a broad range of foods against a reference method, GB 4789.15-2016.

Methods: The validation study for Neogen® Soleris® Direct Yeast and Mold (DYM) test method consisted of a method comparison study, including inclusivity and exclusivity test and sensitivity test. For the inclusivity and exclusivity test, 30 yeast and mold isolates and 31 bacterial isolates were evaluated. In a paired-sensitivity test, six categories□one food item was tested per category of foods were evaluated and compared to the GB 4789.15-2016

National Food Safety Standard – Food Microbiological Examination: Enumeration of Molds and Yeasts. For each matrix, 20 test portions were spiked at a fractional positive level, one test portion at high inoculation level, and one uninoculated. The rLOD was calculated as the log-transformed ratio of detection limits between the reference and alternative methods.

Results: Thirty inclusive strains were tested within 48 hours, and 31 exclusive bacteria all produced no detection within 48 hours. The rLOD following a paired comparison study were from 0.63 to 1.45, which met the acceptability limit of 1.5 according to GB 4789.45. These results indicate that the rapid method is an effective alternative to the China GB reference plating procedure.

Significance: Compared to conventional plating methods (5 days), the alternative method offers rapid results in 48 hours or less for the detection of yeast and mold in a broad range of foods. It offers a fast and reliable detection method on an easy-to-use platform.

P1-139: EFFICACY OF ROBOTIC SWABBING TO MONITOR THE HYGIENE OF FOOD-CONTACT SURFACES IN THE POULTRY INDUSTRY

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Environmental monitoring in poultry processing plants relies on manual swabbing, which can introduce variability in microbial recovery.

Purpose: This study aims to evaluate the efficiency of a robotic arm swabbing system for recovering bacteria from stainless steel (SS) surfaces when compared to manual swabbing.

Methods: Stainless steel coupons (929 cm^2, 144 in^2) were spot inoculated with ~6 log of *Escherichia coli* C3000 and allowed to dry for 80 min under ambient conditions. Surfaces were sampled using pre-moistened sponge samplers mounted on a robotic arm operating with controlled top-to-bottom and side-to-side motion and a sustained pressure of 1.5 N. For human participants (n=5), the surfaces were also sampled using the same procedure. After sampling, sponges were stomached in 1×PBS for 1 min, serially diluted, plated in duplicate on CHROMagar™ *E. coli*, and incubated at 37°C. Recovery efficiency was calculated as the percentage of total inoculated CFU recovered. Experiments were completed two and three

times for robotic arm and human participants, respectively.

Results: For human participants, recovery averaged 75% and ranged from 47 to 90% of the applied inoculum, while a minority of runs exhibited lower recoveries indicative of operator-related variability resulting in statistical differences in recovery across participants ($P<0.001$). Meanwhile, the robot arm system produced a mean recovery value of 82% with a range of 80–84%, and these were tightly clustered with low intra-pressure variance, demonstrating high repeatability of the robotic swabbing procedure.

Significance: Robotic swabbing provides a consistently higher recovery of bacteria from SS surfaces found in poultry processing plants, compared with manual swabbing performance. This automated approach may reduce operator-dependent variability, strengthen sanitation verification, improve detection of hygiene failures, and support more robust preventive food safety controls.

P1-140: QUANTIFYING THE EFFECT OF LOSS OF TEMPERATURE CONTROL ON BACTERIAL VIABILITY DURING -80°C STORAGE

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Maintaining microbial culture viability is essential for food safety research. Freezer failures can expose cultures to conditions for which quantitative survival data remain limited.

Purpose: This study examined survival of *Escherichia coli* TVS 353 and *Listeria innocua* SLCC 3379 when exposed to temperature deviations from -80°C and repeated freeze-thaw cycling.

Methods: Twenty percent glycerol stocks of *E. coli* and *Listeria innocua* were stored at -80°C, and viability was assessed during 14 daily freeze-thaw cycles. Additional studies held cultures at -20°C, 4°C, room temperature (~22°C), and on dry ice (-80°C). At each time point, at least three replicates of one tenth mL aliquots were collected daily, serially diluted, and plated on TSA. Survival was quantified using linear regression.

Results: *E. coli* exhibited modest viability loss during freeze-thaw cycling at -80°C (slope -0.043; R^2 0.43) and at -20°C (slope -0.038; R^2 0.56), while declines at 4°C were minimal (slope -0.010; R^2 0.03) after 14 days. *Listeria innocua* showed consistently greater sensitivity across these same conditions, with steeper declines during -80°C freeze-thaw (slope -0.107;

R^2 0.81), at -20°C (slope -0.077; R^2 0.71), and at 4°C (slope -0.036; R^2 0.30). Room-temperature storage produced the most rapid losses for both organisms, especially *L. innocua* (slope -0.591; R^2 0.85) compared with *E. coli* (slope -0.213; R^2 0.62). *E. coli* maintained near-complete stability (slope -0.002; R^2 0.02), whereas *L. innocua* showed a measurable but moderate decline (slope -0.116; R^2 0.99) after 3 days under dry ice storage.

Significance: These results quantify the consequences of power outages and various remediation measures on culture viability. These findings will aid in management of culture collections after loss of temperature control.

Award Application: Developing Scientist Award

P1-141: EXPLORATORY ANALYSIS OF PHENOTYPIC MICROARRAYS TO ELUCIDATE SUBLETHAL STRESS IMPACT ON SALMONELLA ENTERICA GROWTH KINETICS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Throughout the course of processing, *Salmonella* Hadar is exposed to a variety of stress conditions. Investigating the impact of primary sublethal stress on *S*. Hadar tolerance to downstream stressors may provide insight on best practices during processing to enhance food safety.

Purpose: This study evaluates the growth kinetics of *S.* Hadar using phenotypic microarrays after lactic acid stress.

Methods: *S.* Hadar was grown overnight (20°C; 200 rpm; 20 h) in tryptic soy broth and 50 µL was transferred to a tryptic soy agar (TSA) plate or a TSA plate pH adjusted to 5.13 ± 0.3 with filter sterilized lactic acid. Plates were incubated at 37°C for 18 h. Cells were transferred to Biolog cell inoculating fluid IF-0a at 85% transmittance. A 1:200 dilution was made in Biolog cell inoculating fluid IF-10a, and 100 µL/well was dosed into Biolog PM 9 and PM 10 microplates assessing osmotic/ionic and pH response, respectively. Plates were shaken (100 RPM; 5 min) and incubated in the Biolog Odin VIII instrument (37°C; 24 h). Optical density (590 nm) was measured every 10 min. Results were measured as average area under the curve.

Results: Several substrates resulted in notable differences ($p < 0.05$) in the area under the curve for sublethally stressed and nonstressed *S.* Hadar cells. For example, in the presence of 6% sodium formate or pH 4.5 + L-Tryptophan, stressed cells displayed enhanced survival. Conversely, nonstressed cells were more tolerant of 10% ethylene glycol and 9% NaCl presence.

Significance: There is evidence that sublethally stressing *S.* Hadar with lactic acid may confer enhanced survival or susceptibility of the strain compared to control. Further evaluation of the phenotypic microarray approach and continued exploration of industry relevant conclusions based on this data is warranted.

Award Application: Developing Scientist Award

P1-142: MICROBIAL DIVERSITY ON DECLINE? TRENDS IN LISTERIA MONOCYTOGENES FROM FOOD SOURCES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: It is critical to monitor *Listeria monocytogenes* (*Lm*) isolated from Food Safety and Inspection Service (FSIS) inspected products to detect changes in genetic diversity and population structure.

Purpose: Monitoring these metrics enables the identification of emerging strains and pathogenicity shifts, providing insights into persistence and control measure effectiveness.

Methods: Sequences of *Lm* (2017–2025) obtained from routine, risk-based contact and non-food contact surface samples, as well as ready-to-eat product samples, were analyzed to assess the frequency of Sequence Types (STs). The Shannon Diversity Index (SDI) was calculated for STs to evaluate year-to-year changes and significance was tested using the Kruskal-Wallis method. For temporal trends, Spearman's rank correlation was applied on min-diff proportions of isolates with <= 20 SNPs, considered closely related to a clinical strain.

Results: Between 2017 and 2025, a total of 574 *Lm* strains were analyzed. SDI values ranged from 2.85 (2017) to 2.49 (2024). While index values showed a declining trend, differences

were not significant. The proportion of isolates with <=20 SNPs declined from 72% (2014) to 61% (2025) suggesting a moderate downward trend – approaching significance (*p*= 0.0581).

Significance: Although SDI differences were not significant, trends suggest a gradual decline in microbial diversity (2017–2025). While the decline in min-diff values <=20 is close to significant, they are an indirect indicator of clinical relevance, and results may reflect lower clinical sampling compared to FSIS sampling. The decline in diversity may reflect industry practices that reduce certain STs but allow others to persist and emphasize the need for proactive, continuous genetic monitoring. FSIS public datasets with *Lm* subtypes support research on persistence mechanisms linked to higher human exposure risk and inform industry strategies by identifying the most common subtypes for targeted control.

P1-143: ASSESSING OPTICAL DENSITY AS A PROXY FOR VIABLE CELL COUNTS IN FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Cell count is essential in microbiological research. Using $\log_{10}$ CFU remains the standard for quantifying viable cells; however, optical density (OD_{600}) is an alternative to monitor bacterial populations. The outcomes from both techniques differ, and little information is available to understand the numerical correlation between them.

Purpose: To establish growth parameters and evaluate the correlation between OD_{600} and $\log_{10}$ CFU/mL during the growth of two foodborne pathogens.

Methods: Bacterial growth was monitored for *E. coli* O157:H7 and *Salmonella*. Cultures were incubated at 37°C and samples collected every 5 min during early exponential phase until the third doubling time (dt), followed by hourly sampling every 4h until 24h. At each time point, OD_{600} was measured and serial dilutions plated on non-selective agar for enumeration. Exponential-phase data was used to calculate growth rates (μ) and dt, and linear regression was performed to evaluate the relationship between OD_{600} and viable cell counts.

Results: For *E. coli* O157:H7, OD_{600} growth curve showed a dt of 26.22 min and a μ of $1.5848h^{-1}$. Log_{10} CFU/mL modeling resulted in $\mu=1.4292h^{-1}$ and dt=29.16min. A strong

correlation between both measurements was observed (r=0.9765; R^2=0.839). For *Salmonella*, OD_{600} modeling resulted in μ=1.1629h^{-1} and dt=35.76min, while $\log_{10}$ CFU/mL modeling yielded μ=1.1506h^{-1} and a dt=36.12min. OD_{600} and $\log_{10}$ CFU/mL growth curves showed comparable trends throughout incubation, with a strong linear association between measurements (r=0.9685; R^2=0.8831).

Significance: Quantifying the correlation between OD_{600} and $\log_{10}$ CFU helps with the interpretation of bacterial growth. While $\log_{10}$ CFU/mL provides a direct measure of viable populations, OD_{600} measurements allow rapid monitoring but reflect total biomass rather than viability. Results of this study serve as a guide for researchers to estimate the correlation between each technique.

P1-144: PATHOGENIC CAMPYLOBACTER SPP. CIRCULATING IN LIVESTOCK AND WILDLIFE IN THE CALIFORNIA CENTRAL COAST AGRICULTURAL REGION

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Livestock and wildlife can be sources of pathogenic *Campylobacter* spp. The California (CA) Central Coast Agricultural Region contains hundreds of square miles of rangeland and riparian corridors that harbor livestock and wildlife populations, which are

located adjacent to fresh produce production.

Purpose: To identify pathogenic *Campylobacter* spp. circulating in livestock and wildlife near Central Coast fresh produce production areas during a CA Longitudinal Study and to better understand the potential sources of produce contamination.

Methods: From May 2022 to March 2025, livestock (n=255) or wildlife (n=19) fecal samples (~25 g each) were collected. Samples were enriched for *Campylobacter* according to the FDA BAM *Campylobacter* Chapter 7 protocol, with minor modifications, including different antibiotics for early samples and the addition of R&F *Campylobacter jejuni/C. coli* Chromogenic Plating medium as a secondary enrichment. Colonies were screened by microscopy for *Campylobacter*-like morphologies. Suspected *Campylobacter*-positive isolates were cultured and further analyzed by whole genome sequencing.

Results: Among livestock fecal samples, 46.7% (119/255) were positive for at least one pathogenic *Campylobacter* spp. (*C. jejuni*, *C. coli*, *C. hyointestinalis*, *C. fetus*, or *C. californiensis*). Among wildlife fecal samples, 10.5% (2/19) were *Campylobacter*-positive (*C. upsaliensis* in coyote and *C. hyointestinalis* in feral pig). Several samples were positive for multiple sequence types and/or species of *Campylobacter*. Furthermore, new sequence types of pathogenic *Campylobacter* spp. were also identified.

Significance: The pathogenic *Campylobacter* spp. identified in livestock and wildlife populations within the CA Central Coast Agricultural Region represent potential sources of *Campylobacter* contamination.

P1-145: FOOD-CONTACT SURFACE TYPE SHAPES MULTI-SPECIES BIOFILM STABILITY AND LISTERIA MONOCYTOGENES TRANSFER POTENTIAL

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Food-contact surfaces can influence not only biofilm persistence but also the structure and transfer dynamics of embedded pathogens. In leafy produce environments, understanding how different materials affect multi-species biofilm behavior is critical to assess cross-contamination risks.

Purpose: To evaluate how surface type influences multi-species biofilm formation and the stability of *Listeria monocytogenes* in the presence of native environmental microbiota (NEM).

Methods: Biofilms were formed under low-shear flow for 24h on stainless steel (SS), rubber (R), and polycarbonate (PC) using mono- or multi-species inoculum (5-strain *L. monocytogenes* cocktail ± NEM). Cell counts were measured at 5h and 24h. Transfer to spinach was assessed through five consecutive 1-minute contacts. Cell surface hydrophobicity (CSH) was evaluated by microbial adhesion to hydrocarbons. Three replicates were run in all essays.

Results: *L. monocytogenes* counts were higher in multi-species biofilms across all surfaces and timepoints ($p<0.05$), reaching 5.55±0.07 log CFU/coupon on SS at 24h vs. 3.61±0.01 in mono-species biofilms. NEM also persisted longer in multi-species biofilms. On SS and R, multi-species biofilms sustained *L. monocytogenes* transfer through all five contacts (□2.7 log CFU/coupon), while mono-species biofilms dropped below detection (<1.70) after early

contacts. Transfer rates were higher in mono-species biofilms, but residual rates were greater in multi-species biofilms on SS and R. CSH was also higher in multi-species biofilms, especially on SS.

Significance: NEM may promote *L. monocytogenes* persistence on certain surfaces while delaying its initial release. SS and R favored both retention and structural protection, highlighting the need to consider surface type and microbial interactions in sanitation strategies.

P1-146: ISOLATION OF ANTIFUNGAL-RESISTANT CANDIDA FROM VINEYARDS ACROSS MULTIPLE WINE GRAPE PRODUCTION SEASONS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Fungicide usage in crop production can be a driving factor in the propagation of fungi with multi-drug resistance (MDR), including emergent human pathogens. The U.S. CDC recognizes *Candida auris*, a rapidly spreading MDR yeast implicated in nosocomial infections, as a critical health threat with environmental and potentially agricultural origins.

Purpose: As grapevine production requires intensive pesticide management, this study aimed to screen for *C. auris* and related MDR yeasts in vineyards across Maryland over two wine grape production seasons.

Methods: Grapevine berry clusters were collected during twelve total sampling events that took place at four MD vineyards in 2024 and 2025. Following a modified CDC protocol, grape juice was enriched in Salt Sabouraud Dulcitol Broth containing fluconazole at 37°C for up to one week. Positive samples were plated on CHROMagar™ *Candida* agar and incubated at 37°C for 48 hours. Putative *Candida* isolates with unique morphologies (n=18 in 2024, n=7 in 2025) were selected and processed for DNA extraction and ITS2 amplicon sequencing for taxonomic identification.

Results: All grapevine isolates were negative for *C. auris*, however ITS sequencing revealed several key *Candida* relatives. *Candida tropicalis, Meyerozyma guilliermondii,* and *Hanseniaspora uvarum* were identified in 2024 and 2025, and *Pichia kudriavzevii* (formerly *Candida* krusei) and *Zygoascus meyerae* were also identified in 2024. This work will be extended with whole genome sequencing to provide additional insights regarding potential health risks of the identified strains.

Significance: Fungicide usage in agriculture operations has potential to exert selective pressure that drive the spread of MDR pathogens. Environmental sites such as wine grape vineyards can be a valuable model system for exploring emergence of MDR *Candida* with public health implications.

P1-147: GENOMIC AND PHENOTYPIC ANALYSIS OF SALMONELLA TYPHIMURIUM BACTERIOPHAGE-INSENSITIVE MUTANTS SELECTED BY FLAGELLA AND O-ANTIGEN TARGETING PHAGES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Receptor-specific bacteriophages can generate bacteriophage-insensitive mutants (BIMs) with distinct genetic changes and phenotypic trade-offs relevant to food-safety.

Purpose: To compare whole-genome variants and key phenotypes of two *Salmonella* Typhimurium BIMs selected by a flagella-targeting phage (STP-3) and an O-antigen-targeting phage (STP-4).

Methods: MF-19 (STP-3) and MO-14 (STP-4) were isolated after three exposure cycles (MOI 100); resistance was confirmed by spot tests (no plaques) (n=3 independent isolates). WGS with variant-impact annotation was performed versus parental strains. Phenotypes included antimicrobial susceptibility (20 drugs), motility, PM array profiling, and biofilm on SS, LDPE, and HDPE (ANOVA, α=0.05).

Results: MF-19 carried 63 mutations, including five high-impact variants linked to flagella and cell-envelope functions, whereas MO-14 carried 30 modifier-impact mutations with no high-impact variants. Susceptibility changed for 7/20 drugs in MF-19 (increased susceptibility) and 10/20 drugs in MO-14 (increased resistance). MF-19 lost swimming and showed 47% lower swarming; MO-14 lost swarming and showed 33% lower swimming. PM array profiling showed loss of two (MF-19) or four (MO-14) carbon sources and impaired growth at pH 9.5 (MF-19) or pH 4.5 (MO-14). Biofilm on SS, LDPE, and HDPE was comparable to or slightly higher than parental strains (ANOVA, $P<0.05$).

Significance: Receptor-dependent BIMs showed distinct mutational patterns with shifts in antibiotic response, motility, metabolism, and biofilm, informing resistance-aware phage use in food systems.

P1-148: FORMATION OF THE VBNC STATE IN SALMONELLA TYPHIMURIUM UNDER PLASMA-ACTIVATED WATER FACILITATED BY OUTER MEMBRANE PROTEIN PAGC

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Plasma-activated water (PAW) is an emerging non-thermal sterilization technology, but its potential to induce the viable but non-culturable (VBNC) state in *Salmonella* presents a challenge to food safety.

Purpose: This study aimed to verify the role of PagC in *Salmonella* Typhimurium during PAW-induced VBNC state.

Methods: Wild-type (WT) and *pagC* deletion mutant (Δ*pagC*) *S*. Typhimurium were treated by PAW, and flow cytometry was used to determine the proportion of VBNC cells. Transcriptomic and proteomic analysis were performed to evaluate the function of PagC. Physiological properties including ROS levels, ATP content and arginine content, were also measured to verify the results of the omics analysis. Three independent biological replicates were performed for every experiment. Statistical analysis was performed using Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results: The proportion of VBNC Δ*pagC* (76.4%) significantly decreased compared to WT (84.0%) under PAW stress ($p < 0.05$). Transcriptomic and proteomic analysis revealed the

disruption of energy metabolism, glutathione metabolism and arginine biosynthesis in Δ*pagC* compared with WT, indicating the important role of PagC in the response to PAW stress. Physiological analysis showed that the ROS level in Δ*pagC* (103,127 RFU/OD_{600}) was significantly higher than WT (67,821 RFU/OD_{600}) after PAW treatment ($p < 0.05$). *pagC* deletion also led to a decrease in ATP level after PAW treatment (from 2.59 to 1.28 nmol/mg protein, $p < 0.05$), indicating that Δ*pagC* lacked energy for stress response.

Significance: These findings identify PagC as a critical regulator in *Salmonella*'s response to PAW treatment, providing a target for preventing VBNC formation and optimizing PAW applications to ensure food safety.

P1-149: ECO-FRIENDLY ANTIFUNGAL AGENTS FROM PLANT BIOWASTE: TARGETING FUSARIUM OXYSPORUM AND ASPERGILLUS FUMIGATUS

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Category: General Microbiology

Abstract:

Introduction: The extensive use of synthetic fungicides in clinical and agricultural systems has heightened concerns about antifungal resistance and environmental sustainability. To address these challenges, this study explores the antifungal potential of bioactive compounds such as phenolics and lectins, derived from plant-based biowaste, using a non-thermal, membrane-based separation process. The goal is to develop eco-friendly alternatives to conventional fungicides for managing *Aspergillus fumigatus* and *Fusarium oxysporum*, pathogens that threaten human health and global food security.

Purpose: This research evaluates the antifungal efficacy of biowaste sources, including marjoram seed, moringa seed, morro seed, strawberry seed, and citrus peel albedo.

Methods: Biowaste samples were pre-treated with hot water (80°C, 2 min) or acidic conditions (pH 3.4, 2 h), followed by membrane-based extraction of bioactive compounds. Antifungal activity was assessed using agar well diffusion and microtiter plate assays against *A. fumigatus* and *F. oxysporum*. The assay was conducted in five replicates.

Results: Compared with extracts derived from other materials, the citrus peel albedo extract showed significant ($p < 0.05$) antifungal activity against *F. oxysporum*, demonstrating MIC and MFC values of 6,250 µg/mL and 12,500 µg/mL, respectively, although its effectiveness against *A. fumigatus* was comparatively lower. Moringa seed extract selectively inhibited *F. oxysporum* (MIC: 25,000 µg/mL). Other extracts showed negligible antifungal effects even at 100,000 µg/mL.

Significance: This study highlights biowaste-derived compounds as promising, sustainable antifungal agents. The use of membrane-based extraction reinforces environmentally responsible bioactive recovery, supporting safer agricultural practices and public health.

P1-150: INACTIVATION OF CRONOBACTER SAKAZAKII DURING RECONSTITUTION OF INFANT CEREALS

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: *Cronobacter sakazakii* is desiccation resistant and can survive in dry foods for extended time periods. Recently, recalls due to possible *C. sakazakii*-contamination of dry infant cereals have occurred. If present in the dry cereal, *C. sakazakii* may not be inactivated during the reconstitution process.

Purpose: To determine the inactivation of *C. sakazakii* in two different formulations of infant cereal during reconstitution at 70, 85, or 100ºC.

Methods: Rice- and oat-based infant cereals were dry-inoculated with *C. sakazakii* at 8 log CFU/g. Cereals were reconstituted according to manufacturer instructions (1 tb. cereal [3.4/4.0 g for oat/rice] and 60 mL liquid), in triplicate, with water or reconstituted milk-based powdered infant formula (RPIF) heated to 70, 85, or 100°C. Cereals were mixed, left on the counter, and sampled at 1, 3, 5, 10, 30 and 60 min by removing 1 mL and placing on ice. Samples were serially diluted and plated for enumeration. Two independent trials were conducted. Population reductions were compared using ANOVA; $p<0.05$ considered significant.

Results: *C. sakazakii* was inactivated by >5 log CFU/mL in both types of cereals reconstituted with water or PIF at 85 or 100°C after only 1 min. When 70°C water was used for reconstitution, *C. sakazakii* populations were reduced by 4.62±0.80 and 4.51±1.07 log CFU/mL in rice- and oat-based cereal, respectively. When RPIF was used for reconstitution, the reduction was significantly greater in rice-based cereal (2.31±0.62 log CFU/mL), but similar in oat-based cereal (3.67±0.47 log CFU/mL). Regardless of the cereal formulation, reconstitution liquid, or temperature used, no significant further population reduction was observed from 1 to 60 min.

Significance: Results of this study indicate that reconstitution temperature plays a role in the inactivation of *C. sakazakii* in infant cereals and that using RPIF instead of water for reconstitution may provide a protective effect for the pathogen.

P1-151: TWO NOVEL REAL-TIME PCR ASSAYS FOR THE RELIABLE AND FAST DETECTION OF LISTERIA FROM VARIOUS FOOD MATRICES AND ENVIRONMENTAL SURFACES

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: The genus *Listeria* contains several species but, *Listeria monocytogenes* is the only known pathogenic strain. Several other species play a role as indicator for *Listeria monocytogenes* contamination. Regulations of *Listeria* in foodstuff varies but, usually state zero tolerance, because listeriosis is one of the most serious foodborne diseases, which affects mostly risk groups like the elderly, pregnant women, and infants.

Purpose: This study presents a validation of two novel real-time PCR kits designed for rapid and reliable detection of *Listeria* while improving user convenience to reduce errors.

Methods: The performance of the BACGene GO *Listeria* methods was validated according to current AOAC Appendix J Guidelines and best practices, and in harmonization with ISO 16140-2:2016 guidelines. The reference method was ISO 11290-1:2017. The validation comprised inclusivity and exclusivity testing; matrix studies for 25 g of deli salad, raw ground beef, raw milk, raw milk cheese, bagged lettuce, smoked salmon, frozen scallops, and 10 cm x 10 cm of stainless steel, and ceramic tile, as well as robustness, product consistency and cycler comparison studies.

Results: Comparison between presumptive and confirmed results, or between candidate and reference methods, using the probability of detection statistical analysis from AOAC

“Appendix J” showed no significant differences regardless of matrix or PCR platform. Inclusivity and exclusivity tests showed 100% specificity, as well as robustness, product consistency and cycler variability tests all gave the anticipated results.

Significance: Classical methods of detection rely on cultivating *Listeria* on agar plates. Although they are still widely used, these methods demand several days to reach a result. However, most industries require considerable faster results, showing the demand for rapid methods like PCR Assays.

P1-152: INACTIVATION OF SALMONELLA ENTERICA AND ESCHERICHIA COLI O157:H7 ON MUNG BEAN SPROUTS BASED ON PHỞ SOUP BROTH TEMPERATURE

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Presentation type/Format: Poster

Category: General Microbiology

Abstract:

Introduction: Recalls of mung bean sprouts due to *Salmonella enterica* or *Escherichia coli* O157:H7 have recently occurred. Mung bean sprouts are often served as garnishes on noodle soups in restaurants. It is not known the extent to which pathogens would be inactivated on mung beans if submerged in the hot soup broth.

Purpose: To determine the inactivation of *S. enterica* and *E. coli* O157:H7 on mung bean sprouts in phở soup broth at different temperatures.

Methods: Mung bean seeds were inoculated with *S. enterica* or *E. coli* O157:H7 at 7 log CFU/g. Sprouts were grown from contaminated seeds at 25°C for 7 d with daily rinses. Triplicate 10-g sprout samples were placed in bowls and submerged in phở broth at 70, 80, 90 or 100°C. Bowls were kept at room temperature for 60 min. Samples were removed at intervals of 1, 3, 5 10, 30 and 60 minutes during treatment and pathogens were enumerated. Differences in population reductions were compared via ANOVA; $p<0.05$ considered

significant.

Results: The maximum inactivation of *S. enterica* and *E. coli* O157:H7 on the sprouts occurred within the first 10 min, regardless of initial broth temperature. The use of 90 or 100°C broth resulted in >5 log CFU/g reductions of both pathogens; reductions of 4–5 log CFU/g occurred with 80°C broth. With 70°C broth, *S. enterica* was reduced by a greater extent than *E. coli* O157:H7 after 1 min: reductions of 3.84±0.65 and 1.63±0.63 log CFU/g, respectively. After 10 min, *E. coli* O157:H7 was significantly reduced by an additional 1.47 log CFU/g, whereas no further reduction occurred for *S. enterica*.

Significance: Results from this study provide information on the inactivation of *S. enterica* and *E. coli* O157:H7 on mung bean sprouts which are used as garnishes on noodle soups. These data may assist in the development of food protection policy including suggested serving temperatures of noodle soup broth at retail.

P1-153: QUANTIFICATION OF PFAS IN FOOD AND PACKAGING BY LC-MS/MS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Per- and polyfluoroalkyl substances (PFAS) are synthetic fluorinated chemicals known for strong resistance to heat and chemical degradation. Because they break down slowly, PFAS persist in the environment, and people may be exposed through food, drinking water, dust, or skin contact. Studies continue to associate certain PFAS with potential health concerns, including elevated cholesterol, liver effects, endocrine disruption, and possible cancer links.

Purpose: To protect public health, regulators have begun limiting PFAS exposure. In April 2024, the U.S. EPA established the first national drinking-water standards for six PFAS, while the USFDA has worked with industry to remove PFAS-based grease-proofing agents from food packaging. Therefore, reliable methods for PFAS quantification are needed.

Methods: This method describes a validated LC-MS/MS procedure for PFAS quantification. Food testing follows AOAC SMPR 2023.003 with 30 PFAS; packaging testing references AOAC SMPR 2025.001. The workflow includes homogenizing 5–20 g of sample, adding isotopically labeled internal standards, extracting with acidified acetonitrile, and performing clean-up steps such as QuEChERS and solid-phase extraction. Detection uses multiple reaction monitoring with retention-time confirmation, and quantification is based on calibration curves with dilution and mass corrections.

Results: Method performance—including linearity, accuracy, precision, and LOQ—meets AOAC SMPR 2023.003 requirements. Typically, repeatability for each PFAS compound (n=4) is below 30% and reproducibility (n=8) is below 40%. LOQs for the four EPA-regulated PFAS meet drinking-water targets: PFOA and PFNA at 0.001 ppb, and PFOS and PFHxS at 0.003 ppb.

Significance: This method will enable regulators to effectively monitor PFAS levels in food, supporting regulatory compliance and helping reduce public exposure. Ultimately, it will contribute to protecting public health and promoting a safer food supply.

P1-154: VALIDATION OF A USDA-FSIS MULTI-ANALYTE ICP-MS METHOD FOR TRACE METALS IN MEAT, POULTRY, FISH, AND PROCESSED PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Metal contamination in food remains a significant public health concern. To support the National Residue Program and initiatives such as FDA's Closer to Zero, USDA-FSIS modernized its metals method to enhance detection capabilities and eliminate reliance on dual-instrumentation (ICP-OES and ICP-MS). The updated ICP-MS method expands applicability to multi-ingredient products and enables the simultaneous detection of sodium in a broad range of matrices.

Purpose: This study aimed to validate a quantitative ICP-MS method for 18 metals in various FSIS-regulated products. The validation focused on calculating the minimum level of applicability (MLA), establishing robust recovery, precision, and linearity in complex matrices.

Methods: The method employed microwave-assisted nitric acid digestion followed by ICP-MS analysis. Each sample batch included blanks and recoveries spiked near the MLA. Method validation included 3 data sets, conducted across 6 different matrices (beef, pork, poultry, goat, *Siluriformes*, and processed products). Calibration standards and quality controls were prepared using certified reference materials. MLA values were calculated as the lowest spiking level that consistently met method recovery and Horwitz equation-based acceptance precision limits in all matrices.

Results: The method achieved acceptable recoveries for all 18 metals across varied matrices, with MLAs ranging from 10 ng/g (e.g., Cd, Tl, W) to 40,000 ng/g (Zn), depending on the analyte. Precision (%RSD) was generally <15% at the MLA level, and all analytes exhibited strong linearity (R^2 ≥ 0.9975 for all calibration curves).

Significance: This validated method strengthens FSIS's ability to more accurately detect trace metals in various FSIS-regulated products. Establishing analyte-specific MLAs allows the agency to detect and interpret low-level findings with greater confidence, while the recovery and precision data support the method's scientific defensibility.

P1-155: TARGETED DETERMINATION OF ZEARALENONE AND ITS MODIFIED FORMS IN COMMERCIAL FOODS USING LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Zearalenone (ZEN) is a non-steroidal mycotoxin regulated primarily in its parent form but can be structurally modified during food processing or metabolism, generating derivatives that may revert to ZEN and, in some cases, exhibit comparable or higher estrogenic activity.

Purpose: This study aimed to develop and validate a simultaneous analytical method for ZEN and 12 of its modified forms using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and to assess their occurrence in commercially available foods.

Methods: A total of 345 food samples, including grains, grain-based products, fermented soybean products, and beers, were collected from supermarkets, local markets, and online platforms between 2024 and 2025. Samples were extracted with an acetonitrile-based solvent and purified using solid-phase extraction to reduce matrix interferences. Quantitative analysis was performed by LC–MS/MS equipped with electrospray ionization in multiple

reaction monitoring mode.

Results: The method was validated in four representative matrices (white rice, snacks, soybean paste, and beer) by evaluating linearity, limits of detection and quantification, accuracy, and precision. All validation parameters met AOAC performance criteria, demonstrating satisfactory linearity, sensitivity, and precision. Application of the method to 345 commercial food samples showed that 67 samples (19.4%) contaminated with at least one ZEN-related compound, and 20 samples exhibited co-occurrence of ZEN and one or more modified forms.

Significance: This study provides a validated targeted LC–MS/MS method and occurrence data that support improved exposure assessment and safety management of zearalenone and its modified forms in food.

P1-156: RAPID DETECTION OF AFLATOXINS IN GROUND MAIZE USING A HANDHELD MULTIMODE SPECTROSCOPIC DEVICE

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Aflatoxins contamination in food and agricultural products poses serious health risks and leads to substantial economic losses.

Purpose: This study investigates the potential of a novel handheld multimode spectroscopic device for the rapid classification of aflatoxins-contaminated maize samples.

Methods: The handheld spectrometer was employed to measure both fluorescence spectra (with excitation at 365 nm and 405 nm) and reflectance spectra in the 400–1900 nm range. Classification models were developed using partial least squares-discriminant analysis (PLS-DA) and support vector machines (SVM), and the performance of the handheld device was compared to that of a hyperspectral imaging (HSI) system.

Results: A total of 273 ground maize samples were tested for aflatoxin contamination, with concentrations ranging from 0 to 585 µg/kg and a mean of 95.7 µg/kg. The ground maize samples were divided into two groups: group 1 for negative samples (aflatoxins

concentration at zero or below cut-off of 10 μg/kg, n=146) and group 2 for positive samples (concentration range 12–585 μg/kg, n=127). Samples in each group were randomly assigned to calibration and validation sets, with 80% of the dataset being used for training, and 20% used for validation. The results demonstrated that both handheld spectroscopy and HSI achieved maximum classification accuracies of 85.5% and 98.2%, respectively, in fluorescence and reflectance spectroscopy, effectively distinguishing contaminated maize samples. Compared to laboratory-based HSI systems, the handheld spectrometer offers significant advantages, including portability, ease of use, and lower cost.

Significance: These findings support its potential as an effective tool for rapid, on-site screening of agricultural products.

P1-157: OCCURRENCE PROFILING OF THIRTY-SEVEN PARENT AND MODIFIED MYCOTOXINS IN SOUTH KOREAN MARKET FOODS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Modified mycotoxins (MMs), including metabolism- and processing-related derivatives, often co-occur with their parent compounds and may contribute to overall dietary exposure. However, occurrence data remain limited, as most analytical methods target few matrices, focus on limited modified forms, or primarily quantify parent mycotoxins.

Purpose: This study aimed to develop a single-run, liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the simultaneous determination of 37 parent and MMs, including major *Aspergillus* and *Fusarium* toxins, and to apply the validated method to assess their occurrence profiles in plant-derived foods.

Methods: Method performance was evaluated in four matrices (white rice, maize, peanut, and sesame) for linearity, limits of detection and quantification (LOD and LOQ), accuracy, and precision following Codex performance criteria and relevant EU guidance. The method was then applied to 414 samples across six food categories.

Results: The method showed acceptable performance with linearity (R^2 > 0.99), accuracy (70.2–117.4%), and precision (0.1–19.5%). No mycotoxins above the LOQ were detected in oats or red beans, whereas at least one mycotoxin was detected in most other matrices. β-Zearalenol was the most frequently detected MM and was present in all pepper samples. Up to nine mycotoxins co-occurred in sorghum, most commonly zearalenone and fumonisin B1. Modified-to-parent concentration ratios were generally 1, but exceeded 1 in selected matrices, including brown rice, red rice, and perilla seeds. Positive correlations (r = 0.63–1.00) were observed for AFB1–AFM1, ZEN–zearalanone, and ZEN–α-zearalenol in matrices where both analytes were quantifiable.

Significance: This study establishes a validated single-run LC–MS/MS method across multiple matrices and occurrence profiling data that support dietary exposure assessment of MMs.

P1-158: DEVELOPMENT OF A RAPID AND SIMPLE SURFACE-ENHANCED RAMAN SPECTROSCOPY METHOD FOR POLYSTYRENE NANOPLASTICS QUANTIFICATION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Plastic food packaging can release nanoplastics, resulting in direct human exposure and necessitating accurate measurement. Existing methods have limitations: FTIR and Raman are largely restricted to microscale detection, while fluorescence and flow-based methods lack polymer specificity. Although Surface-enhanced Raman spectroscopy (SERS) enables nanoplastics detection via plasmonic hotspots, current methods rely on complex, lab-based substrate synthesis, limiting adoption by the food industry and regulators.

Purpose: This study aims to develop a rapid, simple SERS method to quantify polystyrene nanoplastics (PS NPs) relevant to food packaging migration.

Methods: 50 nm carboxyl-modified PS NPs were used to simulate packaging-derived nanoplastics, while 50 nm citrate-capped gold nanospheres (AuNPs) served as the SERS substrate. PS NPs suspensions were prepared in water and acetone (0.00098-980 μg/mL), with water samples tested as reference. 50 μL of PS NPs water or acetone suspension was mixed with 50 μL of AuNPs (0.25 mg/mL) for 10 s, and 2.5 μL was deposited onto aluminum foil, air-dried, and analyzed by Raman spectroscopy at six distinct positions. The characteristic PS Raman peak at 999 cm^{-1} was used to construct calibration curves across six

concentrations for PS NPs quantification.

Results: Without enhancement, PS NPs showed a detection limit of 32 µg/mL, which improved 15-fold to 2.1 µg/mL with the incorporation of AuNPs. Acetone increased the hydrodynamic diameter of 114.4 ± 0.2 nm (121%), enhancing hotspot formation for SERS. Combining acetone with AuNPs broadened the quantification range to 0.0098-980 µg/mL (R^2 = 0.95) and improved the detection limit by 2100-fold to 1.0 ng/mL. This method enables rapid (<20 min) sample preparation using commercially available reagents.

Significance: This method presents a novel approach for rapid assessment of nanoplastics, supporting food packaging migration testing for industry and regulatory surveillance.

Award Application: Developing Scientist Award

P1-159: IS YOUR NEXT SIP SAFE? A SIMPLE, RAPID METHOD FOR MEASURING VOLATILE PFAS IN JUICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Juices are widely consumed globally and may represent a pathway for human exposure to per- and polyfluoroalkyl substances (PFAS), a class of persistent and potentially harmful contaminants.

Purpose: Increasing public concern and regulatory scrutiny underscore the need for robust analytical methods, and this study presents a novel workflow for the accurate and precise quantitation of volatile PFAS in complex juice matrices.

Methods: Thirteen PFAS targets (PFIs, FTIs, FTACs, FTMACs, FTOHs, and FASAs) were analyzed using headspace solid-phase microextraction coupled with triple quadrupole gas chromatography–mass spectrometry (HS-SPME GC/MS/MS). Quantitation employed an eight-point internal calibration curve (1–2000 ng/L) with mass-labeled internal standards added at 100 ng/L. Samples were adjusted to 2% (w/v) salinity with sodium chloride, vortexed, and analyzed using an AOC-6000 Plus autosampler. Method performance was evaluated using

laboratory control samples (LCS; n=4) and four fortified juice matrices (100 ng/L; n=3 per matrix). The juice samples included two brands that included multiple flavors.

Results: Method blanks showed no analytical interferences. Calibration curves demonstrated strong linearity for all analytes (R^2 ≥ 0.993), with calibration verification control recoveries of 70–130%. Mean LCS recoveries ranged from 83–115% with %RSDs of 0.6–6.8%. Isotope dilution was used to improve quantitation of the targets in complex matrices. Across 12 juice samples from four juice matrices, recoveries ranged from 69–120% with %RSDs <12%, meeting all performance criteria. The results demonstrated the method's reliability for PFAS analysis in diverse juice matrices.

Significance: As regulatory oversight expands, reliable methods for accurate PFAS quantitation in beverages are increasingly important. This workflow offers a simple, rapid, precise, accurate and robust approach for routine monitoring of volatile PFAS in diverse and complex juice matrices.

P1-160: COMPREHENSIVE ANALYSIS OF MULTIPLE MYCOTOXINS IN WHEAT PRODUCTS AND THEIR DIETARY RISK ASSESSMENT

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Wheat is susceptible to fungal infection, and some fungi produce multiple mycotoxins that contaminate wheat and its products, posing health risks to consumers. The co-occurrence of mycotoxins complicates detection and risk assessment, creating a major challenge for food safety.

Purpose: To establish a rapid, sensitive method for simultaneous detection and quantification of multiple mycotoxins in wheat samples, supporting regulatory compliance and accurate risk assessment.

Methods: A QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)-based extraction with ultrasonic assistance, combined with ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), was optimized for simultaneous determination of 16 mycotoxins in wheat samples. The method was applied to commercial wheat products to quantify contamination and assess dietary exposure, clarifying potential consumer health risks.

Results: The optimized QuEChERS extraction with UHPLC-MS/MS enabled efficient, sensitive detection of 16 mycotoxins in wheat samples. Seven mycotoxins (nivalenol, deoxynivalenol, 15-acetyldeoxynivalenol, HT-2, T-2, zearalenone, ochratoxin A) were identified in wheat/flour.

In processed products, deoxynivalenol and T-2 toxin appeared in scallion pancakes, noodles & dough skin, and oatmeal & flour; deoxynivalenol was also found in instant noodles, noodles, and vermicelli, while T-2 toxin was present in toast, bread, and wheat rolls. All concentrations were below Taiwan Food and Drug Administration (TFDA) limits, and also showed low dietary risk.

Significance: This study established a reliable and rapid analytical framework for detecting multiple mycotoxins in wheat samples. It also confirmed that the mycotoxin levels in commercially available wheat products comply with TFDA regulations, and showed that the contamination levels pose a low risk to consumers.

P1-161: DEVELOPMENT AND COMPARISON OF UNIVARIATE, MULTIVARIATE, AND NONLINEAR REGRESSION MODELS FOR ARSENIC-LEAD PEAK OVERLAP CORRECTION IN ENERGY DISPERSIVE X-RAY FLUORESCENCE SPECTROSCOPY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Trace quantification of arsenic (As) and lead (Pb) in high-risk foods by energy-dispersive X-ray fluorescence spectroscopy (ED-XRF) is hindered by spectral overlap at characteristic emission lines.

Purpose: This study developed and compared three quantitative models to correct for spectral overlap and enable accurate quantifications of trace As and Pb in rice and rice-based foods.

Methods: Calibration standards were prepared by pelletizing milled rice (4.0 ± 0.2 g) spiked with varying concentrations As and Pb (~0 – 1000 ppb) and analyzed by ED-XRF. Resultant spectra were used to generate calibration models via univariate linear regression (ULR), multivariate linear regression employing partial least squares regression (PLSR), and nonlinear regression utilizing artificial neural networks (ANNs). Validation samples included a certified reference material, commercial rice-based foods (n = 11), and commercial rice-based foods spiked with Pb (n = 12).

Results: The ULR model boasted the highest calibration linearity (R^2_{As} = 0.99, R^2_{Pb} = 0.94), lowest validation error (+4.85% and -5.28 ppb in As and Pb, respectively), and most accurate quantifications in the commercial samples. While not as consistent as the ULR model, the PLSR model accurately quantified As in samples with low Pb content (median As error = 13.85%, while the ANN yielded the best predictions of As in samples with high Pb content (average As error = 14.11%). The strong performance of the ULR model highlights the importance of interpretability in models with few spectral components, whereas the situational strengths of the PLSR and ANN models indicate the usability of more complex models at distinct As-Pb concentrations.

Significance: These results offer insights into the comparisons of each developed model and show the ability of ED-XRF coupled with appropriate data modeling techniques to enable accurate quantifications of As and Pb in rice-based foods at trace levels.

P1-162: STRAIN-DEPENDENT VARIABILITY IN SALMONELLA QUANTIFICATION FROM GROUND BEEF MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Current routine *Salmonella* testing in ground beef relies mainly on detection methods that generate either presence/absence results or estimates contamination levels using relative qPCR assays.

Purpose: This study aimed to investigate strain-dependent variability in *Salmonella* quantification associated with fast- and slow-growing strains in ground beef samples.

Methods: The growth curves for 12 *Salmonella* strains, spanning six serotypes that are a priority for the beef industry, were characterized using an optical density-based method. Growth curve data were used to classify strains into fast- and slow-growing categories, which were then used for inoculating ground beef samples. Ground beef (88% lean, 12% fat; 325 ± 5 g) was inoculated in duplicate with nutritionally stressed *Salmonella* at 1 and 10 CFU/g. Samples were enriched at 35°C for 6 hours in modified buffered peptone water. Enrichments were collected and centrifuged to obtain bacterial pellets, and then diluted cells were directly used for a partition-based dPCR assay targeting the *invA* gene.

Results: The paired analysis conducted between dPCR-derived concentrations and inoculum counts revealed no significant difference for slow-growing *Salmonella* strains (paired *t*-test, $P = 0.1904$, $n = 12$). In contrast, fast-growing strains exhibited significantly higher quantification by dPCR compared to inoculum (paired *t*-test, $P = 0.0043$, $n = 12$). Across samples, the quantification assay estimated higher contamination levels, especially for fast-growing strains. This difference may be caused by nutritional factors present in the ground beef samples.

Significance: This study demonstrates that the quantification of *Salmonella* in ground beef matrices can vary in a strain-dependent manner. The data from this study call for the inclusion of both fast- and slow-growing strains for revalidating the accuracy of all commercially available *Salmonella* quantification assays.

Award Application: Developing Scientist Award

P1-163: SALSURE QUANT: ENRICHMENT-FREE SALMONELLA SPP. QUANTIFICATION AND DETECTION OF SALMONELLA SEROTYPES OF CONCERN BY A NOVEL, HIGH THROUGHPUT, DNA MICROARRAY

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* contamination in poultry products is a significant health concern. The new guidance and industry's need for a Performance Standard calls for the quantification of *Salmonella* species down to 10 CFU/mL and detection of serovars of concern. The poultry industry is seeking a more accurate, rapid and cost-effective quantitative testing solution.

Purpose: This study describes the evaluation of an array-based, enrichment-free quantification and screening assay to Quantify *Salmonella* spp., and simultaneously, detect and discriminate serovars of greatest concern, from poultry carcass rinsate.

Methods: PCR primers and array hybridization probes were designed for analysis of *Salmonella* spp. and two internal quantitative standards so that the entire set can be

multiplexed as a single Endpoint PCR reaction. The reaction is analyzed as a single, open, array-based test in each well of a 96 well plate. To determine quantitative accuracy whole bird rinse samples were prepared via USDA MLG 4.15, and artificially inoculated with select *Salmonella* serovars at 1000, 100, 10, and 1 CFU/ml. We compared our unenriched method to the published MLG 4.15 enrichment approach.

Results: The assay was validated both internally and externally on standardized chicken carcass rinsates, demonstrating 100% specificity of *Salmonella* spp., *S.* Typhimurium and *S enteritis* serovars. Quantification of *Salmonella* spp. demonstrated results of 1573.89±145.33, 118.96±34.51, 6.72±4.54, and 1.16±0.35 CFU/ml (n=5 at each level; 3 technical replicates per sample).

Significance: The unenriched, multiplexed assay provides cost-effective, same-day results, significantly improving the turnaround time and accuracy to the MPN reference standard that is needed to meet the QC/QA needs of the poultry industry in the context of USDA guidance.

P1-164: SALSURE DETECT, SALVAC, AND SEROX: UNENRICHED VS. ENRICHED AOAC PERFORMANCE TEST METHOD FOR THE DETECTION OF SALMONELLA SPP. IN CHICKEN CARCASS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: SalSure Detect, SalVac, and SeroX are qualitative tests for the detection of *Salmonella* spp plus the Elanco AviPro® Megan® Vac1 and a broad range of *Salmonella* serovars. The test involves the extraction of nucleic acids from samples, followed by a labeling PCR amplification step, and lastly the amplified product is hybridized to a DNA microarray. Detection of *Salmonella* spp, Megan Vac1, and select serovars is determined by fluorescence.

Purpose: The objective of this study was to validate the three methods at 0 and 24-HR enrichment following an approved AOAC protocol, in Chicken Carcass Rinsates to demonstrate low level detection of *Salmonella* spp. with and without enrichment.

Methods: The evaluation followed study designs from the *AOAC OMA Appendix J Guidelines*, the reference method for the qualitative matrix study follows MLG 4.15, and carcass sampling is described in FSIS Directive 10,250.1 with the use of neutralized Buffered Peptone Water (nBPW) for rinsing carcasses as directed in FSIS Notice 08-19.

Results: The results showed no statistically significant difference, at the 95% and 90% confidence intervals (N=5 High Level; N=20 Low Level; N=5 Uninoculated Carcass), between the candidate method, presumptive methods positive results and the confirmed cultural positive results at all contamination levels and enrichment time points.

Significance: This study provides data that demonstrates the methods being evaluated are reliable for detection of *Salmonella* species in chicken carcass from 0–24 hours enrichment. This highly sensitive method provides results within <6–8 hours of sample acquisition, compared to qPCR or cultural methods where results are obtained in 2–7 days. This method also provides the option of enrichment, flexibility for end users and matched enrichment guidelines to current regulations.

P1-165: QUANTITATIVE MULTIPLEX QPCR FOR SAME-DAY DETECTION AND QUANTIFICATION OF SALMONELLA KPI SEROVARS IN POULTRY MATRICES USING A VALIDATED CQ-TO-CFU ALGORITHM

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Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella enterica* remains a leading cause of foodborne illness associated with poultry, requiring rapid quantitative detection methods suitable for routine laboratory use.

Purpose: This study evaluated the quantitative performance, limit of quantification, and matrix robustness of a multiplex qPCR platform for same-day detection of *Salmonella* key performance indicator (KPI) serovars in poultry matrices.

Methods: Five *Salmonella enterica* serovars [*S*. Enteritidis, *S*. Hadar, *S.* Muenchen, *S.* Typhimurium, and *S*. Typhimurium monophasic variant (1,4,[5],12:i:-)], were cultured and adjusted to a 0.5 McFarland turbidity standard (~1.5 x 10^8 CFU/mL), combined into defined chicken and turkey KPI mixtures, and serially diluted. Dilutions were inoculated into chicken rinsate or ground turkey (24 samples) to achieve contamination levels from ~ 10^3 to <10 CFU per gm or per mL. Plate counts were also performed. DNA was extracted using a chemical lysis method and analyzed using a five-channel multiplex PCR thermocycler. Quantification was estimated using serovar-specific Cq-to-CFU regression algorithms and the results were

compared to culture enumeration and to a commercial molecular assay.

Results: The multiplex qPCR analyses demonstrated strong quantitative agreement with culture enumeration across both matrices (R^2 = 0.92), with amplification efficiencies ranging from 74% to 98%. Limits of quantification in chicken rinsate ranged from 11 CFU/mL for *S.* Typhimurium monophasic variant (1,4,[5],12:i:-) to ~ 56 CFU/mL for *S. enterica*, while ground turkey samples exhibited comparable performance with slightly higher LOQ limits for *S.* Muenchen. All KPI serovars were reliably detected at contamination levels of 15 CFU per 27g or per mL with no evidence of matrix inhibition.

Significance: This study demonstrates that multiplex qPCR combined with Cq-to-CFU algorithms enables rapid, accurate, and quantitiative detection of multiple *Salmonella* serovars in poultry matrices.

P1-166: ADVANCING SALMONELLA DETECTION: PERFORMANCE EVALUATION OF QRED ENRICHMENT MEDIA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Effective enrichment is critical for detecting *Salmonella* in food and environmental samples, enabling recovery of low-level or injured cells and minimizing the impact of inhibitory matrixes. Quantitative Rapid Enrichment Dehydrated (QRED), a newly developed medium, supports improved *Salmonella* growth compared to conventional media while suppressing competing microflora. QRED is the enrichment component of Neogen's Quantitative *Salmonella* Molecular Detection Assay; the present study focuses solely on the performance of QRED as an enrichment medium.

Purpose: This study assessed QRED's performance for quantitation characteristics of *Salmonella* versus the reference growth medium, Buffered Peptone Water (BPW).

Methods: Media growth promotion was assessed by inoculating four *Salmonella* serotypes at (0.5–5 CFU/mL) in triplicate into QRED or BPW and enumerating *Salmonella* populations after 6 hours of incubation at 41–42°C. QRED media was prepared either with sterile water or by

autoclaving. Performance of media and preparation methods were evaluated by enumeration (n=10) and growth curve analysis (n=4), and were repeated seven times on different days. Inclusivity and exclusivity testing included 45 additional *Salmonella* strains and non-target organisms.

Results: *Salmonella* counts of SK 9263, SI 51741, ST 14208, ST 13311 were 0.68–1.09 Log_{10} CFU/mL higher in QRED compared to BPW after 6 hours of incubation, showing significantly better growth ($P < 0.05$) in QRED. Regardless of preparation method, QRED produced higher counts and shorter lag times ($P < 0.05$) compared to BPW. QRED supported robust growth of 45 additional *Salmonella* serotypes and effectively inhibited exclusivity organisms *B. cereus* and *E. faecalis*.

Significance: QRED offers a robust, user-friendly solution for rapid *Salmonella* recovery. It provides repeatable quantitation results with excellent selectivity and can provide quantitation for poultry products when paired with a molecular detection assay.

P1-167: AUTOMATED DNA EXTRACTION ENHANCES SALMONELLA QUANTIFICATION ACROSS DIVERSE FOOD MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Consistent DNA recovery is critical for accurate *Salmonella* quantification using molecular methods. Manual extraction introduces variability that can compromise reproducibility across complex food matrices.

Purpose: To validate an automated DNA extraction platform integrated into an MPN–qPCR workflow, specifically using GENE-UP® QUANT *Salmonella*, for accurate *Salmonella* quantification in multiple food types.

Methods: Four matrices—beef trim, MicroTally® beef cloth, chicken carcass rinse, and ground turkey—were inoculated at four target levels: 0 CFU/g/ml, 10–100 CFU/g/ml, 50–150

CFU/g/ml, and 800–1,200 CFU/g/ml, chosen to represent negative, low, medium, and high contamination scenarios. For each matrix, 20 samples were tested following enrichment, processed using the automated system, and analyzed by real-time PCR. Performance was assessed by comparing quantitative results to inoculation levels and reference MPN estimates using mean log differences, confidence intervals, and hypothesis testing.

Results: Automated extraction delivered results statistically equivalent to the reference method across all matrices and inoculation levels. Mean log MPN differences were negligible, and 90%/95% confidence intervals remained within predefined equivalence limits. No significant differences were detected ($p > 0.05$), confirming robust performance under varied conditions.

Significance: This workflow combines accuracy with efficiency—reducing manual steps and extraction time without sacrificing reliability. Adoption of automated extraction can streamline *Salmonella* quantification, supporting faster, more reproducible food safety testing.

P1-168: VALIDATION OF TWO ENZYME-LINKED FLUORESCENT IMMUNOASSAY AND TWO REAL-TIME PCR METHODS FOR DETECTING LISTERIA SPP. AND LISTERIA MONOCYTOGENES IN ENVIRONMENTAL AQUEOUS CHILLING SOLUTIONS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: VIDAS® UP *Listeria* (LPT) and VIDAS® *L. monocytogenes* Xpress (LMX) (ELFA methods), and GENE-UP® *Listeria* spp. (LIS) and GENE-UP® *L. monocytogenes* (LMO) (PCR methods) are automated assays for rapid detection of *Listeria* spp. and *Listeria monocytogenes* in food and environmental samples.

Purpose: To validate these methods for detecting *Listeria* spp. and *Listeria monocytogenes* in brine against USDA MLG Ch. 8.

Methods: Two sample sets (60 replicates/method) were tested: 5 uninoculated, 20 low-level,

and 5 high-level inoculated samples per set. Samples were analyzed using the two ELFA methods, the two PCR methods and USDA MLG Ch. 8. All enrichments were culturally confirmed.

Results: dPOD analysis ($P < 0.05$) showed no significant differences between candidate and reference methods. The low level dPODs (95% CI) were −0.10 (−0.37, 0.19) and 0.05 (−0.24, 0.33). The high level dPOD (95% CI) was 0.00 (−0.43, 0.43).

Significance: The ELFA and PCR methods are equivalent to the USDA reference method for detecting *Listeria* spp. and *Listeria monocytogenes* in brine.

P1-169: QUANTITATIVE EXTRACTION AND DETECTION OF SALMONELLA FROM PRE-HARVEST AND POST-HARVEST POULTRY SAMPLES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Quantitation of microbial pathogens in animal samples presents unique challenges for molecular assays due to high biological and chemical complexity of such samples.

Purpose: We developed a high-throughput sample prep system for culture-independent detection and quantitation of *Salmonella* that delivers results for up to 48 samples in 4 hours.

Methods: The method utilizes a unique combination of solubilizing and anti-viscosity agents to remove the impurities interfering with cell detection and enumeration in complex samples. All samples were tested in at least two technical replicates, with or without added bacterial targets to account for natural contamination. We compared the detection of increasing levels of *Salmonella* spiked into media, or the following samples: whole carcass rinsates from different stages of processing (hot rehang and postchill, N=20), parts rinsate (thighs, breast, tenders and wings, N= 6), ground turkey (N=10) and boot socks from hatchery and broiler housing (N=18).

Results: We show robust detection and quantification of *Salmonella* at levels 10 to 10^6 CFU/mL with R2 of 0.944 across all sample types and cell input levels. The detection of *Salmonella* was found to be statistically equivalent to media control for bootsock samples, while a statistical difference of the detection in meat samples was 0.65 and 0.75 quantification cycle

(Cq) values in rinsates and ground turkey, respectively. When solubilizing and anti-viscosity agentswere substituted with PBS buffer, the detection efficiency dropped by 32- and 256-fold for postchill and hot rehang rinsate samples, respectively.

Significance: The presented sample prep method enables rapid, high-throughput enumeration of *Salmonella* in pre-harvest and post-harvest samples without culturing.

P1-170: PERFORMANCE OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD TO SCREEN MOST PROBABLE NUMBER TUBES FOR ENUMERATION OF SALMONELLA IN POULTRY MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The Center for Disease Control and Prevention reported that from the 1.35 million human *Salmonella* infections, 23% are attributed to poultry consumption, which has a significant impact on humans. Enumeration of *Salmonella* in poultry enables food processors to monitor the effect of interventions to reduce *Salmonella* levels, which is necessary to validate and verify process controls.

Purpose: To evaluate the performance of the Molecular Detection Assay 2 – *Salmonella*, based on loop mediated isothermal amplification and bioluminescence reporting to screen tubes utilized for most probable number during *Salmonella* enumeration.

Methods: Portions of 325g of ground turkey meat and 30mL test portions of chicken carcass rinse in BPW were spiked at 0CFU/g 1CFU/g, 10CFU/g, and 100CFU/g (n=20 samples per inoculation level and matrix type) using a cocktail of four *Salmonella* serotypes. Ground turkey samples were homogenized with 400mL of BPW before diluting for MPN. Three

dilutions per level were prepared in BPW with five replicates each to determine MPN according to USDA FSIS MLG 2.05. Tubes were also analyzed using the USDA FSIS MLG 4.13 and the LAMP-Bioluminescence method for detection of *Salmonella*. MPN was calculated with both methods. The *p*-value of a paired *t*-test was used to compare the two methods at each level of inoculation per matrix.

Results: There was no significant difference between LAMP and MPN methods in all inoculation levels evaluated ($p>0.05$). The paired *t*-test of the MPN at each level for each method had a *p*-value >0.05. The log mean difference at each level was <0.5 log CFU/g.

Significance: LAMP offers a cost-effective, accurate, and rapid method for screening tubes during the enumeration of *Salmonella* utilizing MPN, minimizing labor due to agar preparation and interpretation. Rapid methods for *Salmonella* detection support poultry producers to verify *Salmonella* intervention effectiveness and evaluation of process controls.

P1-171: HIGH-RESOLUTION MULTIPLEX DDPCR FOR QUANTITATIVE DETECTION OF SALMONELLA SEROVARS IN POULTRY MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Accurate quantification of *Salmonella* serovars in poultry products is essential for meeting emerging regulatory standards and improving food safety surveillance.

Purpose: This study evaluated a Droplet Digital PCR (ddPCR)-based workflow for simultaneous detection and quantification of multiple *Salmonella* serovars in mixed contamination scenarios.

Methods: A multiplex ddPCR panel targeting serovars Enteritidis, Infantis, Muenchen, Typhimurium, Hadar, and Dublin was developed using Bio-Rad's QX600 platform. Poultry matrix samples were inoculated with mixtures containing up to four serovars per sample, with relative proportions systematically varied to simulate realistic contamination profiles. A total of 128 multiplexed observations were analyzed. Measured concentrations were compared to inoculated levels using regression analysis, and performance metrics included

slope, intercept, correlation coefficient, and root mean square error.

Results: ddPCR demonstrated strong agreement between inoculated and measured concentrations, with a regression slope of 0.984 and intercept of -1.56, indicating proportional accuracy and negligible constant bias. The correlation coefficient was 0.995, R^2 was 0.990, and RMSE was 19.4, confirming quantitative integrity across the tested range, even under high-order multiplexing.

Significance: This approach provides a rapid, serovar-specific solution for accurate quantification of mixed *Salmonella* populations, supporting compliance with FSIS performance criteria and enabling improved surveillance programs focused on serovars of greatest public health concern.

P1-172: DEVELOPMENT OF A PCR ASSAY FOR SALMONELLA THOMPSON

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The effective control of foodborne salmonellosis relies on the rapid and reliable detection and identification of the pathogen. Developing improved detection tools focused on the most common *Salmonella* serovars can translate to considerable alleviation of the health burden attributed to *Salmonella*. We have developed a PCR assay for the rapid identification of colonies of *Salmonella enterica* serovar Thompson, which is a common serovar.

Methods: Genomic analyses of publicly available sequences of *Salmonella* Thompson revealed the presence of a unique, Thompson-specific fragment which was used to design a pair of oligonucleotides, *STho-F* and *STho-R,* for the PCR amplification of a 808 bp DNA fragment.

Results: Using crude DNA extracts, the 808-bp fragment was detected in 72 out of 73 isolates of *S.* Thompson (sensitivity = 98.6% n = 73 isolates) but not in any of the non-*Salmonella* organisms assessed (n =100; 100% specificity) nor in non-Thompson *Salmonella* serovars (n=100; 100% specificity). The sensitivity (inclusivity) and specificity (exclusivity) indices of the PCR assay for *S*. Thompson met the standard requirements for food safety testing. The inclusion of the Thompson primer pair in a multiplex PCR previously developed for three other common serovars of *Salmonella* resulted in the correct designation of colonies belonging to serovar Enteritidis (n=100), Heidelberg (n=100) and Typhimurium (n=100),

Thompson (n=72) indicating primers compatibility and perfect inclusivity and exclusivity scores for all the four *Salmonella* serovars tested in a single multiplex PCR.

Significance: Rapid and cost effective detection of *S.* Thompson should contribute to efforts to reduce foodborne salmonellosis and mitigate against the increasing prevalence of antimicrobial resistance shown by the organism.

Award Application: Developing Scientist Award

P1-173: RAPID PATHOGEN SCREENING IN BEEF: 4-HOUR MOLECULAR DETECTION OF SALMONELLA AND STEC USING MICROTALLY CLOTH SAMPLING WITH GENE-UP®

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Rapid screening for foodborne pathogens can improve containment decisions in beef processing. This study assessed a 4-hour molecular workflow on MicroTally cloth sampling for *Salmonella* and *E. coli* O157:H7.

Purpose: To determine detection performance of a 4-hour GENE-UP® workflow on the cloths at low and high inoculum levels for *Salmonella* and *E. coli* O157:H7, and benchmark against reference methods.

Methods: The cloths were inoculated at low (~1 CFU, n=20), high (~5 CFU, n=5), and uninoculated (n=5) levels. Samples were enriched for 4 h at 42°C in 200 mL BPW with BTB supplement, followed by automated extraction and molecular assays for *Salmonella* and STEC targets. Reference culture methods (MLG 5C.02 and 4.13) were performed for confirmation. ASAP and XLD plates were examined after extended incubation for culture confirmation. Statistical analysis was performed at 95% confidence.

Results: For both pathogens, molecular assays showed no statistically significant difference

from reference methods ($p > 0.05$ for all comparisons). All inoculated samples were detected, and controls were negative, yielding 100% agreement with culture confirmation.

Significance: A 4-hour molecular screen using automated extraction provides equivalent detection to reference methods, enabling processors to prioritize containment and root-cause investigations ahead of culture confirmation.

P1-174: PREVALENCE OF STEC VIRULENCE GENES AND TOP-7 SEROGROUP MARKERS ON BOVINE HIDES IN BRAZILIAN BEEF PLANTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen associated with beef products. Little is known about STEC prevalence on bovine hides in Brazil, but they are recognized in the literature as an important STEC reservoir, representing a potential source of cross-contamination during slaughter.

Purpose: This study evaluated the prevalence of STEC virulence genes and major serogroups on bovine hides collected after slaughter in three commercial beef plants in Brazil.

Methods: A total of 32 cattle hides were sponge sampled after bleeding. Samples were enriched in BPW (41.5°C for 8 hr) and analyzed by real-time PCR using iQ-Check STEC VirX and SerO II kits for screening (presence/absence of *stx* and *eae* genes) and identification of top-7 serogroups, respectively.

Results: Both virulence genes (*stx*1 and/or *stx*2 and *eae*) were detected in 31 bovine hide samples (96.9%). Top seven STEC serogroups were frequently identified, including multiple serotypes contamination in the same sample. O157:H7 was detected in 15 samples (46.9%) from two slaughterhouses. Higher detection rates were found for O45 (31 samples; 96.9%), O26 (30; 93.8%), O121 (28; 87.5%), O103 (26; 81.3%) and O145 (23; 71.0%). O111 was detected in only two samples from one slaughterhouse. A statistically significant predominance of non-O157 serotypes was observed in one plant (Fisher's exact test, two-tailed, $p < 0.001$), whereas no significant differences were observed in the other plants ($p > 0.05$).

Significance: This high prevalence of STEC virulence gene markers and top-7 serogroups on bovine hides reinforces the importance of strict pre-slaughter sanitation interventions and good manufacturing practices (GMP). The rapid method used represents a valuable tool for presumptive hazard identification and supports the implementation of targeted interventions to reduce STEC contamination during beef processing.

P1-175: SINGLE-STEP ENRICHMENT APPROACH FOR DETECTION OF SALMONELLA SPP. AND ESCHERICHIA COLI O157:H7 IN BOVINE OFFAL

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The control of *Salmonella* spp. and *Escherichia coli* O157:H7 is essential for beef exporters, as international regulations require their absence in meat products. Bovine offal has increased in commercial relevance; however, it represents a challenging analytical matrix due to potential interferents that may compromise microbiological detection.

Purpose: This study evaluated the performance of a single-step enrichment approach using BAX® System MP Media combined with the BAX® Real-Time *Salmonella* and BAX® *E. coli* O157:H7 EXACT assays for the detection of these pathogens in bovine offal.

Methods: Method verification was conducted according to DOQ-CGCRE-089 (ABNT-INMETRO, 2017). Thirty bovine offal samples (10 tongue, 10 heart, and 10 liver), previously tested and confirmed negative for the target microorganisms, were inoculated with *Salmonella* Typhimurium (ATCC 14028) and *E. coli* O157:H7 (ATCC 35150) at 10–100 CFU/25 g, in the presence of background microbiota (~10^4 CFU/25 g). Samples were enriched with 225 mL of

pre-warmed BAX® System MP Media and incubated at 41.5°C for 16 h. After enrichment, DNA was extracted and analyzed by qPCR using BAX® System assays for *Salmonella* spp. and *E. coli* O157:H7, according to the manufacturer's instructions. Ten non-inoculated samples were included as negative controls.

Results: No false positive or false negative results were observed. Sensitivity, specificity, and overall agreement were 100% for both pathogens across all matrices.

Significance: The results demonstrate that a single-step enrichment approach is suitable for screening *Salmonella* and *E. coli* O157:H7 in bovine offal, reducing analytical workload and accelerating result release.

P1-176: ASSESSMENT OF EXTENDED SAMPLE HOLDING TIME IMPACT FOR MESOPHILIC BACTERIA QUANTIFICATION IN BEEF CARCASS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: The holding time between sample collection and laboratory analysis is a critical factor to achieve reliable microbiological results. For beef carcass swabs, official guidelines typically recommend a maximum holding time of 24 hours. However, logistical constraints often affect timely delivery, raising the need for data-proven efficacy of extended hold periods, such as 48 hours, to prevent sample rejection without compromising analytical integrity.

Purpose: To evaluate the impact of extending refrigerated sample holding time from 24 to 48 hours on quantification of aerobic mesophiles microorganisms in beef carcass swabs.

Methods: Seventy sponge swab samples were collected from five slaughterhouses and transported under refrigeration (2 to 8°C). Samples were kept under refrigeration, and aliquots were processed for mesophilic count (AOAC 990.12) at two distinct time intervals: 24 hours post-collection (T0 – reference holding time) and 48 hours post-collection (T1– extended holding time). Data was analyzed using a paired *t*-test to compare the two scenarios.

Results: No statistically significant variations ($p > 0.05$) were observed between the reference holding time (T0) and the 48-hour test interval (T1). Microbial populations remained stable under refrigeration conditions for the interval tested.

Significance: The extended holding time of 48 hours is comparable to the reference holding time of 24 h and does not impact on the analytical results for mesophilic bacteria in beef carcass swabs. Data showing that use of extended holding time does not interfere in analytical results brings flexibility to the logistic chain, reducing sample rejection and overall analysis cost. Further research is recommended to assess the impact of extended holding times on other indicators, such as *E. coli* and Enterobacteriaceae.

P1-177: INDEPENDENT LABORATORY EVALUATION OF THE EZ-CHECK SALMONELLA SPP. METHOD FOR THE DETECTION OF SALMONELLA SPP. IN A VARIETY OF FOODS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The EZ-Check *Salmonella* assay is a simple and rapid qualitative test allowing for the detection of DNA sequences specific to *Salmonella* spp. using real-time PCR in food products, animal feed, and environmental samples. Up to 94 samples can be processed at one time, with minimal risk of contamination and an easy-to-use procedure. The use of this test allows for results to be obtained within a few hours following enrichment.

Purpose: The purpose of this AOAC® Independent Lab Study was to conduct a method comparison for a variety of food matrices and environmental surfaces.

Methods: The new method was compared to the FDA/BAM and USDA/MLG reference methods for the detection of *Salmonella* spp. following an unpaired study design for a variety of foods and environmental surfaces which included 22 total matrices and an alternative confirmation pathway.

Results: All 100 *Salmonella* spp. inclusivity strains were detected, and all 30 exclusivity organisms were excluded. Qualitative statistical analysis was conducted according to the

Probability of Detection (POD) statistical model for qualitative methods. No statistically significant difference was observed for 30 samples (5 control, 20 low, 5 high) for all food types using 375 g test portions and environmental surfaces. Identical results were obtained for the reference and alternative confirmation procedures.

Significance: This proprietary method allows for fast and reliable detection for *Salmonella* spp. from a variety of food matrices and environmental surfaces. The alternative confirmation procedure provides an additional pathway for the isolation and further confirmation of *Salmonella* spp.

P1-178: VALIDATION OF NEW LISTERIA LYOPHILIZED PCR ASSAYS FOR FOOD AND ENVIRONMENTAL TESTING

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The EZ-Check *Listeria* spp*.* and *Listeria monocytogenes* assays are simple and rapid qualitative tests allowing for the detection *L.* spp*.* and *L. monocytogenes* using real-time PCR in food products and environmental samples with ready-to-use lyophilized PCR reagents. Results can be obtained within a few hours following enrichment on multiple thermocyclers.

Purpose: This study aimed to validate the real-time PCR assays for rapid, qualitative detection of *Listeria* in accordance with ISO 16140-2:2016 protocol.

Methods: The new method was compared to the ISO 11290 reference method for the detection of *L.* spp. and *L. monocytogenes* following an unpaired study design for a broad range of foods and environmental surfaces including dairy, meats, seafood, produce, eggs, multicomponent foods, ready-to-eat items, and environmental surfaces and an alternative confirmation pathway. Both manual and automated extractions were evaluated.

Results: Sensitivity for the EZ-Check *Listeria* spp. assay was 78.0% (592 samples), and for *L. monocytogenes* 74.0% (569 samples), with relative trueness above 74% for both. No false positive or false negative results were found with both alternative methods. Relative limits of

detection (RLOD) were 1.19 for all matrices, below the acceptability threshold of 2.5. All 55 *Listeria* spp. and 50 *L. monocytogenes* inclusivity strains were detected, and all 30 exclusivity organisms were excluded with no false positives/negatives or PCR inhibition observed.

Significance: The assays demonstrated equivalent or superior performance to the reference method and robust reproducibility across platforms and workflows. These findings support the EZ-Check *Listeria* spp. and *Listeria monocytogenes* PCR assays as rapid, sensitive, and specific tools for routine food safety monitoring, enhancing *Listeria* detection across a broad spectrum of sample types, contributing to improved public health protection.

P1-179: INDEPENDENT LABORATORY EVALUATION OF THE EZ-CHECK LISTERIA SPP. AND LISTERIA MONOCYTOGENES METHODS FOR THE DETECTION OF LISTERIA SPP. AND LISTERIA MONOCYTOGENES IN A BROAD RANGE OF FOODS AND SELECTED SURFACES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The EZ-Check *Listeria* spp. and *Listeria monocytogenes* assays are simple and rapid qualitative tests allowing for the detection *Listeria* spp*.* and *Listeria monocytogenes* using real-time PCR in food products and environmental samples. The kits ready-to-use lyophilized PCR reagents contain oligonucleotides (primers and probes) specific for *Listeria* spp. and *L*. *monocytogenes*, as well as DNA polymerase and nucleotides. Results can be obtained within a few hours following enrichment on multiple thermocyclers.

Purpose: The purpose of this AOAC® Independent Lab Study was to conduct a method comparison for a broad range of food matrices and environmental surfaces.

Methods: The new method was compared to the FDA/BAM, USDA/MLG, and ISO 11290 reference methods for the detection of *Listeria* spp. and *L. monocytogenes* following an unpaired study design for a broad range of foods and environmental surfaces including 26

matrices and an alternative confirmation pathway.

Results: All 55 *Listeria.* spp. and 50 *L. monocytogenes* inclusivity strains were detected, and all 30 exclusivity organisms were excluded. Qualitative statistical analysis was conducted according to the Probability of Detection (POD) statistical model for qualitative methods. No statistically significant difference was observed for 30 samples (5 control, 20 low, 5 high) for all food types and all test portion sizes (25 g, 125 g, 375 g) evaluated and environmental surfaces. Identical results were obtained for the reference and alternative confirmation procedure.

Significance: This proprietary method allows for fast and reliable detection for *L.* spp. and *L. monocytogenes* from a broad range of food matrices and environmental surfaces. The alternative confirmation procedure provides an additional pathway for the isolation and further confirmation of *L.* spp. and *L. monocytogenes*.

P1-180: INDEPENDENT LABORATORY EVALUATION OF THE SOLERIS® E. COLI TEST FOR THE DETECTION OF E. COLI IN SELECT FOODS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The Soleris® *E. coli* test is an automated, growth-based method for detecting *Escherichia coli*. The 5.0 mL test vial (EC-104) contains a peptone–yeast extract base with lactose as the carbon source and selective agents including bile salts, sodium lauryl sulfate, and Gram-positive inhibitors. Detection is based on acidification from lactose utilization, using a bromocresol purple pH indicator. Selectivity is achieved through a concentrated inhibitory system and incubation at 43.5°C. Results are available within 18–24 h.

Purpose: The purpose of this AOAC® Independent Lab Study was to conduct a method comparison for two food matrices.

Methods: The new method was compared to the ISO 7251:2005 reference method for detection and enumeration of presumptive *E. coli* following a paired study design for cocoa powder and an unpaired study design for pasteurized liquid egg including an alternative confirmation pathway.

Results: All 50 *E. coli* inclusivity strains were detected, and all 30 exclusivity organisms

excluded. Qualitative statistical analysis was conducted according to the Probability of Detection (POD) statistical model for semiquantitative methods. No statistically significant difference was observed for 30 samples (5 control, 20 low, 5 high) of cocoa powder for dilute-to-specification detection (7/20 pos at fractional level) and pasteurized liquid egg for presence/absence detection where 9/20 were positive vs 8/20 for the ISO method. Identical results obtained for the reference and alternative confirmation procedure.

Significance: This novel method allows for fast and reliable detection of *E. coli* from select foods. The alternative confirmation procedure provides an additional pathway for the isolation and further confirmation of *Escherichia coli.*

P1-181: HARMONIZED VALIDATION STUDY OF A NOVEL FILM-BASED METHOD FOR QUANTITATIVE DETERMINATION OF BACILLUS CEREUS GROUP IN A BROAD RANGE OF FOODS AND ANIMAL FEED

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The Petrifilm® *Bacillus cereus* (BC) Count Plate is a selective, differential, sample-ready culture system for quantitative determination of the *B. cereus* group in foods and animal feed. The method uses proprietary nutrients, cold water-soluble gelling agents, chromogenic indicators, and a lecithinase substrate to support colony enumeration, with results available in 20–24 hours. The *B. cereus* group includes *B. cereus*, *B. anthracis*, *B. cytotoxicus*, and related species; the method does not differentiate among species.

Purpose: This harmonized AOAC OMA/Microval validation was to compare the alternative method to three reference methods in a method comparison study (MCS) and multi-lab study organized by an Expert Laboratory.

Methods: The alternative method was compared to FDA/BAM Chapter 14, USDA/MLG Chapter 12, and ISO 7932:2004 using a paired study design. Six food and feed categories were evaluated: pasteurized milk and dairy, dried cereal, fruits/nuts/seeds/grains, multi-

component foods, RTE and RTRH meat and poultry, infant formula and cereals, and animal feed, using 50 g test portions.

Results: All 52 *B. cereus* group isolates were detected, and all 33 exclusivity organisms were excluded. Statistical analysis was conducted according to both ISO and AOAC statistical models and showed no statistically significant difference between methods in all 6 categories for the MCS and the rice flour for the multi-lab validation study of 12 labs across 6 countries. Relative trueness results met acceptability limits for all 27 food types, and the Accuracy Profiles fell into the acceptability limits (+/- 0.5 log) for the 6 categories. The difference of means showed equivalence (between -0.5 to +0.5 log) at 90% confidence.

Significance: This proprietary method is an easy-to-use, fully validated alternative method for enumerating *Bacillus cereus* group (up to 50 g test portions) in a broad range of foods, animal food and feed using one plate.

P1-182: WITHDRAWN

WITHDRAWN

P1-183: RAPID DETECTION OF SALMONELLA SPP. USING THE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY – BIOLUMINESCENT IN PRIMARY PRODUCTION BOOT SOCKS AND BOOT SOCKS WITH HIGH AMOUNTS OF LIME POWDER COLLECTED FROM A BRAZILIAN CENTER-WEST REGION FARM

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Sanitary measures and verification steps throughout poultry production are necessary for monitoring and controlling *Salmonella* spp. Broiler litter sampling using boot socks may interfere with target detection, therefore a robust, rapid and reliable testing method is needed to support corrective actions.

Purpose: This study aimed to compare the performance of a LAMP-bioluminescent assay with the ISO 6579-1:2017 reference method for *Salmonella* detection in pre-slaughtering boot sock samples.

Methods: Boot socks samples (n=20) were collected from farms located in the Brazilian center-west region in a paired study. Samples consisted of broiler litter (e.g., soil, feathers, feces, wooden shavings, and lime powder) and sterile boot socks moistened with Buffered Peptone Water (BPW ISO). A wild type of strain of *Salmonella* serovar Minnesota was artificially inoculated into the sample matrix to generate 15 fractionally positive samples (0.2–2 CFU/portion) and 5 high-inoculum samples (5 CFU/test portion). Enrichment was

performed by adding 225 mL of double strength BPW ISO to each test portion according to AOAC PTM 091501. Samples were incubated at 37°C for 20–26 hours, then diluted 1:10 in BPW ISO after enrichment, and both sample sets were analyzed with a LAMP-Bioluminescent assay. Confirmation of enriched samples was performed using ISO 6579-1 :2017. Performance acceptability criteria were analyzed based on ISO 16140-2:2016, determining Sensitivity and Relative Trueness.

Results: Sensitivity and Relative Trueness were both 95%. Comparison between the Neogen Molecular Detection Assay 2 *Salmonella* and the ISO 6579-1:2017 reference method showed no statistically significant difference.

Significance: The alternative LAMP method enables reliable and rapid detection of *Salmonella* spp. in primary production boot sock samples.

P1-184: AUTOMATED LIQUID HANDLING SYSTEM PRECISION TRANSFERENCE EVALUATION THROUGH A PATHOGEN MOLECULAR DETECTION ASSAY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The implementation of automated liquid handling systems represents a critical advancement in molecular biology workflows, as these platforms significantly enhance experimental reproducibility by ensuring high-precision volumetric accuracy and establishing robust barriers to mitigate cross-contamination, which are essential for the integrity of high-sensitivity assays such as molecular biology.

Purpose: Determine level of cross-contamination and results of precision of automated liquid handling system compared to a manual transference within the pathogen detection workflow utilizing the Molecular Detection System.

Methods: *Salmonella* spp. was cultivated into a high population (8 $\log_{10}$ CFU/mL) and lysate with a commercial rapid kit (N=1056) were prepared alternating positives and negatives (N=528, each) to determine the level of cross-contamination through the positive results

obtained from the blank wells. Manual pipetting transfer of the culture to the lysate was considered the reference, and it was utilized as comparison.

Results: The automated liquid handling system presented 0% cross-contamination index, being 100% correlated to manual transfer, being its configurated protocol synergistic to the evaluated rapid pathogen molecular detection assay.

Significance: The Loccus Extracta® Assist 200 Liquid Handling System enabled precise aliquot transfer during the pathogen detection workflow of the Neogen® Molecular Detection System.

P1-185: PERFORMANCE COMPARISON OF AN ALTERNATIVE RAPID YEAST AND MOLD COUNTING PLATE AND THE ISO 21527-1:2008 METHODS FOR ENUMERATION OF MOLDS AND YEASTS IN HIGH-FAT DAIRY PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Microbial contamination analyses in finished products are an important tool for the food industry, as the use of fast and reliable methodologies enables quicker product release and efficient quality control.

Purpose: To evaluate the performance of an alternative rapid counting method for enumeration of yeast and molds in the high-fat matrices butter and cream cheese, in comparison with the traditional method based on plate counts using DRBC agar.

Methods: For each matrix, 10 replicates were analyzed in both alternative and reference methods. Samples were artificially contaminated with *Candida albicans* ATCC 10231, and the appropriate sample preparation was followed (ISO 6887-1:2017 and ISO 6887-4:2017). For butter, 41 mL of BPW were added to 50 g of sample, heated in a water bath (45°C for 15 min), and mixed in a stomacher homogenizer for 60 s. After phase separation at room temperature, 1 mL of the lower phase was used to prepare a 1:10 dilution. For cream cheese,

10 g of sample were added to 90 mL of BPW supplemented with Tween 80 (2.6 g/L). After dilution, 1 mL of each sample was inoculated onto the alternative rapid plates and DRBC agar and (3 agar plates per sample) using the surface plating technique. The alternative rapid count plates were incubated at 28°C ± 1°C for 48 h, and DRBC plates were incubated at 25°C ± 1°C for 5 days. After incubation, plates were enumerated and results were log-transformed. A paired *t*-test was conducted to determine statistical differences between enumerations ($p < 0.05$).

Results: The *t*-test shows no significant differences between the Neogen® Petrifilm® Rapid Yeast and Molds (RYM) and the reference method ($P > 0.05$), for enumeration of yeast and molds in high-fat dairy matrices butter and cream cheese.

Significance: The rapid method enabled reliable enumeration of yeast and mold in high-fat food samples within 48 h for dairy products, with results comparable to ISO standards.

P1-186: EVALUATION OF DIFFERENT DNA CONCENTRATION TECHNIQUES FOR SALMONELLA DETECTION AND QUANTIFICATION IN POULTRY PROCESSING MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Conventional *Salmonella* quantification workflows, including the GENE-UP *Salmonella* Quant protocol, rely on centrifugation-based DNA concentration steps that can introduce variability and increase processing time.

Purpose: To initiate a comparison of DNA concentration techniques for *Salmonella* detection and quantification in poultry rinse samples using the GENE-UP *Salmonella* Quant protocol as the reference workflow.

Methods: Poultry rinse samples were inoculated across five contamination levels (0–4 log CFU/mL) and analyzed using a microfiltration-based DNA concentration workflow with embedded extraction (PathoTrak). Thirty samples were analyzed across inoculation levels (n = 30). Quantification results were expressed as log CFU/mL and summarized using descriptive statistics (mean ± SD).

Results: *Salmonella* was detected in ~80% of samples. Mean (± SD) concentrations increased

with inoculation level, with approximate values of 0.80 ± 0.05, 1.10 ± 0.15, 1.40 ± 0.30, 1.75 ± 0.45, and 2.40 ± 0.05 log CFU/mL for levels 0–4. Variability increased at intermediate levels (2–3 log CFU/mL), while low levels (0–1 log CFU/mL) clustered near the lower quantification range.

Significance: These preliminary results suggest microfiltration-based DNA concentration may be a promising alternative workflow for *Salmonella* quantification compared with centrifugation-based preparation.

Award Application: Developing Scientist Award

P1-187: EVALUATION OF INDIVIDUAL AND COMPOSITE MICROBIAL SAMPLING SCHEMES FOR PATHOGEN CONTROL AND PRODUCT LOTTING IN SECONDARY PROCESSING OF POULTRY PRODUCTS

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Presentation type/Format: Poster

Category: Meat, Poultry and Eggs

Abstract:

Introduction: Poultry processors commonly use rinsate sampling of individual product portions to verify microbial control, but interest in composite sampling strategies requires evaluation of whether these approaches accurately represent contamination within production-defined lots.

Purpose: To compare individual and composite sampling schemes for the enumeration of microbial indicators and *Salmonella* outcomes in poultry products.

Methods: Chicken tenders were sampled across two production days using three schemes: Individual Rinse (IR), Meat Composite (MC), and Rinse Composite (RC). Thirty-two sequential product lots (~2,000 lb combos) were collected per day (n = 192 samples). Aerobic counts (AC), Enterobacteriaceae, *Salmonella* prevalence, and *Salmonella* concentrations (log CFU/mL)

were evaluated. Differences among schemes were analyzed using Friedman tests with Wilcoxon post-hoc comparisons, and prevalence using Cochran's Q with McNemar tests (α = 0.05).

Results: Sampling scheme influenced microbial outcomes. On Day 1, median AC values were higher for IR (3.3 ± 0.6 log CFU/mL) compared with MC (2.2 ± 0.5) and RC (2.4 ± 0.5) (Friedman $P = 1.08 \times 10^{-8}$), with Wilcoxon tests confirming higher IR values versus MC ($P = 9.01 \times 10^{-6}$) and RC ($P = 1.39 \times 10^{-4}$). *Salmonella* concentrations also differed (Friedman $P = 0.0021$), with higher values in IR (1.3 ± 0.4 log CFU/mL) compared with MC (1.05 ± 0.2, $P = 0.0237$) and RC (1.05 ± 0.25, $P = 0.0126$). On Day 2, AC distributions were similar across schemes ($P = 0.430$), but *Salmonella* prevalence differed (Cochran's Q $P = 0.0195$), with higher detection in IR than MC (McNemar $P = 0.0200$).

Significance: Individual rinsate sampling was generally more sensitive for detecting and quantifying microbial contamination, while composite approaches may dilute contamination signals and underestimate variability within production lots.

Award Application: Developing Scientist Award

P1-188: EVALUATION OF TWO METHODS FOR DETECTING SALMONELLA IN COCOA MATRICES

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Category: Laboratory and Detection Methods

Abstract:

Introduction: *Salmonella* contamination in cocoa and chocolate products poses a significant food safety risk. Reliable detection methods are essential for public health protection.

Purpose: This study aimed to validate and compare the performance of a chromogenic media and a real-time PCR method for detecting *Salmonella* spp. in cocoa and chocolate products, following the EN ISO 16140-2:2016 standard.

Methods: Sensitivity study was performed assessing 72 (chromogenic) and 76 (PCR) artificially contaminated cocoa and chocolate matrices (375 g samples). The RAPID'*Salmonella* method relies on chromogenic agar for presumptive detection, while the EZ-Check *Salmonella* method employs a 2-step-PCR-based protocol. Each method was tested against the ISO 6579-1:2017 reference method. Enrichment was performed with 375 g sample + 3375 mL (1/10 dilution) pre-warmed (41.5°C) BPW incubated for 18–24 hr at 41.5±1°C for both alternative methods. Relative limit of detection (RLOD) was assessed with 25 raw cocoa powder samples contaminated with *Salmonella* Give. Statistical analyses followed ISO 16140-2:2016 and ISO 16140-2:2016/Amd1:2024 guidelines.

Results: The RAPID'*Salmonella* method resulted in 8 negative deviations (ND) and 7 positive deviations (PD) (sensitivity of 80.0%). The EZ-Check *Salmonella* method gave 8 ND and 6 PD (sensitivity of 78.9%). Both methods complied with acceptability limit (TND-PD>AL=3). The RLOD values were also below the acceptability threshold (2.5) for both methods (0.873), indicating equivalent detection limits with the reference method. All positive samples were confirmed by both protocols. No significant differences were observed after storage of enrichment broths for 72 hr at 5°C.

Significance: The two validated *Salmonella* methods provide reliable alternatives for *Salmonella* detection in cocoa and chocolate products, supporting food safety and public health objectives.

P1-189: EVALUATING A CHROMOGENIC AGAR METHOD FOR ENVIRONMENTAL DETECTION OF CRONOBACTER SPP. IN INFANT FORMULA AND CEREAL FACILITIES

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Category: Laboratory and Detection Methods

Abstract:

Introduction: *Cronobacter* spp. contamination in infant formula production environment poses a significant food safety risk. Reliable detection methods are essential for public health protection.

Purpose: This study evaluated the performance of a chromogenic media based alternative method (RAPID's*akazakii* agar), for detecting *Cronobacter* spp. in environmental samples collected from infant formula and cereal production sites, in accordance with ISO 16140-2:2016 and ISO 16140-2:2016/Amd1:2024.

Methods: An unpaired study design was used to compare the alternative method with the ISO 22964:2017 reference method. Sensitivity, relative trueness, false positive/false negative ratios, and RLOD were assessed using both artificially and naturally contaminated samples. The stability of enrichment broth and agar plates after refrigerated storage for 3 days at 2–8°C was also evaluated.

Results: For the environmental samples category, the alternative method demonstrated a

sensitivity of 83.3% and a relative trueness of 88.3%, with no false positives and no false negatives reported. The difference of deviations (TND-PD) observed was consistent with the unpaired sampling design. RLOD value was 1.107 and was below the acceptability limit of 2.5, indicating equivalent detection capability to the reference method. Refrigerated storage studies showed no impact on the results.

Significance: The RAPID'*sakazakii* method study on environmental samples demonstrated compliance with all data requirements and interpretative standards outlined in ISO 16140-2:2016 and ISO 16140-2:2016/Amd1:2024. The method is considered equivalent to ISO 22964:2017 for the detection of *Cronobacter* spp., supporting its suitability for routine industrial monitoring.

P1-190: CULTURE-INDEPENDENT AND RAPID SALMONELLA DETECTION IN POULTRY RINSATE USING FIBER-OPTIC SURFACE ENHANCED RAMAN SPECTROSCOPY

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Enhancing food safety within poultry production relies heavily on the swift and precise identification of foodborne pathogens. Traditional detection methods are limited by

lengthy enrichment and the high organic load in chicken carcass matrices, underscoring the need for culture-independent diagnostics.

Purpose: This study validates a fiber-optic-based surface-enhanced Raman spectroscopy (SERS) label-free sensor for the detection of *Salmonella* directly from chicken rinsate without the need for enrichment or extensive processing.

Methods: A completely randomized design was used to evaluate sensor performance. Raw chicken rinsate was filtered using a 70 µm cell strainer to remove debris. The rinsate was spiked with serial dilutions of *Salmonella* Typhimurium (ATCC 13311) and *S.* Enteritidis (ATCC 13076). Optimal sensitivity was determined using 1-ml samples (N=15) at concentrations ranging from 1 to 10^^{3} CFU/mL with two replicates/concentration. Samples were loaded into a testing chamber, and SERS spectra were measured before and after to obtain the background signal and total relative intensity. OceanView software (V2, Ocean Optics, USA) was utilized for initial spectral acquisition and peak identification, while OriginPro 2025b was employed for detailed peak analysis and comparison to determine consistency across samples.

Results: SERS spectral peaks corresponding to *Salmonella*'s molecular fingerprints were detected within 10 minutes without enrichment. Significant differences in Raman intensity were observed among concentration levels. For *S.* Typhimurium, significant Raman peaks were detected at 495 ± 5cm^{-1}, 608 ± 4cm^{-1}, 808 ± 7cm^{-1}, and 1052 ± 7cm^{-1}, confirming the high degree of molecular fingerprint sensitivity in the low cell range.

Significance: Reducing reliance on slow conventional tests can improve poultry processing efficiency. Pre-market *Salmonella* detection in chicken rinsate delivers significant food safety and public health gains.

P1-191: IMPROVING RECOVERY OF SALMONELLA IN SOIL AND SEDIMENT

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Preharvest traceback of foodborne outbreaks frequently involves recovering pathogens from complex environmental samples where detection can be challenging.

Purpose: To conduct a single-laboratory validation of an optimized method for recovering *Salmonella* in soil and sediment, compared with a previously validated reference method.

Methods: This study was conducted in accordance with FDA guidelines for the validation of qualitative detection methods for microbial species. Four soil types were used: silt, sand, clay and sediment. The reference method (RM) and alternate method (AM) differed in test portion size (RM – 25g, AM – 100g) and the sample-to-broth ratio of modified Buffered Peptone Water used as the preenrichment (RM – 1:9, AM – 1:5). A comparison was also made between Hektoen enteric (HE) agar and HE agar containing novobiocin (HE+N). Following preenrichment, all test portions underwent FDA qPCR analysis using two platforms (QuantStudio 5, 7500 Fast) and two DNA extraction methods (Maxwell RSC Cultured Cells

DNA Kit, BioSprint 96 One-For-All Vet Kit). A total of 240 samples were evaluated.. The limit of detection (LOD) was calculated using the non-parametric Spearman-Kärber approach. Results were statistically analyzed using Fisher's Exact Test for pairwise comparisons.

Results: HE and HE+N showed no false positives or negatives, but HE+N yielded more *Salmonella*-like colonies in negative samples, suggesting novobiocin is unnecessary in HE agar. The average LODs were 1.69 CFU/100g and 1.73 CFU/25g for the AM and RM, respectively. The relative LOD (LOD_{AM}/LOD_{RM}) was 0.97, indicating that the AM is more sensitive in isolating *Salmonella.* No significant differences were seen between the soil types, DNA extraction methods, or qPCR platforms.

Significance: This study provided an optimized and validated method for the recovery of *Salmonella* from soils and sediments, improving environmental monitoring capabilities for field grown food commodities.

P1-192: CHARACTERIZATION OF A NOVEL SALMONELLA BACTERIOPHAGE FOR FOOD SAFETY APPLICATIONS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Millions of salmonellosis infections are linked to contaminated food. Due to their high specificity, bacteriophages can be employed as control agents across a variety of food products.

Purpose: This study aims to characterize a novel *Salmonella*-specific bacteriophage and its application as a biocontrol agent in food products.

Methods: A novel lytic *Salmonella* bacteriophage, BAM-25, isolated from wastewater, was purified using double-layer agar plating and ultracentrifugation. Whole-genome sequencing (WGS), genome assembly and transmission electron microscopy (TEM) were performed to characterize the phage. BAM-25's host range was evaluated against 60 bacterial species, including 15 *Salmonella* strains, using a modified double-layer agar method. Optimal MOI was tested at 0.0001–1000, thermal stability at 25–90°C, and lytic activity at MOIs of 0.001–1000 over 12 h. All experiments, except host range study, were performed in triplicate and analyzed in RStudio.

Results: WGS results revealed double-stranded DNA with a size of 114360 bp. Whole-genome BLASTn analysis indicated close relatedness to phages within the family

Demerecviridae and genus *Epseptimavirus*. TEM images showed an icosahedral capsid with a diameter of 90.1 ± 5.7 nm and a long non-contractile tail of 178.5 ± 9.3 nm. BAM-25 showed host specificity towards five *Salmonella enterica* serovars (*S*. Enteritidis, *S*. Typhimurium, *S*. Poona, *S*. Infantis, and *S*. Choleraesuis). It significantly inhibited *Salmonella* growth at MOIs of 0.01 in 12 h, reaching maximal propagation at a MOI of 0.01 (P 0.05), whereas MOIs of 1 produced significantly lower titers. BAM-25 remained stable at 25–60°C with no significant loss in titer ($P > 0.05$), but viability declined significantly at 70°C (P 0.05), with complete inactivation at 80°C.

Significance: These findings demonstrate the potential of phage BAM-25 as a highly specific and thermally stable biocontrol agent for *Salmonella* in food safety applications.

Award Application: Developing Scientist Award

P1-193: A NOVEL CHECKPOINT-ENCODED NANOPORE DETECTION OF SALMONELLA SEROVARS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Unlike conventional complex sequencing workflows, nanopore sensing offers a rapid, single-molecule detection of pathogens. Herewith, a checkpoint-encoded ID-probe nanopore assay targeting *Salmonella* serovar-specific markers was evaluated for electrical fingerprint detection without sequencing.

Purpose: This study aims to design and validate a PCR–nanopore workflow for serovar-level detection of *Salmonella* using ID-probe-tagged genetic markers.

Methods: ID-tagged primers targeting marker genes for four *Salmonella* serovars, Typhimurium, Enteritidis, Muenchen, and Hadar were designed with an oligo-ethylene glycol spacer separating the ID sequence from the primer region. PCR-amplified target marker genes produced amplicons containing two hybridization sites for a universal encoder oligonucleotide. During nanopore testing, duplexes formed between the ID tag and encoder were translocated through an MspA nanopore. The nanopore sequentially unzipped the ID/encoder duplex and amplicon, generating checkpoint-dependent current states that served as electrical identifiers for the target molecule.

Results: Amplification of marker genes was verified using QIAcuity digital PCR, and ID-tagged amplification was confirmed by gel electrophoresis. Purified, encoder-annealed amplicons tested at 0.5 ng/μL in the nanopore chamber produced a mean nanopore event rate of 0.6 signatures per min. Each event displayed three reproducible current states corresponding to two checkpoint sequences followed by amplicon unzipping. Control experiments confirmed that the multi-state nanopore current patterns were specific to the ID-tagged constructs for each serovar.

Significance: These findings demonstrate the feasibility of a novel checkpoint-encoded electrical fingerprint detection of *Salmonella* markers without sequencing and support development of simpler, portable single-molecule diagnostics for food safety monitoring.

P1-194: RAPID DETECTION AND VIABILITY ASSESSMENT OF SALMONELLA ENTERITIDIS AND SALMONELLA TYPHIMURIUM IN TURKEY RINSE USING FIBER-OPTIC SERS SENSOR

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* contamination in poultry products remains a significant food safety concern, highlighting the need for rapid detection tools that can identify low-level contamination and differentiate between live and dead cells.

Purpose: This study aims to evaluate a fiber-optic Surface Enhanced Raman Spectroscopy (SERS) sensor for detecting *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) in

turkey rinse, and for distinguishing viable from non-viable cells.

Methods: Turkey rinse samples, inoculated with 10–1000 CFU/mL concentrations of single strains of SE, ST and mixture were analyzed using the fiber-optic SERS platform. Spectral acquisition and processing were performed with OceanView 2.0.14-EZ software to assess detection sensitivity, repeatability, and background interference. *Salmonella*-inoculated samples were treated with peracetic acid (PAA) at 10–100 ppm for 10 min or subjected to thermal treatment at 95°C for 30 min prior to analysis to assess the ability of the sensor to differentiate viable from non-viable cells.

Results: The sensor successfully detected both SE, ST and mixture in turkey rinse at concentrations as low as 10 CFU/mL within a 10-minute analysis time. It also produced reproducible SERS spectra with characteristic *Salmonella* peaks. Distinct spectral changes were observed following PAA and heat treatments, including loss or weakening of key Raman peaks, enabling clear differentiation between live and dead *Salmonella* cells. Increasing PAA concentrations resulted in progressive reductions in peak intensity.

Significance: These results demonstrate that the fiber-optic SERS sensor provides rapid, sensitive detection of *Salmonella* in turkey rinse while simultaneously assessing cell viability. This approach shows strong potential for in-plant screening and real-time food safety monitoring in poultry processing environments.

Award Application: Developing Scientist Award

P1-195: PERFORMANCE COMPARISON OF A RAPID CULTURE-BASED METHOD AND THE CONVENTIONAL ISO METHOD FOR DETECTION OF SALMONELLA SPP. IN EGG-CONTAINING MATRICES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: The increasing demand for ready-to-eat products with short shelf lives underscores the need for rapid and accurate detection of *Salmonella* spp., a major foodborne pathogen frequently associated with egg-containing matrices.

Purpose: To evaluate the performance and feasibility of a rapid alternative method and a combined approach compared with the conventional ISO reference method for detecting *Salmonella* spp. in egg-containing matrices.

Methods: Kimbap and shell egg samples were analyzed using a commercial rapid alternative

method (Petrifilm® *Salmonella* Express System) and a traditional agar-based reference method (ISO 6579-1:2017), as well as a combined approach involving conventional enrichment followed by the rapid system. Samples (n = 26) were artificially inoculated with *Salmonella* Enteritidis at fractional levels adapted to each matrix: uninoculated controls, low (0–5 CFU/test portion), and high (5–10 CFU/test portion). Results were confirmed using a LAMP-bioluminescent assay. Relative levels of detection (RLOD) were determined using an unpaired study design for the alternative method and a paired study design for the combined method.

Results: Using the traditional agar-based method as the reference, the RLOD values for kimbap and shell eggs were 1.5 and 0.5 while the alternative method and the combined approach yielded values of 1.5 and 0.8, respectively. These RLODs were within the acceptable thresholds (<2.5, unpaired study and <1.5, paired study), demonstrating that both alternative methods are comparable to reference ISO method.

Significance: These findings support the suitability of rapid detection approaches for *Salmonella* spp. in egg-containing products and demonstrate their practical utility in food safety testing where timely and reliable results are essential.

P1-196: A NOVEL HIGH-RESOLUTION, SINGLE-TUBE PCR-NGS ASSAY FOR COMPREHENSIVE DETECTION, GENOTYPING, AND SURVEILLANCE OF 28 LISTERIA SEROTYPES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Listeria monocytogenes* causes severe foodborne infections. While non-*monocytogenes* species rarely cause human disease, genotyping them is valuable for environmental monitoring, contamination control, and food safety surveillance. WGS is expensive, labor-intensive, requires pure isolates and bioinformatics expertise, and takes 8–10 days.

Purpose: To present a novel high-resolution, cost-effective single-tube PCR assay with a streamlined workflow, capable of detecting, serotyping, and subtyping of selective *Listeria* serotypes, identifying multiple serotypes within a single sample including user-friendly data analysis software.

Methods: The MLSTnext *Listeria* NGS assay was developed to detect and genotype known *Listeria* serotypes. The assay amplifies multiple polymorphic loci from all serotypes in a single-tube PCR reaction followed by NGS. Performance was evaluated using 15 reference serotypes and 85 samples containing single or mixed populations of up to four serotypes. Data were analyzed by ChapterDX software containing 65,000 reference genomes,

representing essentially all publicly available *Listeria* genomes in NCBI database.

Results: The *Listeria* assay accurately detected and serotyped all 15 reference serotypes and correctly characterized all 85 test samples, including reliable detection of multiple co-existing serotypes within the same sample. The assay demonstrated 100% specificity and LOD of <10 copies. Moreover, the assay differentiated *L. monocytogenes* serotype to strain level. Distinct loci profiles enabled high-resolution traceability and source tracking for *L. monocytogenes*, *L. marthii*, *L. innocua*, *L. welshimeri*, *L. ivanovii*, and *L. seeligeri*, supporting contamination surveillance and outbreak management.

Significance: The novel *Listeria* assay provides high-resolution, user-friendly, and cost-efficient alternative to WGS, delivering results within 11 hours after pre-enrichment, without requiring pure isolates or bioinformatics expertise.

P1-197: NOVEL LISTERIA SPP. DETECTION METHOD FOR EFFECTIVE ENVIRONMENTAL MONITORING PROGRAM

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: An effective *Listeria* environmental monitoring program (EMP) is critical to reduce the risk of listeriosis and ensure food safety. We developed a novel enrichment-free molecular detection assay (MDA) *Listeria* Right Now (LRN), that detects *Listeria* from environmental surfaces using a proprietary sampler, enabling flexible sampling up to 12"x12" and delivering results under 2 hours.

Purpose: To evaluate the performance of LRN for detection of *Listeria* from environmental surfaces, compared to the FDA BAM reference method.

Methods: Stainless steel (SS), plastic and sealed concrete surfaces were inoculated with 0 (n=10), low (n=40) or high (n=10) levels of *Listeria.* Inoculated surfaces were allowed to dry for

approximately 1h before sampling. Detection of *Listeria* from the environmental surfaces was compared in an un-paired study for each surface type to an enrichment-based reference method. Assay robustness was assessed by introducing deliberate variations (2–5%) of lysis times, temperatures and lysate volumes added to the reagent tubes against the nominal conditions. The differences of probability of detection (dPOD) and 95% confidence-interval were calculated to compare analytical methods.

Results: The dPOD and 95% confidence-interval analysis showed no statistical difference (plastic) or higher (SS and sealed concrete) detection rates by LRN compared to the reference method at low *Listeria* inoculation level (<10 CFU/surface). LRN agrees with reference method across all surfaces at higher inoculation level (20–40 CFU/surface). Robustness testing demonstrated that slightly altered assay parameters (lysis time, temperature and lysate volume) did not significantly impact results.

Significance: Sensitive and rapid detection of *Listeria* from environmental surfaces in less than 2 hours represents a major advancement in implementing an effective *Listeria* EMP, enabling quick identification and immediate corrective action.

P1-198: IMPACT OF SANITIZER RESIDUES ON THE PERFORMANCE OF LISTERIA RIGHT NOW™ RAPID DETECTION ASSAY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Listeria* Right Now (LRN) is a rapid, enrichment-free molecular assay for detecting *Listeria* from environmental surfaces. Since sanitizer residues often remain after cleaning in food industry environments, it is critical to evaluate the assay's performance under these conditions.

Purpose: This study evaluated both the neutralization effectiveness of the sampling devices and the LRN assay's tolerance to widely used sanitizers in food processing environments.

Methods: Four sanitizers – Bleach, Peraside, PI Quant 20, and Star San were tested. Neutralization effectiveness was evaluated by spiking the sampling devices with *Listeria*, applying the sanitizers to the sampling devices and plating immediately and after 10 minutes of contact time. Assay tolerance was assessed by spraying each sanitizer at the recommended concentration applied to food or non-food contact surfaces, sampling, spiking the sampling devices with *Listeria*, and running the LRN assay. Matrix controls assays were included to check for assay inhibitory effects. The data was analyzed using t-test to determine if there was statistically significant difference when sanitizer residues were

present compared to controls with no sanitizer.

Results: Plating results showed no significant difference ($P > 0.05$) in counts immediately after sanitizer application compared to 10 minutes post-application, and between sanitizer treated samples and PBS controls. Similarly, LRN assay performance was unaffected by sanitizer residues, with no significant difference ($P > 0.05$) between each sanitizer and water control. All matrix control tests were valid in the presence of sanitizers.

Significance: The novel enrichment-free *Listeria* assay demonstrated robustness in the presence of commonly used sanitizer residues. Rapid detection of *Listeria* from environmental surfaces represents a major advancement in implementing an effective *Listeria* EMP, enabling quick identification and immediate corrective action.

P1-199: DNA REMOVAL TO REDUCE FALSE POSITIVES IN FOOD MOLECULAR TESTING

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Category: Laboratory and Detection Methods

Abstract:

Introduction: Molecular methods can detect DNA from non-viable microorganisms, leading to false-positive results in food safety testing. This study evaluates the SureClean™ DNA Removal Kit as a pre-analytical treatment to eliminate extracellular DNA while preserving DNA from viable cells, improving workflow specificity, confidence in results, and efficiency in food testing workflows.

Methods: The efficiency of a DNA removal solution was evaluated using samples artificially contaminated with non-viable DNA from *Salmonella, Listeria, Vibrio, Cronobacter,* and *Staphylococcus* species. PCR was performed before and after treatment, with Ct shifts analysed across 534 samples representing diverse matrices, including seafood, powdered infant formula and environmental samples. Sensitivity impact was assessed using Relative Level of Detection (RLOD) studies in SureTect™ workflows utilising large sample sizes and short enrichment times, comparing Ct shifts and detection rates between treated and

untreated samples.

Results: The DNA removal solution reduced false-positive results by 78.48%, with remaining positives showing greater than 2-log reductions in DNA. Sensitivity assessment across 270 samples demonstrated no impact on performance, with an average RLOD of 1.03 and no statistically significant difference between treated and untreated samples ($P = 0.944$).

Significance: This study shows that the evaluated DNA removal solution effectively improves PCR result accuracy by selectively removing extracellular DNA. The solution performed reliably across diverse, high-risk food matrices, reducing false positives while maintaining assay sensitivity. RLOD studies confirmed unchanged detection performance under challenging SureTect™ conditions, demonstrating the solution’s value as a practical, robust enhancement to molecular food safety testing workflows.

P1-200: FULLY VALIDATED DNA-REMOVAL SOLUTION FOR CRONOBACTER AND LISTERIA PCR ASSAYS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: DNA from non-viable cells can persist in powdered infant formula (PIF), dairy products, and environmental samples due to processing stresses, sanitation activities, or ingredient carryover. This residual DNA can be amplified by molecular detection assays and lead to false-positive results. A reliable solution for removing DNA prior to PCR testing is therefore needed to improve result interpretation while maintaining assay sensitivity.

Purpose: This independent lab study evaluated whether the optional DNA removal step impacts the performance of the Thermo Scientific™ SureTect™ PCR Assay methods for detection of *Cronobacter* spp., *Listeria* spp., and *Listeria monocytogenes* across PIF, dairy, and environmental matrices. The objective was to demonstrate that inclusion of the solution does not reduce analytical sensitivity.

Methods: Performance was assessed through an ISO 16140-2 comparative validation supported by AFNOR Technical Rules and AOAC Appendix J guidelines. Each SureTect assay was tested with and without DNA removal and compared to the corresponding reference methods. Statistical analyses included determination of difference of probabilities of

detection (dPOD) and relative limits of detection (RLOD). A total of 180 samples were tested across the SureTect methods.

Results: Environmental sample dPODs at the fractional level were -0.20 for *Listeria monocytogenes*, 0.15 for *Listeria* spp., and 0.00 for *Cronobacter* spp. when compared to the reference method. For PIF, fractional level dPODs were 0.00 for *L. monocytogenes*, 0.00 for *Listeria* species, and 0.05 for *Cronobacter* species.

Significance: The data demonstrates that incorporating the DNA removal procedure does not affect assay sensitivity and performance is comparable to the reference methods. These validated workflows enable improved confidence in PCR testing by reducing false-positive risks without compromising detection performance.

P1-201: A FULLY AUTOMATED TNGS ASSAY CAN PROVIDE SPECIES-LEVEL RESOLUTION DETECTION OF LISTERIA SENSU STRICTO FOR IMPROVED ROOT CAUSE ANALYSIS OF CONTAMINATION EVENTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: General *Listeria* detection is effective for broad monitoring, but species-level identification within the *Listeria sensu stricto* clade is essential for precise troubleshooting, distinguishing persistent residents from transient arrivals & ensuring sanitation efforts are appropriately scaled.

Purpose: Evaluate accuracy of an automated tNGS assay to detect *Listeria* genus & identify *sensu stricto L.* species, comparing results to known identities & qPCR assay.

Methods: 115 *L. sensu stricto*, 23 *L. sensu latto* & 26 non-*Listeria* (164 total) isolates were grown in BHI broth, incubated overnight at 35°C & analyzed by automated tNGS & FDA BAM Lall qPCR. Samples were loaded onto the automated tNGS platform that performs cell lysis, live/dead cell screening, PCR, library preparation & sequencing. Amplicon sequences were

analyzed by mapping to a reference database for species confirmation & strain typing of *L.* isolates. qPCR was performed according to FDA BAM Protocol.

Results: The automated tNGS assay resulted in 93.9% accuracy and a Cohen's kappa of 0.80 at the genus level, indicating substantial agreement with certified culture collection identifications. The FDA Lall qPCR for *Listeria* genus detection resulted in 83.5% accuracy & Cohen's kappa of 0.56, indicating only moderate agreement. Statistical metrics calculated by excluding the *sensu latto* group (141 strains) revealed that the automated tNGS was 100% accurate, with a 1.00 Cohen's Kappa indicating perfect agreement. qPCR accuracy was greatly improved, resulting in 96.4% accuracy & Cohen's Kappa of 0.89 indicating almost perfect agreement. The discrepant false negative findings for qPCR resulted from only detecting 6 of 11 *L. seelegeri* strains.

Significance: Clear Safety *Listeria* automated tNGS assay provides superior taxonomic sensitivity across the entire *Listeria* genus compared to FDA qPCR, accurate species level identification in 48h, & substantially improves root cause analysis for contamination events.

P1-202: VALIDATION OF THE RAPIDCHEK LISTERIA NEXTDAY PLUS TEST SYSTEM FOR DETECTION OF LISTERIA SPP. IN MATRIXES: HOT DOGS, FROZEN COOKED BREADED CHICKEN, ICE CREAM, STAINLESS STEEL, PLASTIC, RUBBER, AND PAINTED CONCRETE SURFACES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Food processing operations often test for *Listeria* spp. as a marker, in a rapid and cost-effective manner to provide evidence of effectiveness in their process control, of sanitation procedures, and to uncover conditions that allow for the presence, growth, and persistence of *Listeria*.

Purpose: The RapidChek *Listeria* NextDay PLUS test system for the detection of *Listeria* spp. in selected foods including hot dogs, frozen cooked breaded chicken, and ice cream, and on environmental surfaces, including stainless steel, plastic, rubber, and painted concrete was validated using an unpaired study design.

Methods: The method uses a proprietary enrichment medium, a 24 to 48 h enrichment at 30 ± 1^{o}C and detects *Listeria* spp. on an immunochromatographic lateral flow device within 10

minutes. *Listeria* inoculated matrixes were tested by the method, and the cultural reference method. The method was tested with 50 *Listeria* strains and 30 non-target strains.

Results: The candidate method yielded 101 presumptive positives, at 24H or 27H enrichment, while the reference method produced a total of 93 confirmed positive, out of 140 low- level samples per method. Probability of Detection analysis demonstrated no significant differences in the number of positive samples detected by the candidate method and cultural reference method at the 95% confidence level. The method detected all 50 *Listeria* strains and none of the 30 non-target strains when adhering to method enrichment.

Significance: The method performed as well as the reference method in the detection of *Listeria* spp. on environmental surfaces after 24 h of enrichment and in selected food matrixes after 27 h of enrichment, providing the end user with a rapid, ease of use, robust, and reliable tool for monitoring the presence of *Listeria* in food producing environments and final product.

P1-203: VALIDATION OF ROMER LABS RAPIDCHEK® LISTERIA MONOCYTOGENES NEXTDAY™ PLUS TEST SYSTEM FOR THE DETECTION OF LISTERIA MONOCYTOGENES ON SELECT FOOD MATRIXES AND ENVIRONMENTAL SURFACES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Listeria monocytogenes* causes listeriosis, a rare but severe foodborne disease with a mortality rate of 20–30%, particularly among elderly and immunocompromised individuals. Because *L. monocytogenes* is ubiquitous and can contaminate food and food processing environments, especially ready-to-eat foods that receive no further heat treatment prior to consumption, rapid detection methods are essential for food safety monitoring.

Purpose: The aim of this study was to validate the Romer Labs RapidChek® *Listeria monocytogenes* NextDay™ Plus Test System against the USDA-FSIS-MLG and FDA-BAM cultural reference methods for detecting *L. monocytogenes* on selected food matrixes and environmental surfaces using an unpaired study design.

Methods: The RapidChek® method uses proprietary enrichment media with incubation at 30 ±1°C for 44–48 h, followed by detection on an immunochromatographic lateral flow device

within 10 minutes. Multiple *L. monocytogenes* strains were used to spike each matrix. Presumptive positives were confirmed based on the reference and an alternate confirmation method.

Results: The candidate method produced 82 presumptive positives, of which 81 were confirmed by the alternate confirmation method. The respective cultural reference methods produced 74 confirmed positives. All non-spiked samples across all tested matrices were negative for *Listeria monocytogenes* by both methods. Probability of Detection (POD) analysis was performed and for all the matrices evaluated, the difference in POD (dPOD) between the candidate and reference methods included zero at 95% confidence interval, indicating no statistically significant differences between methods.

Significance: The RapidChek® *Listeria monocytogenes* NextDay™ Plus Test System demonstrated performance comparable to USDA-FSIS-MLG and FDA-BAM reference methods while providing rapid results, supporting its use for monitoring *L. monocytogenes* in food and food processing environments.

P1-204: PERFORMANCE EVALUATION OF EASY PLATE™ LS FOR SELECTIVE DETECTION OF LISTERIA SPECIES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Listeria monocytogenes* is a major foodborne pathogen causing listeriosis through contaminated food. Environmental monitoring for *Listeria* species is important for controlling *L. monocytogenes*. A newly developed ready-to-use (RTU) medium, Easy Plate™ LS, is convenient and environmentally friendly and allows for rapid enumeration in just 24 hours.

Purpose: The selectivity of the RTU medium was evaluated in comparison with conventional agar media and other RTU media, using *Listeria* strains and food-derived non-target strains.

Methods: For the inclusivity study, 28 *L. monocytogenes* strains and 20 strains of other *Listeria* spp. were tested. For the exclusivity study, 28 non-target strains were tested. Each strain was enriched overnight and diluted in BPW. The evaluated RTU medium was compared with three conventional media (ALOA, PALCAM, and a chromogenic agar) and two RTU media (RTU-A and RTU-B). All media were inoculated with approximately 100 CFU per plate and incubated at 35 or 37°C for 24, 28, and 48 hours.

Results: All *L. monocytogenes* strains grew on every medium within 24 hours. For *Listeria* spp., the evaluated RTU medium supported growth in 75% (15/20) of strains at 24 hours and 85% (17/20) at 28 hours, whereas RTU-A and RTU-B showed only about 50% growth at 24 hours. ALOA supported growth in 90% (18/20), while the other agars supported 65–70% at 24 hours. Species such as *L. seeligeri*, *L. ivanovii*, and *L. grayi* often required extended incubation. Regarding exclusivity, the evaluated RTU medium, RTU-A, and ALOA showed no growth of non-target strains up to 48 hours. In contrast, some *Enterococcus* and *Bacillus* strains exhibited growth on other media when incubation was prolonged.

Significance: The evaluated RTU medium shows high selectivity and reliable detection of most *Listeria* species within 24 hours. By removing complex preparation steps, this medium is expected to significantly enhance environmental monitoring programs for *Listeria.*

P1-205: EVALUATION OF ASSURANCE® GDS FOR LISTERIA SPP. TQ II AND ASSURANCE® GDS FOR LISTERIA MONOCYTOGENES TQ II FOR DETECTION OF LISTERIA SPECIES AND LISTERIA MONOCYTOGENES IN FOODS AND ENVIRONMENTAL SAMPLES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Assurance® GDS for *Listeria* Tq II is an automated nucleic acid amplification system, where the target pathogen is concentrated by immunomagnetic separation post-enrichment in Actero™ Enrichment media for the detection of *Listeria.*

Purpose: Alternative method for detection of *Listeria* spp. and *L. monocytogenes* were compared to USDA-*MLG* 8.15 or the FDA *BAM* Chapter 10 reference methods for the detection of *Listeria* species and *L. monocytogenes* in food and environmental samples.

Methods: Using unpaired samples, the performance of the alternative methods were compared to USDA-MLG 8.15 or the FDA BAM Chapter 10. Chicken sausage (RTE) (25 and 125g), deli ham (25 and 125g), salami (25 and 125g), fresh fruit salad (25g), ice cream (25 and 125g), pasteurized cheese (25 and 125g), frozen cooked shrimp (25g), and stainless steel (4”x 4”) were inoculated at three levels; five replicates of non-inoculated material, 20 replicates at

a low-contamination level to yield fractionally positive results, and five replicates at a high-contamination level. All samples were enriched at 30˚C in Actero™ media with 25g and 125g samples tested at 22 and 24h, respectively. Additionally, selected matrices were processed via an automated liquid handling platform (GDS PickPen® PIPETMAX). All analytical outcomes were culture confirmed by the reference methods.

Results: A total of 390 samples were analyzed in this study for the candidate method. The probability of detection (POD) values met the Acceptability Limit of an unpaired study design. Specifically, the study data indicate with 95% confidence that the *Listeria* detection candidate methods are equivalent to the FDA/USDA plate-based reference methods.

Significance: Assurance® GDS for *Listeria* spp. Tq II and *Listeria monocytogenes* Tq II are suitable methods for detecting *Listeria* species and *Listeria monocytogenes* in selected foods and environmental samples.

P1-206: QUANTITATIVE DETECTION OF VIABLE ESCHERICHIA COLI AFTER CHLORINE EXPOSURE IN TEST AGRICULTURAL WATER USING PROPIDIUM MONOAZIDE QPCR AND DPCR

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Chlorine-based sanitizers are used to improve the microbial quality of agricultural water. Propidium monoazide (PMA) combined with quantitative (qPCR) or digital PCR (dPCR) can quantify viable cells of *E. coli*, a bacterial indicator of agricultural water quality, after chlorine treatment.

Purpose: To determine if PMA-qPCR and PMA-dPCR can detect viable *E. coli* levels post-chlorine treatment in irrigation water.

Methods: Calcium hypochlorite was added to Test Agricultural Water (TAW) to achieve target levels of free chlorine. Cultures of *E. coli* TVS353, a surrogate for enterohemorrhagic *E. coli*, were serially diluted in TAW to achieve 1–8 log CFU/mL as viable cells. Non-viable *E. coli* cells were generated by treating *E. coli* TVS353 (8 log CFU/mL) in TAW with 100 ppm free chlorine

for 5 min. Live/dead cells were mixed and treated with 50 μM PMAxx Dye. Two different primer sets, uidA405 and EC23S857, were utilized in qPCR and dPCR on extracted DNA. Two independent experiments (two replicates each) were conducted. The sensitivity of PMA-qPCR and PMA-dPCR was evaluated based on two-phase linear regression models constructed using target DNA concentrations and viable cell concentrations of *E. coli*.

Results: For PMA-qPCR with primer sets uidA405 and EC23S857, the adjusted R^2 was 0.97 and 0.98, respectively. For PMA-dPCR with primer sets uidA405 and EC23S857, the adjusted R^2 was 0.96 and 0.95, respectively. Based on the breakpoint from each condition, primer set EC23S857 showed a lower limit of detection (2.6 logCFU/mL for qPCR; 3.4 logCFU/mL for dPCR) compared to uidA405 (4.1 log CFU/mL for qPCR; 3.5 log CFU/mL for dPCR). Both PCR methods were able to detect viable cells above 4 log CFU/mL when mixed with dead cells but did not reliably detect viable cells at or below 3 log CFU/mL.

Significance: PMA-qPCR and PMA-dPCR detected viable *E. coli* levels after chlorine treatment in irrigation water. Detection limits were more affected by primer set choice for qPCR.

P1-207: EVALUATION OF BIOMÉRIEUX'S GENE-UP PATHOGENIC E. COLI ASSAY FOR DETECTION OF TOP7 STEC IN AUSTRALIAN MANUFACTURING BEEF

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Australian manufacturing beef exported to the USA is routinely tested for the presence of Top7 Shiga toxin-producing *Escherichia coli* (STEC). Current diagnostic workflows in Australia primarily rely on molecular detection of *stx*, *eae*, along with molecular or antigenic detection of O-antigen genes in enrichment broths. Broths identified as potentially positive for Top7 STEC are then culture-confirmed using methods approved by the Department of Agriculture, Fisheries and Forestry. However, commercial testing results indicate that approximately 10% of these potentially positive broths are confirmed by culture.

Purpose: bioMérieux has developed the GENE-UP Pathogenic *E. coli* (PEC) assay which uses alternative STEC markers to improve the likelihood that screen-positive broths yield culture-confirmed Top7 STEC.

Methods: A collection of 253 Australian manufacturing beef enrichments that initially tested potentially positive (PP) for Top7 STEC by PCR were culture confirmed and evaluated using the PEC assay.

Results: Culture confirmation was achieved in 25 of the 253 enrichments (9.9%). A total of 125 of 253 (49.4%) were PEC positive and would have resulted in a 51% reduction in the number of PPs requiring culture confirmation. Of the 25 samples culturally confirmed, 24 (96%) were PEC positive.

Significance: Adding PEC to traditional PCR screening improved confidence that screen-positive broths contained Top7 STEC. Adoption of this approach by industry could enhance testing efficiency, reduce costs, and accelerate product release to market.

P1-208: MAGNAFLOW PRELIMINARY RESULTS: IN-LINE SAMPLING FOR THE SELECTIVE CAPTURE OF E. COLI O157:H7 IN PRODUCE WASH WATER

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Current produce testing relies on grab sampling (<0.1% of total volume) followed by 24–48 hours of cultural enrichment, creating a detection gap during which contaminated product ships. No existing method detects foodborne pathogens directly from flowing wash water without enrichment, putting them at risk.

Purpose: To evaluate the preliminary performance of the MagnaFlow, a novel, scalable device that integrates proprietary millifluidic channels with polymer surfaces for the selective capture of *E. coli* O157:H7 from large-scale wash water in produce processing plants.

Methods: Device performance was evaluated using *E. coli* O157:H7 (ATCC 35150) across four concentration ranges (1–9, 10–99, 100–999, and 1,000–9,999 CFU/mL). 48 runs were performed at an average flow rate of 2.59 L/min, yielding effective post-dilution concentrations of approximately 0.0004–3.9 CFU/mL. Flows were identically spiked with 1mL samples. 18 negative controls (PBS) were randomly interspersed among 30 positive runs. Sample identity was masked, surfaces were gram-stained and examined using microscopy at 60x by a blinded lab technician.

Results: The device achieved 63% sensitivity (19/30; 95% CI 46–81%) and 72% specificity (13/18; 95% CI 52–93%) across all concentration ranges. Notably, detection was achieved at effective concentrations as low as 0.0004 CFU/mL, where PCR without enrichment, immunoassay, and culture methods produce no detectable signal. Residual carryover between runs was identified as a source of false positives; flushing protocols and polymer optimization are ongoing.

Significance: These results demonstrate the first enrichment-free capture and detection of *E. coli* O157:H7 directly from wash water, enabling same-day pathogen detection with 100% volume coverage. In-line isolation and capture of pathogens in produce wash water fundamentally addresses sampling gap and turn-around times in current produce safety testing. This lowers recall, reshelving, and spoilage risks.

P1-209: DETECTION OF E. COLI O80 AND O80:H2 IN FLOUR-BASED FOOD PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Detection of *E. coli* O80 and O80:H2 in flour-based foods is important, as epidemiological studies have linked these strains to severe pediatric cases and outbreaks in Europe.

Purpose: The study evaluates two new PCR assays that was developed to enable rapid and specific detection of these emerging pathogenic strains in relevant food matrices.

Methods: Two PCR primer-probe solutions for *E. coli* O80 and O80:H2 were designed. Performance was first evaluated by Cq values across serial dilutions and matrix type and compared with iQ-Check STEC VirX PCR Kit. Robustness was then assessed on artificially spiked extracts (spinach and beef steak). Two O80 and one O80:H2 assays were selected and tested on 30 food samples (flour, raw dough, and multicomponent foods) enriched in BPW with Selective STEC Supplement. Test portions of 25 g or up to 375 g were incubated for 18–26 hr at 41.5 ± 1°C. DNA was extracted using the Easy II protocol, with Free DNA Removal

Solution (FDRS) treatment. Detection assayswere run on a CFX Opus 96 with the SerO II FAST APF. Inclusivity/exclusivity was performed on 9 *E. coli* O80 and 10 non-O80 STEC strains.

Results: The new PCR assays achieved 100% specificity, detecting all 9 *E. coli* O80 strains and excluding all 10 non-O80 STEC strains. Sensitivity was confirmed on artificially inoculated food samples at 1~6 CFU/sample, with detection limits comparable to the iQ-Check STEC VirX method. Across all 30 contaminated samples, alternative PCR methods achieved sensitivities of 76.5–77.8% after confirmation (O80), relative trueness of 73.3–76.7%. Detection limits (LOD_{50}) for O80 in flour-based matrices were 0.429–1.067 CFU/test portion for alternative methods and 0.598 CFU/test portion for the reference method, with RLOD values well below the acceptability limit of 2.5.

Significance: The newly developed PCR assays provide a rapid and highly specific method for detecting emerging *E. coli* O80 and O80:H2 strains in flour-based foods.

P1-210: DEVELOPMENT OF A DIGITAL PCR ASSAY FOR THE DETECTION OF STEC IN ENVIRONMENTAL SAMPLES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: In Digital PCR (dPCR), reactions occur in samples dispersed in numerous nano-partitions, enabling the linkage of amplified multiple gene targets within a single bacterial cell after statistical analyses.

Purpose: To develop a multiplex dPCR assay for Shiga-toxin producing *Escherichia coli* (STEC) with the QIAGEN QIAcuity dPCR platform, targeting *stx*1, *stx*2 and *eae* virulence genes.

Methods: The *stx* primers/probes were derived from BAM chapter 4A qPCR assay. The multiplex assay was optimized (primer/probe concentrations and PCR cycle conditions) using a known concentration of DNA from *E. coli* O157:H7 to assess PCR efficiency and limit of detection (LOD). DNA extracted from enrichment samples of sprout spent irrigation water (SSIW) spiked with low levels of *E. coli* O157:H7 (0.04 CFU/mL) were tested using the dPCR assay and compared to the BAM STEC qPCR assay on the ABI 7500 Fast platform (two experiments, n=20/experiment). The dPCR assay was further evaluated using live cell STEC

cultures.

Results: Annealing temperature was a critical parameter for the multiplex dPCR assay, especially for *stx*2 amplification. Following gradient runs (52–62°C), 60°C was selected as the optimal annealing temperature. Regression analysis of test results and DNA concentrations showed high efficiency (R^2> 0.99). The dPCR assay LOD was comparable to the BAM 4A qPCR assay (~10^3 CFU/mL) and both assays showed equivalent performance for detecting positive samples in spiked SSIW samples. When testing pure STEC cultures, fresh cultures (3-hour incubation) demonstrated more efficient amplification with dPCR than 20-hour incubation cultures, with detection of DNA copy numbers increased by 2.4–4.8% across the three targets over a triplicate.

Significance: This multiplex STEC dPCR assay simultaneously detects virulence factors *stx*1, *stx*2, and *eae* from DNA and cultures and may serve as a complementary tool to existing qPCR assays used to detect high-risk STEC in environmental samples.

P1-211: MEHEC ENRICHMENT PROTOCOLS TO DETECT STEC IN MEAT, RAW MILK, DAIRY PRODUCTS, AND ENVIRONMENTAL SAMPLES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Assurance® GDS method offers rapid, flexible solution to detect STEC (Shiga toxin-producing *Escherichia coli*), major foodborne pathogens known for their ability to cause severe illness, often associated with serious complications such as hemolytic uremic syndrome.

Purpose: To evaluate a protocol using a shortened enrichment (6–8 h) and a wider testing window (up to 24 h) by using MPX Top 7 STEC and MPX ID for Top STEC or EHEC ID for *E. coli* O157:H7 detection methods.

Methods: Sensitivity and Relative Limit Of Detection (RLOD) studies were conducted by an independent laboratory according to ISO 16140-2. STEC strains representatives to the Top 5 were inoculated between 0.6 and 13 CFU/sample into raw and ready-to-cook meats (up to 25 g), raw poultry, raw seasoned poultry and heat-treated poultry (up to 25 g), raw milk and

dairy products (up to 25 g) and production environmental samples (up to 25g/mL or sample device). Following an enrichment at 41.5°C for 6 or 8h and 24h, MPX Top 7 STEC screening method was applied to 1 mL of enriched sample followed by MPX ID or EHEC ID confirmation methods. A comparison with the ISO/TS 13136 reference method was conducted on the same categories.

Results: For each category, 60 samples were analyzed for the sensitivity study. For the RLOD study, raw ground beef, raw turkey filet, raw milk cheese and process water were respectively inoculated by *E. coli* O145, O103, O111 and O26 at a low and a high level of inoculation. Uninoculated samples were also tested following the appropriate enrichment protocol. Acceptance limits between -6 and 3 for sensitivity or between 0.871 and 2.357 for RLOD for an unpaired study were met (acceptance criteria are respectively 3 and 2.5), with no significant differences versus the ISO reference method.

Significance: Assurance® GDS reliably detected STEC across diverse matrices under shortened enrichment conditions.

P1-212: HARMONIZATION OF USP GENERAL CHAPTERS 62 AND 2022 METHODS FOR ESCHERICHIA COLI DETECTION: A COMPARATIVE VALIDATION STUDY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: USP chapters <62> and <2022> harmonize incubation time and temperature for detecting *Staphylococcus aureus, Salmonella* spp., and *Pseudomonas aeruginosa*, but not for *Escherichia coli.* To address this gap, we compared *E. coli* detection results using USP <62> and USP <2022> to support method harmonization.

Purpose: To support alignment of incubation parameters for *E. coli* detection between USP chapters <62> and <2022>.

Methods: The two *E. coli* detection methods were compared using a fractional inoculation study per validation guidelines in AOAC Appendix J. Forty test portions were evaluated using a common primary enrichment (10mL tryptic soy broth): 5 uninoculated, 20 low-level inoculated, 5 high-level inoculated, 5 at 1/3x low-level inoculum, and 5 at 3x low-level inoculum. Samples were tested using USP <62>, USP <2022>, and single-modification

variants standardizing secondary enrichment volumes (1mL into 10mL MacConkey broth) and incubation time (18–24 h). Probability of detection (POD) and differences (dPOD) were calculated. Statistical significance was assessed at the 5% level, with acceptance defined as no significant difference between methods.

Results: All dPOD results met acceptance criteria, demonstrating no statistically significant differences at the 95% confidence interval between USP <62> and USP <2022>. The harmonized method showed slightly improved recovery compared to USP <62> (15 vs. 14 positives at low-level inoculation) and equivalent recovery to USP <2022> (15 positives).

Significance: Based on the data, USP officially adopted the harmonized incubation parameters for *E. coli* detection, ensuring consistency across chapters <62> and <2022>. This simplifies laboratory workflows, improves consistency, and reduces incubation time, enhancing operational efficiency for laboratories and clients.[

P1-213: AN IMPROVED METHOD FOR SUBTYPING ESCHERICHIA COLI O157:H7 ISOLATES USING MULTIPLE-LOCUS VARIABLE-NUMBER TANDEM REPEAT ANALYSIS (MLVA)

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Category: Laboratory and Detection Methods

Abstract:

Introduction: *Escherichia coli* O157 is a major foodborne pathogen of concern to the food industry. Subtyping is essential for identifying sources, guiding targeted control strategies, and reducing transmission.

Purpose: To develop an improved MLVA method for subtyping *E. coli* O157 using published loci (Hyytia-Trees et al., 2006, 2010), which ensures reliable and reproducible results.

Methods: DNA was amplified in two multiplex PCR reactions using fluorescently labeled forward primers targeting eight loci. PCR fragments were separated on an ABI 3730XL Genetic Analyzer and analyzed with GeneMapper™ software.

Results: PCR primers were modified based on updated genome sequences to eliminate mismatches in primer-binding regions, balance annealing temperatures, and improve amplification efficiency. The assay was evaluated using 74 isolates from food, human, and environmental sources. Seven to eight loci were amplified in *E. coli* O157 (NM) isolates (n=51), whereas only two to four loci were amplified in non-O157 (NM) STEC isolates (n=13), with fragment sizes outside predicted ranges. No amplification occurred in other species (n=10), except *Salmonella* Enteritidis, which amplified two loci. MLVA analysis of the 51 *E. coli* O157

isolates identified 40 distinct types, with related isolates clustering together and unrelated isolates forming separate groups. All non-O157 STEC isolates exhibited unique, incomplete MLVA profiles. Hunter–Gaston diversity indices ranged from 0.59 to 0.91 for individual loci, with a combined index of 0.99. A direct conversion of fragment size to VNTR copy number minimized interpretation variability. The method demonstrated high data quality and reproducibility and was successfully applied to isolates from food production facilities.

Significance: This MLVA method provides timely, actionable subtyping data to support proactive monitoring and targeted prevention of *E. coli* O157 transmission and foodborne outbreaks.

P1-214: REDUCING THE NUMBER OF PRESUMPTIVE-POSITIVE STEC RESULTS IN BEEF SAMPLES BY THE INCLUSION OF A SECONDARY MOLECULAR SCREEN

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: The beef industry requires rapid, accurate detection of Shiga toxin-producing *E. coli* (STEC). The *FSIS Microbiology Laboratory Guidebook* (MLG) STEC method screens for *eae* and *stx* genes and now utilizes a secondary molecular screen to rule out samples where those genes are from separate organisms and thereby reducing the number of samples requiring serotyping and confirmation.

Purpose: To evaluate the bioMérieux GENE-UP® Pathogenic *E. coli* (PEC) assay as a secondary screening tool in beef matrices and determine its ability to reduce the number of samples requiring further testing.

Methods: Beef samples (n=40), including trim, ground beef, and sampling cloths, from material that had previously screened positive for *stx* and *eae* using commercial real-time PCR kits were included in this study. Enrichments were stored frozen at -80°C in 15% glycerol until further testing. All samples were tested by the secondary molecular assay according to kit instructions, aligning with the current FSIS MLG testing workflow.

Results: Of the 40 samples that screened positive for *stx* and *eae*, only 27 were positive by

the secondary screen, reducing further testing by 32.5%. The 13 samples negative by the secondary assay suggest the presence of unlinked genes. All samples underwent confirmation, with 23 of the 27 samples positive by both screening methods confirming. None of the 13 samples that were negative by the secondary screening test confirmed positive by further testing, indicating no false negatives.

Significance: Implementing a secondary molecular screen reduced the number of STEC presumptive samples requiring confirmation by nearly one-third, enhancing laboratory efficiency and allowing for faster release of beef products while adhering to the FSIS MLG testing method.

P1-215: EVALUATION OF A DIGITAL DROPLET PCR ASSAY FOR THE CONFIRMATION OF STEC IN PRODUCE AND BEEF

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Demand for rapid, reliable pathogen detection in food safety continues to grow as markets prioritize speed without compromising accuracy. Culture-based confirmation remains the gold standard but requires extended timelines, creating a need for technologies that enable faster confirmation while maintaining regulatory compliance.

Purpose: To evaluate Droplet Digital PCR (ddPCR) for confirmation of Shiga toxin-producing *E. coli* (STEC) after initial screening for *stx* and *eae* gene targets, as an alternative to time-consuming culture or immunomagnetic separation (IMS)-based methods.

Methods: Samples (n=143), including beef and produce matrices, were enriched and screened for *stx* and *eae* genes by commercial real-time PCR kits according to manufacturer instructions. Samples of enrichments positive for one or both virulence targets were stored in 15% glycerol at -80°C until ddPCR analysis. The ddPCR algorithm assigns four outputs: Positive (*stx*/*eae* linked), Negative: Unlinked (targets detected in different droplets/cells), Negative (one or both targets absent), and Presumptive Positive (linkage cannot be ruled out).

Results: Of the 81 samples screened positive for both *stx* and *eae* by initial PCR. ddPCR confirmed 29 as STEC, demonstrating linked virulence targets. Thirty-four samples were negative due to unlinked targets, indicating the presence of separate non-pathogenic organisms. The remaining 18 samples failed to confirm, as ddPCR did not detect one or both targets. All 62 samples negative by the initial screen were also negative by ddPCR.

Significance: This study demonstrates the utility of ddPCR for confirming STEC by specifically identifying genetic linkage of the *stx* and *eae* genes. This approach provides significant value by reducing false-presumptive results caused by unlinked virulence genes, while potentially allowing for faster product release than traditional confirmation.

P1-216: ADDITION OF A SECONDARY MOLECULAR SCREEN TO REDUCE THE NUMBER OF PRODUCE SAMPLES SCREENING POSITIVE FOR STEC AND REQUIRING CONFIRMATION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Rapid and accurate detection of Shiga toxin-producing *E. coli* (STEC) is critical in produce safety, where short shelf lives demand fast results. While initial PCR screening for *stx* and *eae* genes is highly sensitive, it often yields presumptive positive results due to the presence of unlinked genes in separate non-pathogenic bacteria, leading to lengthy and costly confirmation testing.

Purpose: To evaluate the bioMérieux GENE-UP® Pathogenic *E. coli* (PEC) assay as a secondary molecular screen to reduce the number of STEC-presumptive produce samples requiring confirmation. This assay uses molecular targets typically present in STEC strains positive for both *eae* and *stx*.

Methods: Produce samples were enriched and screened for *stx* and *eae* genes by commercial real-time PCR according to kit instructions. Samples (n=96) positive for one or both targets were frozen at -80°C in 15% glycerol until they were analyzed by the secondary molecular assay according to kit instructions. The results of the initial screen were compared to the secondary assay to assess the potential reduction in confirmation testing.

Results: Forty-one samples initially screened positive for both *stx* and *eae*. Following analysis with the secondary molecular assay, 15 of these samples remained positive. The remaining 26 samples (63.4% of initial positives) were negative, indicating that the virulence targets were likely unlinked. The secondary assay detected 9 samples that were positive only for *eae,* and no samples positive only for *stx* or negative for both targets. Forty-six samples were negative by both methods.

Significance: The secondary molecular screen reduced the number of samples requiring confirmation by 63%. This approach effectively filters out unlinked genetic targets, eliminating much costly, time-consuming confirmation testing and reducing product hold times.

P1-217: A RAPID, SIMPLE PAPER-BASED DNA EXTRACTION AND LAMP-BASED DETECTION PLATFORM FOR ESCHERICHIA COLI O157:H7 ON INHIBITOR-RICH STRAWBERRY SURFACES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Direct detection of foodborne pathogens in fresh berries (e.g., strawberries) by nucleic acid amplification-based methods is challenging due to the presence of PCR inhibitors such as polyphenols and polysaccharides that interfere with nucleic acid amplification and limit rapid, on-site testing.

Purpose: This study aims to develop a simple, kit and equipment–free, paper-based DNA extraction method for the direct detection of *Escherichia coli* O157:H7 in strawberries using a loop-mediated isothermal amplification (LAMP) assay.

Methods: Strawberries were surface-inoculated with *E. coli* strain EDL933 (10^2–10^6 CFU/g) and processed without enrichment. Following lysis with a customized buffer, DNA was captured on mixed cellulose ester (MCE) membranes without centrifugation or kit-based extraction. DNA was retained and eluted from the membrane within 30 s and used directly for PCR and LAMP targeting *E. coli* genes *stx1*, *stx2*, *eae*, *fliC*, and *rfbE*. Inhibitor tolerance was evaluated by comparing amplification performance using standard versus engineered Taq polymerases. Three inoculation concentrations with three biological replicates each were

tested across two independent trials (n = 18 total samples), along with positive and no-template controls.

Results: The optimized paper-based protocol overcame strawberry-derived inhibitors and enabled direct amplification. DNA captured on membranes was amplifiable after 30 s, and LAMP produced visible results within 50 min, with total workflow time under 2 h. In comparison, commercial kits required 3–6 h for extraction and up to 8 h for full workflow. LAMP consistently detected 10^2 CFU/g and yielded faster time-to-positive. Engineered polymerases enhanced amplification in inhibitor-rich strawberry matrices.

Significance: This low-cost, portable workflow enables rapid, on-site detection of *E. coli* in fresh produce, supporting food safety monitoring in low-resource and decentralized settings.

Award Application: Developing Scientist Award

P1-218: COMPARISON OF TWO BROTHS FOR THE RECOVERY OF SHIGA TOXIN-PRODUCING E. COLI FROM 375ML OF SPENT SPROUT IRRIGATION WATER

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Category: Laboratory and Detection Methods

Abstract:

Introduction: The FDA Produce Safety Rule requires testing of spent sprout irrigation water [SSIW] or sprouts for pathogens. One regulatory method is currently available for testing 100 ml of SSIW for one serotype of STEC, namely *E. coli* O157:H7, yet outbreaks linked to sprouts consumption have been attributed to different serotypes of STEC.

Purpose: Our goal is to develop an enrichment protocol for testing all STECs in 375ml SSIW.

Methods: STEC strains from six serotypes historically linked to sprouts were selected. BAM media M192c (a BPW-based broth) was compared to M192a (the current STEC BAM method broth). Both media were tested with and without antimicrobials for STEC enrichment using 20 mL pure cultures or samples inoculated in 375 mL SSIW and aged 3 days at 4°C. After incubation at 42°C for 24 to 48 hours, STEC growth was measured using a densitometer DEN-1, at a McFarland of 565 ± 15 nm, or on selective CHROMagar STEC plates for enriched SSIW cultures.

Results: The final OD values in M192a were approximately thrice those in M192c. The tested

antimicrobials showed no significant effect on growth in either broth except for the O111 strain requiring an extra day to grow in M192c. 375ml alfalfa SIW samples, with an APC of 6.65 log CFU/ml, were inoculated with 3.8 CFU ECO26 or 1.4 CFU EC0111 [10 replicates each], and with 10-fold higher inocula [4 samples each]. After aging, half of the samples were mixed with 375ml of 2x M192c or with 2x M192a, with antimicrobials, before incubation at 42°C for 24 hours and streaked out on selective CHROMagar STEC plates. EC O26 was successfully detected in the M192a samples (7/7) but poorly in M192c (1/7). EC O111 was not recovered in the M192c samples.

Significance: A nutritious broth stimulated growth and enhanced the recovery of stressed or injured STEC. Antimicrobial supplemented M192a is selected to be validated for the recovery of STEC from 375ml of SSIW.

P1-219: RECOVERY OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IS IMPACTED BY ENVIRONMENTAL MATRIX

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: A modified USFDA Bacteriological Analytical Manual (FDA BAM) method incorporating real-time PCR screening for *stx* followed by culture-based isolation was used to recover STEC isolates from environmental samples collected in the California Longitudinal Study. The effect of environmental matrix on successful recovery of Shiga toxin-producing *Escherichia coli* (STEC) from PCR-positive enrichments remains poorly characterized.

Purpose: To compare PCR detection and culture-based recovery of STEC from diverse environmental matrixes and to quantify the number of presumptive colonies needed to achieve reliable recovery from PCR-positive enrichments.

Methods: A total of 946 samples of livestock feces, wildlife feces, sediment, soil, and surface water collected from 2021 to 2025 were enriched and then screened for *stx* by real-time PCR with a BAM method developed for the AB 7500 fast platform. For each PCR-positive enrichment (Ct □40), up to 80 presumptive colonies, recovered on TC-SMAC, Rainbow Agar O157, CHROMagar O157 or CHROMagar STEC with and without supplements were screened for STEC.

Results: PCR and culture-based STEC prevalence, respectively, were 98%/83% for livestock feces (n=52), 56%/24% for wildlife feces (n=91), 47%/15% for surface water (n=88), 30%/18% for sediment (n=116), and 6%/1% for soil (n=599). Environmental matrix strongly influenced culture recovery of STEC. The median number of presumptive colonies required to obtain at least one STEC isolate from PCR-positive enrichments was 16 for livestock feces, 22 for wildlife feces, 48 for water, 40 for sediment, and 58 for soil.

Significance: Increasing colony screening depth improves isolation success and should be considered when designing STEC recovery strategies for environmental monitoring programs.

P1-220: A NOVEL PHAGE AMPLIFICATION–QPCR ASSAY FOR RAPID DETECTION OF ESCHERICHIA COLI O157:H7

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* O157:H7 is a major foodborne pathogen capable of causing severe illness and death, underscoring the need for rapid, sensitive, and viability-based detection methods. Bacteriophages offer high host specificity making phage-based amplification approaches promising alternatives to conventional detection techniques.

Purpose: To develop and validate a rapid phage amplification assay (PAA) coupled with quantitative PCR (qPCR) for sensitive detection of viable *E. coli* O157:H7 in food matrices using a novel *Escherichia* phage, CAM-21.

Methods: Phage CAM-21 was incubated with *E. coli* O157:H7 strain C7927 to allow phage replication within viable host cells. Microspin centrifuge filters with a pore size of 0.20 µm were used to collect the host infected with progeny phages. Progeny phages were quantified by qPCR and confirmed by a double agar overlay assay. Phage DNA was extracted using a virus DNA isolation kit. CAM-21–specific primers were designed using Primer3. qPCR reactions (20 µL) were performed under the following cycling conditions: 95°C for 5 min; 40 cycles of 95°C for 10 s and 60°C for 30 s; followed by 60°C for 15 s. Assay performance was evaluated in broth and in spiked ground beef.

Results: The completed PAA-qPCR method detected viable *E. coli* O157:H7 C7927 at concentrations below 10 CFU/mL within 4 h. In ground beef, the assay detected the

pathogen at 1 × 10^3 CFU/mL within 7 h. The qPCR assay demonstrated specific and reliable detection of CAM-21 progeny phages following amplification.

Significance: This completed phage amplification–qPCR assay enables rapid, sensitive, and viability-based detection of *E. coli* O157:H7. Its successful application in a complex food matrix demonstrates strong potential for practical food safety testing and rapid contamination assessment.

P1-221: DEVELOPMENT OF AN RPA-CRISPR/CAS12A-BASED MOLECULAR PLATFORM FOR DETECTION OF CLOSTRIDIUM PERFRINGENS IN MEAT SAMPLES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Clostridium perfringens* (*C. perfringens*) is a major foodborne pathogen responsible for a wide range of gastrointestinal diseases in both humans and animals.

Purpose: Conventional methods for detecting *C. perfringens* are time-consuming and often lack sufficient sensitivity. Therefore, we developed a rapid, highly sensitive, and specific RPA-CRISPR/Cas12a-based assay for its detection in meat samples. This will enable early detection of contamination, thereby enhancing food safety, quality control, and supply-chain integrity.

Methods: A recombinase polymerase amplification (RPA) assay that targeted a conserved *C. perfringens* gene was developed and optimized, and the resulting amplicons were used to establish a CRISPR/Cas12a detection system. Key parameters, including crRNA and fluorescence probe concentrations, were optimized, and the analytical sensitivity, specificity, and cross-reactivity of the assay against 52 bacterial species, were evaluated. All experiments were performed in triplicate to ensure statistical robustness.

Results: The developed RPA-CRISPR/Cas12a assay detected 1 fg/µL (equivalent to 0.3 genome copies) of *C. perfringens* genomic DNA, demonstrating greater sensitivity than RPA alone; 10 times higher than RPA. Cross-reactivity was not detected with any of the 52 food-associated bacterial species tested, confirming high analytical specificity, and the assay showed high sensitivity (1 to 10 CFU/g) in spiked meat samples using purified DNA. The RPA-

CRISPR/Cas12a assay obtained results within 90 min which was faster than culture-based detection and PCR confirmation (~180 min).

Significance: The newly developed RPA-CRISPR/Cas12a assay has the potential to provide rapid, sensitive and specific detection of *C. perfringens*, in the food industry.

P1-222: RAPID DETECTION AND GENOTYPING OF CAMPYLOBACTER IN SURFACE WATER USING TARGETED AMPLICON SEQUENCING

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Category: Laboratory and Detection Methods

Abstract:

Introduction: Surface water, widely used for irrigation, is commonly contaminated via zoonotic sources such as wild birds and livestock and can serve as a potential route for human exposure to *Campylobacter*. Next-generation sequencing, including targeted amplicon sequencing (TAS), enables rapid screening and strain typing of foodborne pathogens. This study evaluated TAS for detection and typing of *Campylobacter jejuni, C. coli,* and *C. lari*, which cause most human campylobacteriosis cases.

Purpose: To evaluate a custom TAS panel for screening and genotyping *Campylobacter* in

spiked and naturally contaminated surface water.

Methods: A custom TAS panel targeting 87 species-specific and polymorphic loci was developed by ChapterDx. DNA from ATCC strains of *C. jejuni, C. coli,* and *C. lari* (5, 0.5, 0.05 ng; n = 3 per strain), including a mixed *C. jejuni/C. lari* sample, was spiked into DNA (20 ng) extracted from 100 L surface water. Amplicons were sequenced on an Illumina platform. The assay was further evaluated using 48-h enrichment cultures from spiked (34 CFU *C. jejuni*/100L) and naturally contaminated surface water. Sequences were BLAST matched against reference alleles and analyzed using custom software.

Results: TAS identified *Campylobacter* species and strain genotype at 0.05 ng DNA. At 5, 0.5, and 0.05 ng, the number of loci detected for *C. jejuni* were 77, 75 and 59 and for *C. lari* 21, 21 and 15 respectively, with >99% concordance to spiked genotypes. *C. coli* was detected, but strain-level identification was inconclusive. Mixed samples identified loci from both strains. TAS detected and typed *C. jejuni* from enriched spiked (34 CFU) water and confirmed its presence in a naturally contaminated sample.

Significance: The TAS panel enables rapid identification and typing of *Campylobacter* during culture enrichment, providing earlier and more informative results than traditional methods which typically require an isolated colony.

P1-223: ADVANCES IN ELECTROCHEMICAL BIOSENSING FOR FOODBORNE ENTERIC VIRUS DETECTION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Foodborne enteric viruses are a major cause of acute gastroenteritis worldwide, with norovirus and hepatitis A virus (HAV) responsible for many outbreaks linked to fresh fruits, shellfish, and ready-to-eat foods. Their environmental stability, resistance to inactivation, and low infectious dose present difficulties for monitoring and accurate detection in food safety systems.

Purpose: This review critically examines the advances in electrochemical biosensors for detecting foodborne enteric viruses, and their potential to address limitations of conventional molecular and immunological detection methods.

Methods: To evaluate electrochemical biosensing technologies targeting enteric viruses in food and water matrices, a systematic evaluation of peer-reviewed literature from the past decade was conducted. The review analyzes biorecognition strategies, including antibody, aptamer, molecularly imprinted polymer, and other approaches, alongside different electrochemical transduction methods. Key performance parameters, such as limit of detection, assay duration, matrix tolerance, and validation in real samples or spiked buffers, were used to assess these biosensors.

Results: Several platforms report detection limits compatible with the low infectious doses of enteric viruses, and electrochemical biosensors show improved sensitivity and speed.

Nanostructured electrodes and optimized surface functionalization enhance signal transduction, though limitations remain, including matrix interference, limited reproducibility and stability, difficulty distinguishing infectious from non-infectious particles, and insufficient standardization across studies.

Significance: This review highlights the opportunities and limitations of electrochemical biosensors for foodborne virus surveillance and identifies key research gaps to guide development of reliable, field-deployable sensing technologies supporting food safety.

Award Application: Developing Scientist Award

P1-224: DETECTION OF INFECTIOUS HEPATITIS A VIRUS IN AGRICULTURAL SURFACE WATER USING DEAD-END ULTRAFILTRATION AND CELL CULTURE

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Hepatitis A virus (HAV) is one of the common causes of foodborne outbreaks, which often are associated with fresh produce. Contamination of produce can occur through agricultural water during pre-harvest production and post-harvest processing. Detection of infectious viruses in agricultural water can help more accurately identify and confirm routes of contamination for fresh produce during outbreak investigations.

Purpose: The objective of this study was to develop a method to isolate and detect infectious HAV in artificially contaminated surface water. Due to the low levels of viruses in the environment, large-volume sampling with multi-step concentration is required for cell culture analysis.

Methods: Cell culture-propagated HAV HM175/18f strain (2.3E+08 genome copies) was spiked into 10 liters of surface water before dead-end ultrafiltration (DEUF) with Elisio-25H® ultrafilters. Following backflushing, viruses were concentrated by polyethylene glycol (PEG) precipitation and subsequently purified with 0.45 µm filter, 100 kDa Amicon® column, and 0.22 µm filter. Recovery of infectious HAV was determined using FRhK-4 cells. For real-time reverse-transcription digital PCR (RT-ddPCR) quantification, RNA was extracted from culture supernatants and cells using Monarch® Total RNA Miniprep Kit.

Results: The recovery rate of HAV was 2.4% during primary PEG concentration and 1.1% during secondary column purification (n=2). Cytopathic effect (cpe) was observed in FRhK-4 cells infected with HAV recovered from artificially inoculated surface water on day 4 to day 7 post-infection. RT-ddPCR confirmed virus replication in cells.

Significance: Current virus detection mostly depends on molecular techniques, which cannot differentiate live viruses from dead genome fragments. Developing methods to detect infectious viruses in agricultural water will provide more accurate confirmatory tools for the prevention and investigation of foodborne illnesses.

P1-225: METHOD VALIDATION AND EXTENSION OF HEPATITIS AND NOROVIRUS IN VEGETATION USING GENE-UP AND CEERAM TOOLS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: In-house validation of Hepatitis A and Norovirus kits extended laboratory shelf life and preserved funds during lean budgets.

Purpose: The purpose of the study was to validate norovirus and Hepatitis A using the Gene-Up and extending the shelf life of commercially available kits.

Methods: 25g of leafy greens and berries were spiked at 104 and 103 copies/ µL of Hepatitis A, Norovirus GI and Norovirus GII, and 105 copies/µL of internal standard Mengo virus; then extracted according to the bioMerieux Ceeram-tools protocol with reference to ISO 15216-1 procedure and the bioMerieux eGene-Up, lysis and genetic material clean up, and Gene-Up, real-time RT-PCR, instruments. The Crossing point (Cp), R-squared, and slope values of the internal standard were compared and statistically analyzed between in-house laboratory validation results and listed method results. Kits did not exceed three freeze/thaw cycles and

were used one to three months past expiration. Extended shelf-life results were determined by comparing expired kit results with un-expired kit results.

Results: Mengo virus had 100% recovery for all 12 runs. The average R2 was 0.975, Cp was 29.08, and slope was -3.54. In-house validation gave consistent Cp value later than protocol. All analytes had a specificity of 100%. 65 Hepatitis A samples analyzed: accuracy of 86% and sensitivity of 82%. 40 Norovirus GI samples analyzed: accuracy of 53% and sensitivity of 43%. 48 Norovirus GII samples analyzed: accuracy of 81% and sensitivity of 78%. All kits showed no statistical difference between non-expired vs expired kits. A future study to extend the PCR cycles can be done to improve GI results.

Significance: Norovirus and Hepatitis A are responsible for many U.S. military foodborne outbreaks. Internal department testing with shelf-life extension will help determine outbreak origins and deter further illness with limited funding.

P1-226: RAPID SCREENING AND ENHANCED DETECTION OF INFECTIOUS ROTAVIRUS IN FOOD MATRICES THROUGH SAMPLE POOLING AND ICC-QPCR

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Foodborne illness is a major public health concern as reported by the WHO with 600 million annual illnesses causing an economic loss of US$110 billion in low and middle income countries. Rotavirus is a leading cause of foodborne gastroenteritis in children due to its resilience and low infectious dose. Conventional detection methods like cell culture and real-time qPCR poses challenges like long incubation time, inability to differentiate infectious particles, and PCR inhibitors.

Purpose: The current study aims to address these challenges by integrating screening through sample pooling and detection using real-time PCR (ICC-qPCR) assay, for rapid and sensitive rotavirus detection.

Methods: In current study sample matrices including water (15mL), apple juice (15mL), and spinach (5 g) were spiked with a known TCID50 of rotavirus to evaluate detection limit and time. The spiked samples were concentrated to 500 µL and inoculated onto MA104 cell. Following a 24-hour incubation, supernatant was concentrated to 10 µL and heat-treated prior to real-time PCR analysis. For the pooling assessment, 4–5 random samples were

spiked in a batch of 100 samples to determine detection sensitivity and cost-effectiveness for high-throughput screening.

Results: The conventional cytopathic effect assay required log-4 rotavirus titer and 60 hours for detection. Contrarily, ICC-qPCR detected as low as log-1 TCID50 in water, log-2 in apple juice, log-4 in spinach within 24 hours, demonstrating rapid and 1000-fold higher sensitivity over traditional methods. Integrating sample pooling reduced the testing 60%, making high-throughput screening cost-effective.

Significance: The ICC-qPCR combined with pooling is a rapid, cost-effective, and highly sensitive method for detecting log-1 infectious rotavirus in water, required to cause infection, thereby improving outbreak surveillance to reduce global burden of foodborne illness.

P1-227: RATIONAL ENGINEERING OF AN OMPG NANOPORE SENSOR ENHANCES ELECTRO-OSMOTIC FLOW AND SENSITIVITY FOR NOROVIRUS DETECTION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Human noroviruses are the leading cause of foodborne illness globally, yet existing detection techniques, including PCR-based methods and immunoassays, can lack the portability, sensitivity, and rapid response required for on-site food and environmental surveillance.

Purpose: The objective of this study is to enhance the sensitivity of an Outer Membrane Protein G (OmpG) nanopore sensor for noroviruses by increasing electro-osmotic flow through the pore.

Methods: Site-directed mutagenesis was used to generate OmpG mutants containing one, two, and three aspartic acid substitutions within the pore lumen to increase net negative charge. Recombinant OmpG proteins were expressed in *Escherichia coli*, purified, refolded, and reconstituted into planar lipid bilayers. Single-channel electrophysiology recordings were

conducted in 1 M KCl, 50 mM HEPES-KOH, pH 7.4 at □50 mV to measure pore conductance. Conductance values were calculated from at least three independent pore insertions and reported as mean ± standard deviation. Increased conductance was used as a proxy for enhanced electro-osmotic flow.

Results: OmpG mutants exhibited a charge-dependent increase in conductance. The mutant containing three aspartic acid substitutions showed the highest conductance 1.44 ± 0.02 nS (*N* = 8), where *N* represents the number of independent pores, followed by the two-substitutions mutant 1.36 ± 0.04 nS (*N* = 7), and the one-substitution mutant 1.31 ± 0.01 nS (*N* = 7), compared with wild-type OmpG 1.18 ± 0.02 nS (*N* = 6). These results demonstrate that increasing net negative charge within the pore lumen significantly enhances electro-osmotic flow, a key factor influencing nanopore sensing sensitivity.

Significance: Engineering electro-osmotic flow in OmpG nanopores provides a practical strategy to lower detection limits in nanopore-based biosensors for foodborne pathogens, and supports development of portable and sensitive platforms for food safety and public health applications.

Award Application: Developing Scientist Award

P1-228: RAPID SAMPLE PREPARATION PROTOCOL FOR THE DETECTION OF CRONOBACTER SAKAZAKII IN POWDERED INFANT FORMULA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Cronobacter sakazakii* is an opportunistic pathogen that can persist in dry foods, including powdered infant formula, posing a high risk of life-threatening infections in infants and thus representing a critical concern in food safety worldwide.

Purpose: This study aimed to develop a protocol for the detection of *C. sakazakii* in powdered infant formula within 24 hours through the association of chemical and mechanical sample processing.

Methods: Aliquots of *C. sakazakii* ATCC 29544 fresh cells were pipetted into 50 g of milk-based powdered infant formula at 10–10^3 CFU/g in sterile bags and contents were stored at 25°C and 15–25% relative humidity for 21 days. Inoculated formula was enriched in 300 mL buffered peptone water for 4 hours at 36°C and aliquots of 40 mL were collected for sample processing. Two enzymatic treatments were added to aliquoted samples, separately, at 0.025 and 0.05 g/g, totalizing four treatment systems with three replicates each. Treatments were incubated at 40°C for 1 hour and immediately subject to vacuum filtration through nylon membranes. Membranes were plated onto R&F® *Enterobacter sakazakii* Chromogenic agar and incubated for up to 20 hours at 36°C.

Results: Cell enumeration and filtration rate of samples are reported in CFU/g and mL/min,

respectively. One-way ANOVA revealed that one treatment achieved statistically significant ($p < 0.05$) superior filtration rate (1.50 average mL/min), compared to other treatments (0.78, 0.56, and 0.54 average mL/min) and to the absence of treatment (0.52 average mL/min). *C. sakazakii* colonies were successfully detected post final filtration from artificially contaminated samples down to 10^2 CFU/g.

Significance: This study demonstrates the potential application of a shortened and easily reproducible alternative method for detecting bacterial pathogens in powdered infant formula within one day, supporting food safety practices of surveillance and post-contamination investigations.

P1-229: REDUCTION OF UNCONFIRMABLE POSITIVE PCR RESULTS IN SAMPLES RELEVANT FOR POWDERED INFANT FORMULA MANUFACTURE

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Rapid and reliable pathogen detection is critical for powdered infant formula (PIF) manufacturers to ensure product safety and regulatory compliance. PCR-based testing offers sensitive and timely detection of foodborne pathogens. Heat treatment, low-moisture, and disinfectant use can result in residual free-DNA from nonviable organisms, leading to unconfirmable PCR-positive results. These false positives may cause unnecessary investigations, production delays, and product holds.

Purpose: This study evaluated whether use of the SureClean™ DNA Removal Kit prior to PCR could reduce false positives without compromising sensitivity.

Method: PIF and environmental samples (N=280) were enriched, then post-enrichment aliquots were contaminated with free-DNA from multiple strains of *Salmonella* spp., *Cronobacter* spp., and *Listeria monocytogenes* at levels expected to generate false-positive PCR results. Samples were analysed by PCR before and after treatment with the DNA removal kit.

Additional samples (N=150) were inoculated with low levels (<10 CFU) of live organisms, subjected to stress conditions, and tested pre- and post-treatment to assess impact to assay sensitivity.

Results: Of 280 samples contaminated with free-DNA, 261 yielded PCR-positive results before treatment, while only 35 samples (13%) remained positive after treatment. Of 150 samples inoculated with live organisms, 103 were PCR-positive before treatment and 102 remained positive after treatment and were confirmed by culture media techniques.

Conclusion: The use of a free-DNA removal step prior to PCR analysis significantly (*P*=<0.001) reduced unconfirmable PCR positive results in PIF and environmental samples, while maintaining detection of live stressed organisms at low levels. These findings demonstrate that incorporating free-DNA removal into PCR workflows can improve result reliability for PIF manufacturers, reducing investigations and production disruptions without compromising sensitivity.

P1-230: PERFORMANCE OF A MULTIPLEX PCR ASSAY FOR THE DETECTION OF DIVERSE SPOILAGE ORGANISMS IN BEVERAGE PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Beverage producers are under pressure to ensure product quality in the face of complex spoilage risks from diverse microorganisms. A rapid molecular detection method which enables detection in one reaction with a harmonized workflow significantly simplifies and streamlines testing, compared to traditional plating techniques.

Purpose: The purpose of this study was to verify performance of the SureTect™ Beverage Spoilage Multiplex PCR Assay for detection of yeast and molds, *Brettanomyces* spp., preservative-resistant yeasts, lactic acid bacteria and acetic acid bacteria from diverse beverage products.

Methods: Five matrix-microorganism combinations were tested. Matrices included electrolyte hydration, tea-based, sports energy, and juice-based drinks, with sample sizes ranging from 0.5L to 1.5L (in product bottles). A modified Relative Level of Detection (RLOD) study was performed vs. traditional plating methods with samples artificially contaminated

with target organism microbial cocktails to achieve spike levels of 0, 1–5 and 5–20 CFU/sample. Samples were analysed using the PCR workflow following a single harmonized filtration and enrichment for all of the organisms of interest. Additionally, a panel of 183 inclusivity and 140 exclusivity isolates were tested.

Results: The modified RLOD study demonstrated comparable performance of the PCR workflow to the culture plating method across all matrix-microorganism combinations and inoculum levels tested. The time-to-result for the PCR workflow was 4 days shorter than the traditional method. Inclusivity of 98% and exclusivity of 95% was achieved.

Significance: The Beverage Spoilage Multiplex PCR Assay constitutes a comprehensive panel for detection of yeast and molds, *Brettanomyces* species, preservative-resistant yeasts, lactic acid bacteria and acetic acid bacteria spoilage organisms, with a rapid and reliable workflow for the beverage industry.

P1-231: DEVELOPMENT OF AN IMMUNOASSAY FOR THE RAPID DETECTION OF LISTERIA MONOCYTOGENES USING NEWLY GENERATED MONOCLONAL ANTIBODIES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Listeria monocytogenes* is a major foodborne pathogen characterized by a high case-fatality rate and the ability to grow under refrigeration, leading to recurrent outbreaks.

Purpose: This study aimed to generate monoclonal antibodies against *L. monocytogenes* surface antigens and evaluate their applicability in a rapid lateral flow immunoassay (LFIA) for on-site detection.

Methods: Hybridomas were produced following mouse immunization, and two clones (L1C8H6B9 and L1C8G9E4) were selected based on specificity. Cross-reactivity was assessed against representative food-associated bacteria, including *Paenibacillus polymyxa*, *Clostridium putrefaciens*, and *Bacillus atrophaeus*. A prototype half-strip LFIA was constructed using colloidal gold-conjugated L1C8H6B9 as the detection antibody and membrane-immobilized L1C8G9E4 as the capture antibody.

Results: Both antibodies selectively recognized *L. monocytogenes* without cross-reactivity to tested organisms. The LFIA detected *L. monocytogenes* within 15 min, with a visual detection limit of approximately 10^6 CFU/mL and no false-positive reactions.

Significance: These results demonstrate the feasibility of a monoclonal antibody-based LFIA for rapid detection of *L. monocytogenes*. Further optimization of sensitivity may support development of a full-strip device suitable for routine industrial monitoring.

P1-232: AI-DRIVEN OPTICAL MICROBIOLOGY FOR RAPID, INCUBATION-FREE MICROBIAL ANALYSIS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Microbiological quality control is constrained by long time-to-result and logistical burdens related to incubation and reagent handling, limiting timely decision-making in food safety and environmental monitoring.

Purpose: To introduce a disruptive AI-driven optical microbiology technology that uses biophotonic imaging and machine learning to detect and enumerate microorganisms in 10 minutes without incubation, dyes, or reagents, demonstrated on matrices from the food and beverage industry.

Methods: Samples were analyzed using the LOUIS® system, combining membrane filtration, multi-modal optical imaging, and machine learning-based analysis to detect and enumerate microorganisms directly on filtration membranes. Total Viable Count and Yeast & Mold count were performed on two industrial matrices: ingredient water and an alcoholic beverage. At least 50 samples per matrix, naturally contaminated and artificially inoculated, were analyzed across contamination levels ranging from 1-6 $\log_{10}$ CFU/sample (100-500 mL). The ability to distinguish viable from non-viable microorganisms was evaluated on a broad panel representing more than 50,000 cells. Performances were evaluated by comparison with standard culture-based methods on Petri dishes, including negative controls.

Results: The LOUIS® system generated results within approximately 10 minutes, compared to several days for culture-based methods. Quantitative results in both ingredient water and alcoholic beverage matrices showed good agreement with the reference method, with a mean relative bias below ±0.5 $\log_{10}$ CFU/mL, and strong linearity (R^2>0.9). The system discriminated viable from non-viable cells in the alcoholic beverage based on intrinsic optical signatures with >90% specificity.

Significance: These findings highlight the potential of AI-driven optical microbiology to substantially reduce time-to-result while supporting microbiological quality control in food safety and environmental applications.

P1-233: ARTIFICIAL INTELLIGENCE-ASSISTED MALDI-TOF MS FOR ACCURATE IDENTIFICATION OF THE VIBRIO HARVEYI CLADE RELEVANT TO SEAFOOD SAFETY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Accurate discrimination of *Vibrio harveyi* clade species remains challenging in seafood safety monitoring because closely related species show overlapping phenotypic and MALDI–TOF MS database profiles.

Purpose: This study aimed to evaluate an artificial intelligence-assisted MALDI-TOF MS approach for reliable classification of *V. harveyi* clade species relevant to seafood safety.

Methods: A total of 235 *Vibrio* strains were confirmed by PCR and analyzed by MALDI-TOF MS, yielding 1,082 spectra primarily from on-plate protein extraction. Six machine-learning classifiers were trained and evaluated using independent test sets and cross-validation.

Results: Commercial database identification correctly classified 187 of 235 strains, yielding an overall accuracy of 79.57%, with complete misidentification of all *V. campbellii* strains. In contrast, machine-learning models showed markedly improved performance. The random forest model achieved 100% accuracy, precision, recall, and F1 score on both training and independent test sets, with a cross-validation F1 score of 0.998. Gradient boosting, k-nearest

neighbors, and support vector machine models also achieved near-perfect performance (AUC = 1.00), while naive Bayes showed slightly lower but still high accuracy (98.6%). Principal component analysis using all spectral features showed substantial species overlap; however, restricting analysis to the top 10% of features selected by the random forest model resulted in clear species separation. Key discriminatory mass peaks were identified at 7,194.7, 3,609.8, and 9,179.6 m/z, with significant intensity differences among species ($p < 2 \times 10^{-16}$).

Significance: This study demonstrates that AI-assisted MALDI-TOF MS enables rapid and database-independent identification of *V. harveyi* clade species, improving seafood safety monitoring and routine quality control in food protection laboratories.

P1-234: ASSESSING NOROVIRUS INFECTIVITY: RNASE PRETREATMENT ASSAY VERSUS HUMAN CHALLENGE OUTCOMES

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: The RNase pretreatment assay, coupled with qRT-PCR, detects intact viral capsids resistant to norovirus RNA degradation by RNase, serving as a proxy for infectivity. However, it has not been validated against viral particles that infect humans.

Purpose: To validate whether the RNase pretreatment assay detects norovirus particles, capable of causing infection and illness in human volunteers.

Methods: We tested a dilution series of the human norovirus inoculum 8fIIb, GI.1 Norwalk (10^7 to 10^1 genomic copies [gc]), previously used in human challenge studies (n=80), with the

RNase pretreatment assay. Doses were linked to past infection and illness outcomes. Controls included RNase treatment of extracted norovirus RNA (to confirm RNase activity) and heat-treated stool (80°C) prior to RNase (to mimic damaged capsids).

Results: We found no significant difference ($p > 0.05$, Fisher's exact test), at each inoculum dose between the range of human infection (highest 10^7 gc, 10/13 [77%] infected; lowest 10^3 gc, 3/9 [33%] infected) and RNase results (highest 10^7 gc, 6/6 [100%] positive replicates; lowest 10^3 gc,1/6 [17%] positive replicates). The lowest dose that caused infection in human volunteers (33%, 3/9 infected), had inconsistent detection (17%, 1/6 replicates) via the RNase pre-treatment assay. RNase degraded up to 10^5 gc of free RNA (highest gc tested), while heat treatment reduced detection by ~2 $\log_{10}$. These controls suggest that, while the RNase pretreatment assay is effective at eliminating "free" norovirus RNA, 80˚C prior to RNase treatment may not provide full access of norovirus RNA to RNase.

Significance: The RNase pretreatment assay correlates with infectivity at higher doses but may yield inconsistent results at the lowest infectious doses.

Award Application: Developing Scientist Award

P1-235: BEYOND “ALL POSITIVE”: THE ROLE OF FRACTIONAL CONTAMINATION LEVELS IN METHOD VALIDATION AND LOT TESTING

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: In qualitative microbiological testing, method performance can't be adequately assessed using only “all-positive” or “all-negative” results. Reliable evaluation requires knowledge of detection rates at low-contamination levels, which can only be obtained by explicitly testing those levels. At low contamination, microorganisms are randomly distributed among test portions, leading to a mix of positive and negative results.

Purpose: Evaluate the impact of fractional contamination levels on the calculation and

interpretation of key performance indicators (KPI), particularly POD curves and related metrics.

Methods: Validation designs with and without fractional contamination levels were evaluated. Designs including fractional levels contained at least one level with intermediate positivity (~25–75%). Data were analyzed using μKPI (QuoData) to construct POD curves, estimate Level of Detection (LOD), calculate Relative Level of Detection (RLOD) for method comparison, and identify suitable fractional levels. Practical implications were examined using a lot-testing scenario.

Results: Validations limited to high contamination levels yielded 100% positive results, providing no information on the LOD. Including fractional levels enabled reliable POD estimation and sensitivity comparison via RLOD. In the lot-testing example, a highly sensitive method (RLOD ~1) required fewer test portions to reach 95% confidence of lot negativity, whereas a less sensitive method (RLOD ~5) required substantially more. Fractional-level data revealed differences invisible at high contamination levels and supported evidence-based sampling strategies. Suitable fractional levels were identified using μKPI.

Significance: Fractional contamination levels are essential for reliable POD estimation, method comparison, and lot-testing. They prevent overestimation of analytical performance, support transparent method evaluation, and provide defensible guidance for low-level detection and sampling.

P1-236: COMPARISON OF SHORT- AND LONG-READ ITS SEQUENCING FOR FUNGAL DETECTION AND INTERPRETATION IN ENVIRONMENTAL MONITORING

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: Accurate detection and interpretation of fungal communities are important for environmental monitoring and risk assessment, yet differences among sequencing pipelines complicate data comparison.

Purpose: The objective of this study was to compare short-read ITS2 and long-read full-length ITS amplicon sequencing for fungal detection, taxonomic resolution, and interpretability using identical DNA extracts.

Methods: Air (n = 28) and surface swab samples (n = 39) were collected from residential, dormitory, and laboratory environments, along with negative controls (n = 17). DNA from each sample was analyzed in parallel using ITS2 sequencing on the Illumina platform and full-length ITS sequencing on the PacBio platform. Data from both platforms were processed using identical bioinformatic workflows, including denoising with DADA2 and taxonomic assignment with the UNITE database. Cross-platform sequence mapping was used to assess overlap and platform-specific detection.

Results: Full-length ITS sequencing provided higher genus- and species-level taxonomic resolution compared to ITS2 sequencing and showed clearer separation of samples by environment. Overall community patterns were broadly consistent between platforms, with moderate correlation in genus-level relative abundance. Cross-platform mapping indicated that 76% of ITS2 amplicon sequence variants aligned to full-length ITS references, while unmapped variants were primarily low-abundance features. Detection of health-relevant fungal genera varied by sequencing platform and sample type, indicating pipeline-specific biases.

Significance: Long-read ITS sequencing improves species-level resolution relevant to fungal surveillance, while ITS2 sequencing remains effective for community-level profiling. Understanding platform-specific trade-offs can inform method selection for environmental monitoring relevant to food protection and public health.

P1-237: DEVELOPMENT OF A REAL-TIME PCR METHOD BASED ON THE BETA-TUBULIN AND PUBERULIC-ACID-RELATED GENE FOR THE SPECIFIC DETECTION AND QUANTIFICATION OF PENICILLIUM ADAMETZIOIDES, A PUBERULIC ACID-PRODUCING FUNGUS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: *Penicillium adametzioides* produces puberulic acid. It has recently been reported as the cause of an outbreak associated with red yeast-fermented rice tablets and characterized by renal dysfunction, fatigue, and urinary abnormalities. To advance knowledge of the recently recognized mycotoxin-producing fungus, efficient methods for its detection and quantification are required.

Purpose: This study aimed to develop a real-time PCR method based on the β-tubulin and puberulic-acid-related gene for the specific detection and quantification of *P. adametzioides*.

Methods: Three primer candidates were designed based on the whole genome sequences of *P. adametzioides*. Primer specificity was evaluated using five *P. adametzioides* strains and eight other *Penicillium* spp. Genomic DNA was extracted from mycelia cultured for 24 h and used as a template for real-time PCR assays. In addition, a correlation analysis was

performed between Ct values and mycelial dry weight as a proxy for microbial biomass, to construct a calibration curve.

Results: Real-time PCR testing of the primer candidates demonstrated that two primer sets specifically amplified *P. adametzioides*, while no amplification was observed for other *Penicillium* spp. Electrophoretic analysis confirmed that the amplicons were approximately 100 bp in length. Calibration curves relating mycelial weight to Ct values were successfully constructed with the designed primers, yielding Root Mean Square Error values of 0.64–0.73 mg and R^2 values of 0.83 (n=77). These results suggest that the developed calibration curves enable quantification of *P. adametzioides* growth. Furthermore, quantification using *P. adametzioides*-specific PCR primers allows accurate quantification in samples with complex microbial communities.

Significance: This study presents real-time PCR primers for the efficient detection and quantification of *P. adametzioides*, a newly recognized mycotoxin-producing fungal hazard.

P1-238: DEVELOPMENT OF TEST METHODS TO ASSESS THE BIOCIDAL ACTIVITY OF FOAM-BASED PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Disinfectants and sanitizers are commonly used in food production with a foaming additive to increase surface-cling time and performance; however, these products are typically tested as liquids because standard foam test methods are lacking.

Purpose: Develop test methods that can be used to assess and compare the efficacy of various foam-based products with a focus on biofilm.

Methods: The CDC biofilm reactor was used to grow *Pseudomonas aeruginosa* biofilm on 304 stainless steel carriers. In a lab-scale method, carriers were placed in wire mesh drain baskets and treated with peracetic acid (PAA) combined with various foaming additives using a foamer bottle. In an intermediate-scale application, rods from the reactor were clamped to a steel panel and sprayed with foam using a portable 5-gallon foaming unit with multiple wand types with different parameters. All treatments were applied at 20°C for 10 min followed by neutralization (n 6). Bacterial viability was determined by serial dilution plating.

Results: In the lab-scale method, PAA at concentrations ranging from 200–800 ppm combined with a foaming additive achieved log reduction values ranging from 2.9 to 8.2. When the same combinations were tested as liquids and the carriers were submerged, approximately ½ the amount of PAA was required to achieve comparable kill. In the intermediate-scale application even higher levels of PAA (> 400 ppm) were required to achieve low levels of kill (< 2-log). Using both methods an acidic foam additive was compared with a pH neutral foam additive, and the former was more effective by log reduction values ranging from 1.3–5.5 depending on the level of PAA.

Significance: Testing products in their intended use state is vital to understanding their performance, and developing foam-based test methods is necessary as liquid-based test methods may not accurately reflect efficacy as a foam.

P1-239: DOMAIN-ADAPTIVE HYPERSPECTRAL MICROSCOPY FOR CLASSIFICATION OF MAJOR FOODBORNE PATHOGENS ACROSS ILLUMINATION CONDITIONS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Hyperspectral microscopy is a promising rapid method for artificial intelligence (AI)-enabled pathogen classification, yet variability in imaging conditions limits model generalizability.

Purpose: This study evaluates domain adaptation strategies within an AI-enabled hyperspectral microscopy framework to improve pathogen classification across illumination conditions.

Methods: Five bacteria (*Listeria monocytogenes*, *Listeria innocua*, *Salmonella* Enteritidis*, Salmonella* Typhimurium*,* and *E. coli* O157:H7) were cultured in trypticase soy broth with yeast extract at 32°C for 16 h, washed, and resuspended in deionized water. Aliquots of 2 µL were pipetted onto glass slides, air-dried, and rehydrated with 1 µL of deionized water, with three slides prepared per biological triplicate. Non-overlapping regions were imaged using a darkfield hyperspectral microscope at 100× under tungsten halogen and metal halide

illumination, with 50 hyperspectral data cubes per bacterium per condition. Data were reduced by principal component analysis to composite images and used to train a convolutional neural network with adversarial domain adaptation, treating halogen images as the source domain and metal halide images as the target domain. Model performance was evaluated on a held-out target dataset using accuracy, and feature separation was visualized using t-distributed stochastic neighbor embedding.

Results: A dataset of 500 hypercubes across two illumination conditions was established. Models trained with adversarial domain adaptation improved cross-illumination accuracy from ~39% to 82% on the held-out target dataset (n=225, p<0.05). Feature visualization confirmed clearer separation of bacterial classes after domain adaptation.

Significance: These results show that domain adaptation enables AI-enabled hyperspectral microscopy models to remain accurate despite changes in imaging conditions, supporting consistent pathogen classification using more accessible systems.

Award Application: Undergraduate Student Award

P1-240: EVALUATING DIGITAL PCR AS A CULTURE-INDEPENDENT TOOL TO SUPPORT IRRIGATION WATER MICROBIAL ASSESSMENTS

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Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: The FDA's risk-based approach to evaluating irrigation water recommends comprehensive assessments, rather than relying on microbial testing alone. These assessments could benefit from quantifying multiple indicator organisms, but culture-based

methods can be time consuming and labor intensive. Culture-independent methods, such as digital PCR (dPCR), could be used to efficiently monitor a suite of indicators and pathogens simultaneously.

Purpose: This study compared dPCR and culture-based IDEXX methods to assess microbial quality of harvested rainwater (HRW) used for irrigation.

Methods: HRW was collected and stored in a 500-gallon tote and sampled weekly over 10 weeks (n=10). Enterococci (Ent.), *E. coli* (EC) and total coliforms (TC) were quantified using IDEXX Enterolert and Colilert culture-based assays. Water samples were filtered through 0.20 uM membranes and frozen until DNA extraction and dPCR using a QIAcuity were performed. Ent., EC and *Bacteroides* spp. (Bac), in lieu of TC, were quantified via dPCR due to assay availability. Bacterial concentrations were compared using Pearson correlation and fitted linear regression analyses.

Results: TC/Bac, Ent, and EC were significantly correlated (p 0.001, p 0.001, p=0.02) between culture and dPCR methods. dPCR results showed significant correlations between Bac and EC (p=0.003), Bac and Ent (p=0.04) and EC and Ent (p=0.02). Culture and dPCR methods showed positive correlations for TC/Bac (r=0.903), Ent (r=0.702) and EC (r=0.924), with culture results explaining 82%, 49%, and 85% of variability observed in dPCR results, respectively.

Significance: The significant positive correlations observed among dPCR results, and between dPCR and culture method results, support the potential for dPCR to be used in irrigation water monitoring with comparable levels of confidence as culture methods. dPCR may be able to provide unique insights into irrigation water quality and any risks associated with its application.

P1-241: EVALUATION OF INSTADOSE™ GRAVIMETRIC DILUTOR FOR HIGHLY ACCURATE RECONSTITUTION OF CONCENTRATED ENRICHMENT MEDIA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Achieving dilution accuracy using concentrated broth media in food testing is challenging at low sample masses; on-demand preparation of working-strength broth can reduce media-prep burden, but requires accurate dilution with practical dispense times.

Purpose: This study evaluated a gravimetric reconstitution workflow that dispenses and electronically records both concentrate and water masses, enabling rapid, accurate dilution to single-strength solution.

Methods: A gravimetric diluter serially dispensed filtered water and 10x concentrate to produce 1x broth for 10 g sample: 90 g broth, 25g:225g, and 125g:1125g workflows using variable and fixed mass dispense programs. Logged dispense data were used to calculate Total Mass Deviation (TMD) and Reconstitution Deviation (RD), with acceptance limits of ±2% and ±4%, respectively. Replicate testing included 10g (n=450), 25g (n=275), and 125g (n=20) dispenses (total n=745). Dispense times were measured to assess workflow practicality.

Worst-case repeatability (10g and 25g programs) was assessed over three days.

Results: All dispenses (100%, n=745) met acceptance criteria and met ISO 6887-1 dilution tolerances (±2% TMD). Mean TMD ranged from −0.2% to 0.1%, and mean RD from −0.5% to 0.1% (RD limit ±4%). Day-to-day variability remained below the predefined practical threshold (0.5%). Mean dispense times ranged from 9.9–51.1 s depending on the workflow configuration.

Significance: Results demonstrate that the InstaDose™ Gravimetric Diluter gives fast, accurate, reproducible and fully traceable reconstitution of a 10x concentrate to 1x strength for routine food microbiology testing.

P1-242: EVALUATION OF NEOGEN™ MICROBIAL LUMINESCENCE SYSTEM (MLS) FOR REDUCED RUNNING TIME USING A MODIFIED PROTOCOL IN RAPID STERILITY TESTING OF PLANT-BASED UHT PRODUCTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Plant-based UHT products are increasingly prevalent, requiring rapid microbial evaluation methods such as the Microbial Luminescence System (MLS) to ensure product quality.

Purpose: Evaluate the performance of the Microbial Luminescence System using the prescribed protocol and a time-reduced modified method and compare with the traditional

methods.

Methods: A total of 132 samples were tested across three UHT beverage types (Almond, Pistachio, and Oat milk). These were inoculated with a panel of stressed microorganisms (*B. cereus* and *K. aerogenes*) at a level of less than 30 CFU/container. Inoculated beverage containers were pre-incubated at 32°C for 48 or 72 hours. Following incubation, the beverages were evaluated using a modified MLS protocol where the incubation with ATPase was reduced from 900 to 600 seconds compared to the prescribed protocol. Additionally, the samples were analyzed using standard methods, including Colony Count and pH measurement. An additional sensitivity study was conducted for bacteria *B. cereus* and *K. aerogenes* in all three UHT beverage types.

Results: The study demonstrated that the modified Microbial Luminescence System protocol produced results comparable to the default protocol and ISO 4833-1. The Sensitivity for the Alternative Method (SE_{alt}), Sensitivity for the Reference Method (SE_{ref}), and Relative Trueness were all 100%, while the False Positive Ratio for the alternative method was 0%. For the sensitivity study, both protocols successfully detected a level of 10^3 CFU, expected post-enrichment. Importantly, the modified protocol reduced the total time-to-result by approximately 5 minutes per run.

Significance: Modified protocol for Microbial ATP bioluminescence can provide a more rapid result compared to conventional methods and reduce time to result.

P1-243: VERIFICATION OF RAPID MICROBIAL METHODS FOR COMMERCIAL STERILITY OF DAIRY AND ALTERNATIVE DRINK PRODUCTS WITH IDENTIFICATION OF CHANGES IN METABOLIC ACTIVITIES FROM HEAT-RESISTANT SPORE-FORMING BACTERIA

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Heat-resistant spore-forming bacteria impact microbial food quality and safety of UHT products. Research on the microbiological quality and prevalence of these bacteria in UHT products is relevant to establish control strategies.

Purpose: To develop an early warning system for detecting of heat-resistant spore-forming bacteria in UHT products using Soleris® technology and metabolic activities change using sucrose detection.

Methods: A total of 120 samples analyzed, six UHT products (milk, almond, pistachio, soy, coconut milk and coconut drink) were studied. Overnight cultures of *G. stearothermophilus* and *B. sporothermodurans* were used for inoculation. Experiments followed ISO 16140-3:2021. Spiking levels were based on the Soleris® NF 105 validation report No. 2021LR100 (LOD_{50}: 0.821 CFU/test portion). Samples were incubated at 35°C and 55°C for 48 hours. Sucrose content was determined using the Sucrose/D-Glucose kit.

Results: Four of six UHT samples of *G. stearothermophilus* showed $eLOD_{50}$ values of less than 1.0–2.0 CFU/test portion, except milk and coconut drinks, which exhibited growth inhibition. Meanwhile, all UHT samples of *B. sporothermodurans* showed $eLOD_{50}$ values of 1.0–2.3 CFU/test portions. As ISO 16140-3:2021 criteria, all results were passed. Sucrose content demonstrated significant changes in all UHT products, except for coconut drinks in *G. stearothermophilus* and milk for both bacteria.

Significance: The rapid alternative method provided accurate results within 72 hours, suitable for both mesophilic and thermophilic UHT sterility testing. This reduces incubation time by two days, saving storage space and enabling faster detection of metabolic activity changes.

P1-244: EVALUATION OF RAPID AUTOMATED PH MEASUREMENT TECHNOLOGY FOR COMMERCIAL STERILITY TESTING

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: In commercial sterility testing, pH is commonly used as an indicator of microbial spoilage. Microbial growth can alter a product's chemical environment, resulting in measurable shifts from the product's normal, stable pH range. Monitoring these changes provides an early and sensitive marker for potential spoilage.

Purpose: The objective of this study was to evaluate a rapid automated pH measurement system for liquid food and beverage products and to compare its performance with conventional manual pH measurement methods.

Methods: Three phases were conducted to comprehensively evaluate the rapid automated pH system. Phase 1 compared automated and manual pH measurements across a range of product types including broths, protein shakes, plant-based milks and high-viscous matrices such as cheese sauce analyzed in triplicate both directly (n=54) and after acid adjustment (n=54). Phase 2 assessed the potential for cross-contamination between wells (n=48) in a standard 96-well microtiter plate using a broth with contrasting pH values. Phase 3 evaluated the technology's ability to detect pH changes as small as 0.2 units in four matrices that were

artificially inoculated (n=12) with relevant spoilage organisms with results compared to culture.

Results: Collectively, the study demonstrated that the automated pH system provides consistent and precise results, although absolute pH values may differ from those obtained by manual methods. No evidence of cross-contamination was observed with broth, as indicated by very low standard deviations (pH 5.15, SD=0.084; pH 2.74, SD=0.060). The technology was also able to detect pH shifts associated with active microbial spoilage, with the exception of very low-level contamination that did not produce clearly distinguishable pH changes.

Significance: These findings indicate that the rapid automated pH system may be suitable for implementation in commercial sterility testing to indicate microbial spoilage.

P1-245: DETECTION OF LISTERIA FROM READY-TO-EAT DELI HAM USING THE HYGIENA BAX® SYSTEM REAL-TIME PCR ASSAY

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Ready-to-Eat (RTE) meats, including deli meats, are recognized as a high risk food category for contamination with *Listeria monocytogenes* (Lm). Due to the high frequency of these products causing human listeriosis cases, both regulators and industry make continuous efforts to enhance food standards safeguarding these products.

Purpose: This study was designed to evaluate the performance of a real-time PCR-based method compared to the USDA-FSIS reference method for the detection of Lm in three different RTE deli hams including smoked, tavern, and uncured varieties.

Methods: Three unpaired matrix studies were performed following the technical guidelines in AOAC INTERNATIONAL Official Methods of Analysis Appendix J. Each matrix was inoculated with Lm at a low-level expected to yield fractional positive results and at a high-level expecting to yield all positive results**.** Additional samples were reserved for negative controls. After a 72 hour equilibration at 4°C, test portions were divided into test method samples (125 g and 25 g) and reference method samples (25 g). Samples were enriched and analyzed according to their respective method.

Results: For 125 g test method samples, real-time PCR detected Lm in 16/20 smoked, 15/20 tavern and 12/20 uncured low inoculated samples. For 25 g test method samples, real-time PCR detected Lm in 14/20 smoked, 13/20 tavern and 11/20 uncured low inoculated samples. All high-inoculated samples were positive in each set. All presumptive results were identical to culture with 100% sensitivity and 100% specificity.

Significance: Overall, the BAX System Real-Time PCR assay for Genus *Listeria* and Real-Time PCR assay for *L. monocytogenes* demonstrate accurate and reliable results for the detection of *Listeria* species in both 125 g and 25 g samples of RTE deli ham equivalent to the USDA MLG reference method.

P1-246: EVALUATION OF RAPID TECHNOLOGY TO ENUMERATE YEAST AND MOLDS ON DAIRY-BASED FERMENTED MATRICES

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Dairy matrix is nutrient-dense and contains bioactive minerals, proteins, lipids and carbohydrates. Most common types of spoilage cultured dairy products include proliferation of yeast and contamination by molds. Food industry stakeholders must prioritize the critical assessment and implementation of high-sensitivity, rapid diagnostic tools for the precise identification of microbiological hazards.

Methods: Six dairy-based fermented matrices were evaluated; Samples were obtained directly from the production line and then shipped to the laboratory facilities in Queretaro México. Sixty samples were contaminated with one milliliter of overnight fresh culture of two different target microorganisms: Thirty samples with *Candida albicans* ATCC 10231 and thirty samples with *Aspergillus brasiliensis* ATCC 1664. Three inoculum levels were tested: low (1–10 CFU/g), medium (10–0 CFU/g), and high (40–100 CFU/g). Each 10-g sample was diluted with 50 mL of Phosphate buffer 0.1% and then plated on Petrifilm® Rapid Yeast and Mold and Dextrose Potato Agar Acidified. Both methods were incubated at 25°C during 48 and 72 h for

Petrifilm and 60 and 120 h for Agar. Log-counts were compared using *T*-paired at 95% of statistical confidence to determine statistical differences.

Results: In general, no statistical differences (p=.214) were found for the enumeration of yeast at 60 hours of incubation: 2.06 ±0.40 and 2.05±0.42 CFU log for agar and Petrifilm respectively. Mold recovery was not statistical different only for one matrix analyzed (p=.128): 2.26±0.20 and 2.21±0.21 CFU log for agar and Petrifilm, respectively.

Significance: Petriflm Rapid Yeast and Mold can be used as a quick method to detect microorganisms typically related to dairy spoilage in a quick and accurate way.

P1-247: ISO 16140-2 VALIDATION OF A RAPID REAL-TIME PCR METHOD FOR DETECTING ENTEROBACTERIACEAE IN POWDERED INFANT FOODS AND INGREDIENTS

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Powdered infant foods are consumed by a highly vulnerable population, and Enterobacteriaceae contamination, especially *Cronobacter* spp. and *Salmonella* spp*.,* remains a key hazard, motivating rapid and reliable screening of infant foods and related ingredients.

Purpose: To validate an alternative real-time PCR method against ISO 21528-1:2017 using the ISO 16140-2 validation protocol.

Methods: Sixty-four food samples (infant formula/cereals with or without probiotics, and milk-powder ingredients), low-level inoculated or uninoculated, were analyzed in parallel by the alternative and ISO reference methods. For the alternative method, a selective-supplemented enrichment was evaluated on 375 g test portions incubated 18–26 h at 37°C. Two extraction workflows were assessed: direct extraction from enrichment broth or

extraction after a 1:10 dilution in buffered peptone water; the free DNA removal solution procedure was systematically applied to limit detection of free DNA. Presumptive positives were confirmed on VRBG and a second chromogenic Enterobacteriaceae agar. Inclusivity/exclusivity, RLOD, and an inter-laboratory study (10 laboratories; 50-g test portions) were also performed.

Results: Alternative-method sensitivity was 81.8% (direct extraction) and 81.4% (1:10 dilution), versus 65.9% and 67.4% for the reference method. RLOD values ranged from 0.8 to 0.9 (acceptance limit = 2.5), with LOD50 values of 1.0–1.1 CFU/test portion compared with 1.5 CFU/test portion for the reference method. All 50 target strains were detected, and all 30 non-target strains were negative; inter-laboratory RLOD was 0.8. Overall, ISO 16140-2 acceptance criteria were fulfilled, supporting equivalence to the reference method.

Significance: The iQ-Check Enterobacteriaceae assay provides a rapid, robust alternative for screening Enterobacteriaceae in powdered infant foods and related ingredients, with performance equivalent to ISO 21528-1:2017.

P1-248: OPTIMIZATION OF DNA EXTRACTION FOR TUNA SPECIES IDENTIFICATION

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Presentation type/Format: Poster

Category: Laboratory and Detection Methods

Abstract:

Introduction: Canned tuna is susceptible to species mislabeling, and optimizing DNA extraction-incubation time may improve the efficiency of species identification while maintaining amplification success.

Purpose: This study aimed to optimize DNA extraction of canned tuna by comparing the current protocol to one that uses a shorter incubation time.

Methods: A total of 24 tuna samples, 6 samples per species (albacore, skipjack, light, and yellowfin), were analyzed in duplicate for each respective incubation time. DNA extraction was performed on tuna samples using two different lysis incubation times: 2 h and 20 h. PCR was performed on the DNA extracts, followed by gel electrophoresis to determine the success of amplification.

Results: PCR amplification results indicated comparable amplification success for albacore, skipjack, light, and yellowfin tuna DNA extracted using 2-h and 20-h incubation times. Amplification was detected in all albacore tuna samples under both conditions (6/6), while light tuna samples resulted in no amplification under either condition (0/6). For skipjack tuna, amplification was detected in 1/6 samples following 20-h incubation, whereas no amplification was observed following 2-h incubation (0/6). Overall, reducing incubation time from 20 h to 2 h resulted in minimal differences in amplification outcomes among the species evaluated.

Significance: The findings suggest that shorter DNA extraction incubation times may be sufficient to support reliable and efficient PCR amplification for tuna species identification. This optimization in the incubation protocol could reduce the testing time while also maintaining analytical effectiveness in canned tuna authentication.

P1-249: TOLERANCE OF FOOD-SPOILAGE MOLDS TO SINGLE OR MIXED PRESERVATIVES

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Organic acid preservatives are failing to control mold spoilage in processed foods.

Purpose: This study evaluated the minimal inhibitory concentration of preservatives commonly used in food industries against six fungi isolated from spoiled bread and dry-cured meat.

Methods: Potassium sorbate, sodium benzoate and calcium propionate, single or mixed, were confronted with three strains of *Penicillium roqueforti* and three strains of *Aspergillus* series *Circumdati*. After seven days of cultivation on malt extract agar at 25°C for 7 days, a 10^4 spores/mL spore suspension was inoculated in 96 well plates containing yeast extract buffered broth (pH 5.0) and added with the preservatives to be tested. The initial concentration for single preservative was 10%, and 5% for the mixed. The plates were incubated at 25°C and the fungal growth was checked by occeloscope after 7 days.

Results: Differences in the preservative susceptibility profile were observed among the

tested strains. The common bread spoilers *P. roqueforti* were more sensitive to sodium benzoate and very tolerant to calcium propionate. On the other hand, the cured meat spoilers *Aspergillus* series *Circumdati* were more susceptible to potassium sorbate and by far the most tolerant to benzoate among all the tested strains. Differences in the susceptibility to sorbate were observed among some strains of *P. roqueforti* and to propionate among *Aspergillus* series *Circumdati.* No synergistic effect was observed when mixing preservatives; in some cases, an antagonist effect appeared to occur.

Significance: This study shows the high tolerance of food spoilage moulds to organic acids preservatives and provide useful information for decision making on preservative selection to control spoilage problems.

P1-250: ASSESSMENT OF THE DIVERSITY OF FUNGI SPOILING INDUSTRIAL BREADS

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Category: Microbial Food Spoilage

Abstract:

Introduction: Industrial breads are widely consumed globally due to their convenience and stability, having molds as the main spoilage agents influencing the product shelf life.

Purpose: To assess the main molds spoiling industrial breads from different regions of Brazil.

Methods: A total of 220 industrial breads from 44 baking factories (5 per site) were sampled from markets in the 5 geographical regions of Brazil: North (N), South (S), Southeast (SE), Midwest (MW), and Northeast (NE). The breads were stored at room temperature and visually observed until they became moldy or up to 30 days after their expiration date.

Results: 21 out of 44 (47.7%) baking factories showed spoilage problems by fungi, mainly located in N (80%), NE (66.6%) and S (66.6%). The highest diversity of species occurred in NE, followed by S. The well-known preservative tolerant molds from *Penicillium* section *Roquefortorum* (*Penicillium roqueforti* and *Penicillium* paneum) were the most common isolated, spoiling breads in 18.2% of the baking factories, in all the regions. Isolates of xerophilic *Aspergillus* section *Aspergillus* (*Aspergillus chevalieri* and *Aspergillus pseudoglaucus*) were also common spoilers (13.6%), however they were only deteriorating breads from S and

NE. The chalky molds were the third most common spoilers, with a 9.09% of occurrence each, with *Saccharomycopsis fibuligera* present in NE, S and MW; and *Hyphopichia burtonii* only in S. Finally, heat-resistant *Paecilomyces variotii* and *Aspergillus* species with potential to produce mycotoxins (form sections *Flavi, Circumdati* and *Nigri*) were also isolated, among others.

Significance: This knowledge can help to develop products or strategies to improve the shelf-life of industrial breads and increase the safety of consumers.

P2-001: PLANT-BASED MEAT ALTERNATIVES: AN EVALUATION OF POTENTIAL C. BOTULINUM TOXIN PRODUCTION AND METAGENOMIC SURVEY

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Presentation type/Format: Poster

Category: Plant-Based Alternative Products

Abstract:

Introduction: Plant-based meat alternative (PBMA) products contain protein sources of wheat gluten, soybean, pea, or mushroom. Many PBMA products are extruded, a process where *C. botulinum* spores may survive. Insufficient process cooling or storage temperature abuse may allow potential outgrowth of *C. botulinum* spores.

Purpose: Evaluate the risk of *C. botulinum* toxin production and survey metagenomic profiles of PBMA products.

Methods: Eighteen PBMA products were surveyed for toxin production. Samples were weighed into Whirl-Pak bags (25g, in triplicate) and 225mL of Trypticase-Peptone-Glucose-Yeast (TPGY) broth was added. Samples were heat-shocked at 60°C for 15 minutes to inactivate vegetative populations and anaerobically incubated (37°C; 7 days). PBMA challenge studies were performed by inoculating 19 PBMA products at 1% with proteolytic *C. botulinum* cocktail (10^3 spore/mL) and incubating anaerobically (37°C; 7 days). Survey and challenge samples were evaluated for botulinum toxin production using the endopeptidase-MS assay. Shotgun sequencing (Illumina NextSeq2000) was utilized to assess the metagenomic profile

of 16 PBMA products.

Results: Results demonstrated negative toxin production from all 18 PBMA products surveyed. Challenge study results demonstrated positive toxin production from 13 PBMA inoculated products, with 11 products positive for Type A and B toxin and 2 products positive for Type A toxin only. Following metagenomic sequencing, all PBMA products analyzed demonstrated trace amounts of *C. botulinum* DNA*;* however, relative abundance was low (□0.03%). Bioinformatic analyses identified the most dominant microorganisms as *Lactobacillus* and *Lactococcus* spp., *Corynebacterium glutamicum*, *Pseudomonas syringae*, and *Bacillus cereus*.

Significance: This study may aid in understanding the metagenomic profile of PBMA products and potential risk of *C. botulinum* and/or other pathogens if insufficient process cooling or temperature abuse occurs.

P2-002: AUTHENTICATION OF GRASS-FINISHED BEEF USING GENE PROFILING

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Presentation type/Format: Poster

Category: Food Fraud

Abstract:

Introduction: Grass-finished beef is associated with a beneficial nutritional profile, but variability among products and reliance on imports raise concerns regarding the accuracy of producers' claimed grazing practices.

Purpose: This study quantified expression of four candidate genes to assess their viability as biomarkers for differentiating beef by finishing diet.

Methods: Simmental-Angus influenced steers (n = 117) were randomly assigned to 1 of 4 finishing diets: (1) pasture with hay supplementation (GHAY), (2) pasture with baleage supplementation (GBLG), (3) pasture with soybean hull supplementation (GSH), or (4) feedlot-fed with baleage and soybean hulls (BLGSH). RNA was extracted from 25 ± 5 mg of frozen ribeye tissue and standardized to 1.5 ng/μL prior to cDNA synthesis. cDNA template was used for real-time PCR to quantify expression of 4 target genes (NPNT-1, ABCA-1, SCD, SREBP-1), and 1 housekeeping reference gene (RPLP0) for normalization. Non-template negative controls were included in all PCR runs and relative gene expression was calculated using the ΔΔCt method. Target gene expression among dietary treatments were assessed using Kruskal–Wallis tests followed by Dunn’s multiple comparisons, with individual animals

treated as the experimental unit (α = 0.05).

Results: NPNT-1 expression differed among diets, with greater expression in GHAY compared to GSH and BLGSH ($p < 0.01$). ABCA-1 expression did not differ among dietary treatments ($p > 0.05$). Results are reported for NPNT-1 and ABCA-1 only, as data collection for SCD and SREBP-1 is ongoing.

Significance: This study demonstrates the potential of PCR-based methods to authenticate grass-finished beef and support verification of grazing claims, contributing to improved labeling accuracy, producer credibility, and consumer trust.

Award Application: Developing Scientist Award

P2-003: COMPARISON OF DNA BARCODING APPROACHES TO IDENTIFY ROCKFISH (SEBASTES SPP.) ON THE COMMERCIAL MARKET

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Presentation type/Format: Technical

Category: Food Fraud

Abstract:

Introduction: Rockfish (*Sebastes* spp.) are vulnerable to mislabeling due to their visual similarity and variable market value, which can be difficult or impossible once filleted and skinned, further incentivizing seafood fraud.

Purpose: The mitochondrial gene regions, cytochrome c oxidase subunit I (COI) and cytochrome b (*cyt b*), have been reported to differentiate *Sebastes* spp., but there is limited research comparing the performance of these markers in identifying rockfish sold in the United States commercial market, this study evaluated the effectiveness of COI and *cyt b* markers for the species-level identification of rockfish.

Methods: Commercial rockfish samples (n = 69), including fresh, frozen, and cooked products, were collected online and from retailers in Southern California. DNA was extracted from ~10 mg of sample tissue, followed by amplification and sequencing of the COI and *cyt b* regions. All PCR runs included positive and negative controls. The resulting sequences were assembled and compared against reference databases for species-level identification.

Results: While both regions exhibited 97% amplification rates, COI had a higher sequencing

rate (100%) compared to *cyt b* (98.5%). COI and *cyt b* produced the same species-level identification in 23 of 64 samples sequenced by both markers. Using COI, 62 of 67 samples were identified to the species level, while five samples were identified to the genus level. In contrast, *cyt b* yielded species-level identifications for 38 of 66 samples, with 28 samples limited to genus-level identification.

Significance: These results highlight the significant impact of marker choice on species identification of rockfish, with implications for seafood fraud detection and consumer trust.

Award Application: Developing Scientist Award

P2-004: RAPID AND VISUAL DETECTION OF PORK ADULTERATION USING AN EXTRACTION-FREE TOOTHPICK-ASSISTED LAMP ASSAY

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Presentation type/Format: Technical

Category: Food Fraud

Abstract:

Introduction: Pork adulteration of high-value meat products remains a global food fraud concern, reducing consumer confidence and complicating regulatory enforcement, particularly in processed meat products.

Purpose: This study aimed to develop and validate a rapid, extraction-free, and visual loop-mediated isothermal amplification (LAMP) assay for on-site detection of pork adulteration using a toothpick-assisted direct sampling strategy.

Methods: A LAMP assay targeting the mitochondrial cytochrome c oxidase subunit I (COI) gene was developed and optimized using purified DNA to select the best-performing primer set and determine analytical sensitivity. A sterile bamboo toothpick was used for direct tissue sampling, and Tris-HCl buffer (1.5 M, pH 8.8) was incorporated to stabilize the pH-sensitive hydroxynaphthol blue (HNB) colorimetric readout. Assay specificity was evaluated using DNA

from 12 non-target animal species. Practical performance was assessed using pork-beef and pork-mutton adulteration models and validated with 20 commercial meat products. Results were independently verified using qPCR and Sanger sequencing.

Results: Under matrix-free conditions, the assay achieved a detection limit of 0.5 pg/μL with high specificity and no cross-reactivity among the 12 non-target animal species tested. In the extraction-free toothpick format, clear visual discrimination between positive and negative under ambient light enabled detection of pork adulteration as low as 0.05% (w/w) within 20 min without specialized instrumentation. Validation of 20 commercial meat products showed complete agreement among toothpick-LAMP, qPCR, and Sanger sequencing results, supporting robustness for real-world applications.

Significance: This toothpick-assisted and extraction-free LAMP assay provides a rapid, low-cost, and user-friendly approach for on-site detection of pork adulteration, supporting routine food fraud surveillance in resource-limited settings.

P2-005: RAPID RHPCR ASSAY FOR AUTHENTICATING ROYAL RED SHRIMP IN U.S. SEAFOOD MARKETS

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Presentation type/Format: Poster

Category: Food Fraud

Abstract:

Introduction: Seafood mislabeling is a significant concern in the United States with approximately 30% of shrimp products substituted for other imported species, undermining consumer trust, and domestic industry profitability.

Purpose: To develop and validate an RNase H-dependent PCR (rhPCR) assay for accurate identification of royal red shrimp (*Pleoticus robustus*) to mitigate species substitution.

Methods: Genomic and crude DNA was extracted from 57 barcoded shrimp tissue samples representing seven commonly traded species namely, *Litopenaeus setiferus* (n=8), *Litopenaeus vannamei* (n=9), P*enaeus aztecus* (n=4), P*enaeus duorarum* (n=4), *Penaeus monodon* (n=7), *Pleoticus muelleri* (n=5) and *Pleoticus robustus* (n=20). Primer pairs targeting COI gene of *P. robustus* were designed, optimized using extracted DNA and real-time PCR platforms. The selected primer pair was converted to an RNase H2-activated primer labeled with FAM and biotin for conventional PCR and lateral flow detection. Optimal rhPCR conditions included initial denaturation at 95°C for 900s, 35 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30s, and extension at 72°C for 5s. Independent validation used 20 blind samples marketed as royal red shrimp collected by a state's food safety division. All assays were

performed in duplicate.

Results: The rhPCR assay achieved 100% specificity, accurately identified the 20 barcoded and eight market samples as royal red shrimp specimens by having two bands on the lateral flow stick to distinguish it from all non-target species with a total turnaround time of 150 minutes. Detection sensitivity was confirmed at 0.1 ng/µl, ensuring reliable identification at low DNA concentrations.

Significance: This assay offers a rapid, sensitive, and specific tool for regulatory and enforcement agencies to detect species substitution in shrimp products on-site, thereby protecting public health and supporting domestic royal red shrimp markets against economic fraud.

Award Application: Developing Scientist Award

P2-006: A LABORATORY'S ROLE: BRIDGING THE GAP BETWEEN FOOD SAFETY AND FOOD DEFENSE IN THE ARMED SERVICES

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Presentation type/Format: Technical

Category: Food Defense

Abstract:

Introduction: The U.S. Army has combined food safety and food defense into one overarching mission, Food Protection.

Purpose: How the U.S. Army Public Health Command Laboratories can bridge the gap between the Military Food Defense and Food Safety missions.

Methods: Interviews with Army Veterinary Services, Food Safety, and Food Defense officials about the impacts of unintentional contamination on safety and defense of military personnel were conducted. Laboratory testing procedures including emergency military operations, common surveillance, and increased methodology were researched.

Results: Food defense and safety is managed by two different groups in the Army. The laboratory is the connection between these areas. Public Health Command Laboratories have begun work to close the gap to include open communication with officials, training Army field laboratories, updating surveillance programs, and working across multiple agencies.

Significance: The Army oversees food protection for all branches of the U.S. Military, and the laboratory can and has played a large role in fortifying that mission.

P2-007: AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION OF USING PATHOGENESIS-RELATED PROTEINS FOR RESISTANCE AGAINST ASPERGILLUS FLAVUS IN PEANUTS

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Presentation type/Format: Poster

Category: Food Defense

Abstract:

Introduction: Aflatoxin (AFB1) contamination by *Aspergillus flavus* represents a major threat to global peanut (*Arachis hypogaea*) production and food safety. The development of genetically engineered varieties with enhanced resistance to fungal infection offers a promising strategy to reduce AFB1 and improve crop safety.

Purpose: We aim to develop transgenic peanuts through *Agrobacterium tumefaciens*–mediated transformation using pathogenesis-related (PR) proteins, chitinase, and β-1,3-glucanase, and quantification of aflatoxin B1 (AFB1) in genetically modified peanuts.

Methods: Peanut embryos, Georgia-12Y, were co-cultivated with *Agrobacterium* harboring pCAMBARGlu 289 and pCAMBAR Chi11 constructs, driven by a constitutive promoter. Embryos were cultured in Murashige and Skoog medium with 3 mg/L 2,4-dichlorophenoxyacetic acid. Subsequently, shoot and root regeneration were induced in media containing 1 mg/L benzyladenine and 1 mg/L indole-3-butyric acid, respectively. Untreated embryos served as controls, and experiments were conducted in replicates. Cultures were incubated at 28°C for 14 days with 25 mg/L kanamycin and 250 mg/L cefotaxime to suppress bacterial overgrowth. The genomic DNA of the transformants was

amplified by Polymerase Chain Reaction (PCR) using gene-specific primers. Liquid chromatography–mass spectrometry (LC–MS) was used to quantify AFB1 in seedlings.

Results: Embryogenesis was achieved at a 98% success rate. Callus induction and regeneration of shoots and roots indicated potential transgene integration. PCR amplification of transformants confirmed the chitinase gene at approximately ~700 bp, while the GUS assay verified the establishment of the β-1,3-glucanase gene. Quantification of AFB1 in seedlings is currently underway, and data analysis is expected soon.

Significance: These findings support the development of peanut varieties with improved resistance to *A. flavus*, enhancing crop safety and reducing economic losses for peanut growers in Georgia.

P2-008: ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA FROM MEXICAN FERMENTED BEVERAGES AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Food Defense

Abstract:

Introduction: In the Mexican Altiplano, fermented beverages production is expanding and represent a source of diverse lactic acid bacteria (LAB). Beyond their role in fermentation, LAB may offer functional benefits through antagonistic activity against foodborne pathogens.

Purpose: The aim of this study was to asses the in vitro antagonistic activity of LAB isolated from Mexican fermented beverages against *Salmonella enterica* and *Listeria monocytogenes.*

Methods: LAB strains were isolated from the production enviroment of wine (10 strains), pulque (6 strains) and cider microfermentations, using MRS agar with cycloheximide (100 ppm). These LAB were evaluated using the double-layer agar technique. In a button shape, 5 µL of LAB cultures (1×10^9 CFU/mL) were inoculated onto MRS agar and after 24 h incubation at 35°C a layer of Triptic soy agar (TSA) containing five pooled strains of *S. enterica* or *L. monocytogenes* (1×10^6 CFU/mL) was added. After incubation at 35°C for 24 h, inhibition zones were measured. All assays were performed in triplicate, and data were analyzed using ANOVA and Tukey's test (JMP 11.0).

Results: All LAB isolates exhibited antagonistic activity, with inhibition patterns dependent on both the LAB strain and the target pathogen ($p<0.05$). The strongest inhibition was observed against *Salmonella*, with zones up to 34 mm, while cider- and pulque-derived LAB reached inhibition zones up to 31 mm. In contrast, inhibition of *L. monocytogenes* was generally lower, with the highest activity observed among wine-associated LAB (up to 29 mm). Enological strains CU17, CM60, VC59, and V24 demonstrated consistent and broad antagonistic activity against both pathogens.

Significance: These findings highlight fermented beverages from the Mexico as a promising reservoir of LAB with antagonistic potential against major foodborne pathogens, supporting their further evaluation as probiotic and natural biocontrol agents to enhance food safety in fermented and non-fermented foods.

P2-009: APPLYING OPEN SOURCE INTELLIGENCE METHODS TO VERIFY MARITIME TRANSPORT COMPLIANCE FOR BULK EDIBLE OIL IMPORTS: A FRAMEWORK DEVELOPMENT STUDY

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Presentation type/Format: Poster

Category: Food Defense

Abstract:

Introduction: Maritime transport of bulk edible oils presents contamination risks from previous cargoes that current U.S. import verification programs do not systematically address.

Purpose: To develop an Open Source Intelligence (OSINT) framework enabling importers to independently verify vessel cargo history compliance with international edible oil transport standards.

Methods: This framework development study analyzed regulations governing bulk edible oil transport, including Federation of Oils, Seeds and Fats Associations (FOSFA) International previous cargo requirements, Codex Alimentarius transport standards, and Foreign Supplier Verification Programs (FSVP) obligations. Publicly accessible maritime data sources were evaluated, including Automatic Identification System (AIS) vessel tracking platforms, ship registry databases, and port state control records. The framework provides four verification layers: AIS-based voyage reconstruction to identify chemical terminal calls and cargo spacing patterns; corporate registry analysis for vessel ownership verification; port call sequencing to detect undisclosed ship-to-ship transfers; and cross-referencing tank cleaning certifications against vessel movements.

Results: The framework integrates multiple OSINT data streams to enable systematic verification of FOSFA Banned Immediate Previous Cargo compliance. Key detectable indicators include vessel visits to chemical terminals, cargo spacing intervals inconsistent with cleaning requirements, AIS transmission gaps, and draft anomalies. Verification capabilities are accessible through commercial maritime intelligence platforms at cost levels appropriate for mid-sized importers.

Significance: This framework addresses a critical gap between FSVP verification and domestic food defense programs by providing importers practical tools to independently verify maritime transport compliance, transforming supplier self-certification into importer-verified due diligence.

Award Application: Developing Scientist Award

P2-010: EVALUATION OF CLOSTRIDIUM BOTULINUM NEUROTOXIN TYPE B IN LIQUID INFANT FORMULA PROFICIENCY TESTING SAMPLES

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Presentation type/Format: Poster

Category: Food Defense

Abstract:

Introduction: *Clostridium botulinum* neurotoxins cause botulism and have acute toxicity in humans. Recently, a significant nationwide outbreak in infant formula has reinforced the need for reliable, rapid identification of active botulinum toxin in food matrices.

Purpose: To verify method performance of a Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) for *Clostridium botulinum* neurotoxin B (BoNT/B) using homogenous and stable proficiency testing (PT) samples.

Methods: Liquid infant formula (LIF) samples were bulk inoculated with BoNT/B at 150, 200,

and 300 ng/mL, homogenized via manual shaking, and aliquoted into 5 mL test samples. Ten samples were tested to establish homogeneity, and two samples were tested on days 5, 9, 16, and 20 for stability. Extracted toxin was verified to be BoNT/B by using MALDI-TOF/MS to compare the sample spectra to established theoretical mass-to-charge ratio (m/z) values of the BoNT/B peptide substrate's intact peptide and two cleavage products. PT samples were prepared and shipped on dry ice to eight participating Food Emergency Response Network (FERN) laboratories.

Results: The tested inoculation levels of 150, 200, and 300 ng/mL had m/z values for the intact peptide, cleavage product 1, and cleavage product 2 of 4019 ±1, 1760 ± 1, and 2284 ± 1, respectively. These values fell within the theoretical m/z values of the BoNT/B peptide substrate cleavage products. The eight participating FERN laboratories were able to detect all inoculated PT samples with 100% accuracy.

Significance: The current gold standard for detection of *C. botulinum* BoNT is the mouse lethality assay, which takes four days for results. The MALDI-TOF/MS detection method can yield results in as little as four hours. A proficiency exercise using the MALDI-TOF/MS method was 100% successful and demonstrated the capabilities of FERN laboratories should a BoNT outbreak occur.

P2-011: DISINFECTION OF POULTRY PROCESSING WASTEWATER USING ADVANCED OXIDATION PROCESSES

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Given the increasing demand of poultry, poultry processing continues to generate a large volume of wastewater rich in diverse foodborne pathogens that require effective treatment to support safe reuse.

Purpose: This study evaluated the utility of photo-electrochemistry (PEC) combining electrochemical (EC) and photochemical (PC) treatments to disinfect poultry processing wastewater.

Methods: Wastewater from the scald and bird-washer units were treated using independent 7 L and 30 L PEC reactors operated at 70 V and 100 V EC with a fixed 1.60% curcumin for PC. Treatment was conducted for 300 min with aliquots collected every 30 min for analysis of aerobic bacteria (AC), *Enterobacteriaceae* (EB), coliforms (CC), and *E. coli* on Petrifilm™. Furthermore, samples collected at 0, 150 and 300 min were enriched and plated on XLT-4 and *Campylobacter* agar for *Salmonella* and *Campylobacter* detection, respectively. The data was reported as $\log_{10}$ CFU/mL and analyzed using linear mixed-effects model on R software ($p < 0.05$) with 1 CFU/mL detection limit.

Results: Across 43 samples evaluated from wastewater types and reactor scales, PEC treatments at 70 V and 100 V achieved substantial reductions in AC, reducing 5.4-6.2 log CFU/mL initial loads to 2.0-2.9 log CFU/mL within 300 min. Treatments at 100 V reduced AC to 2.7 log CFU/mL, meeting the regulatory threshold (500 CFU/mL) for reuse. EB, CC, and *E. coli* were completely inactivated, with faster disinfection at 100 V (120-180 min) compared to 70 V (180-210 min). Although differences between pathogen reductions were insignificant between EC levels ($p > 0.05$), 100 V showed a trend of quicker and greater microbial reduction across all microorganisms. Furthermore, *Salmonella* and *Campylobacter* were consistently eliminated within 150 min across all treatments.

Significance: These findings demonstrate PEC as a sustainable disinfection strategy that reduced pathogens including *Salmonella* and *Campylobacter* in poultry wastewater.

Award Application: Developing Scientist Award

P2-012: EFFECTIVENESS OF CONSUMER WATER PURIFICATION PRODUCTS IN REDUCING WATERBORNE BACTERIA FROM RECREATIONAL FRESHWATER SOURCES

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Over 3.5 million people die each year due to waterborne pathogens, and millions of hikers in the U.S. rely on water tablets/filters to drink water during hikes.

Purpose: The objective of my experiment was to determine which method (tetraglycine hydroperiodide tablets, sodium dichloroisocyanurate tablets, 4-stage filtration straw, 5-stage filtration straw, and ferric sulfate/calcium hypochlorite sachet) would reduce aerobic plate counts, generic *E. coli*, coliform, and Enterobacteriaceae from three lake water sources in Texas.

Methods: After collecting three-gallon samples from each water source on three separate days, we performed the five water filter/disinfection methods according to their instructions. We plated before and after samples (conducted 1:10 dilutions) on Petrifilm® Aerobic Plate Count, *E. coli*/coliform, and Enterobacteriaceae. Plates were incubated at 35°C/37°C and read at 24 hours or 48 hours, depending on the petrifilm type.

Results: Overall, the ferric sulfate/ calcium hypochlorite sachet and 4-stage filtration straw were most effective at eliminating aerobic bacteria, generic *E. coli*, coliforms, and

Enterobacteriaceae. The 5-stage filtration straw was least effective as it was only effective at eliminating *E. coli*.

Significance: Consumers should be cautious when buying water filters/tablets as some are ineffective at removing waterborne bacteria.

P2-013: HEAT AND DESICCATION RESISTANCE IN EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI (EXPEC)

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: A growing body of evidence has demonstrated that naturalized wastewater *Escherichia coli* (NWEC) and extraintestinal pathogenic *E. coli* (ExPEC) are resilient to wastewater treatment processes such as anaerobic digestion, chlorination and heat, raising concerns about pathogen persistence in the food–water nexus.

Purpose: To evaluate the heat and desiccation resistance of wastewater-derived ExPEC (W-ExPEC) and NWEC to better understand pathogen survival mechanisms.

Methods: Eleven ExPEC strains, containing W-ExPEC and NWEC strains, were suspended in autoclaved distilled water (ADW), 1% peptone, and tryptic soy broth (TSB) and treated at 50–55°C for up to 9 hours. In some experiments, bacteria were acclimatized to high temperature and then subjected to heat shock treatment. A modified most probable number (MPN) spot assay was used to enumerate surviving bacterial cells. Desiccation tolerance was assessed

over the short (24–48h) and long-term (7–21 days). One or two-way analysis of variance (ANOVA) with Bonferroni's post test was used to evaluate the significance of treatment.

Results: W-ExPEC and NWEC strains showed significant resistance to heat, surviving 55°C for up to 2 hours compared to laboratory control strains (ATCC25922 and CFT073 [clinical ExPEC]) that were completely inactivated within the first hour. Remarkably, some W-ExPEC strains survived 52^{o}C for >9 hrs. NWEC and W-ExPEC strains also resisted desiccation for 21 days. Thermal acclimatization increased W-ExPEC's heat resistance by ~1000-fold when compared to immediate heat shock treatment, suggesting that acclimatization induces even greater resistance to heat treatment.

Significance: Our findings suggest that W-ExPEC and NWEC are relatively heat and desiccation-resistant, raising questions about treatment survival during food processing and water treatment.

Award Application: Developing Scientist Award

P2-014: INTEGRATING MICROBIAL INDICATORS INTO PATHOGEN WASTEWATER SURVEILLANCE SYSTEMS OF DIFFERENT POPULATION SIZES

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: SARS-CoV-2 (COVID-19) continues to circulate at low levels with less clinical testing, similar to agents of foodborne disease. Wastewater analysis of microbial indicators may provide insights through quantitative techniques that may be useful for future foodborne illness investigations.

Purpose: This study evaluates use of novel statistical analysis of indicators with COVID-19 concentrations. Leveraging COVID-19 surveillance can improve methods for monitoring foodborne illness.

Methods: Water samples (100 mL, n=137) were collected weekly from two effluent wastewater collection sites in Delaware, one rural (R) and one urban (U). Samples were filtered via vacuum filtration followed by concentration at 3,000xg for 45 minutes at 20°C. Nucleic acid extraction was performed and COVID-19, pepper mild mottle virus (PMMoV), crAssphage (crAss), and *Bacteroides* (*Bac*) presence was analyzed using dPCR (Qiagen QiAcuity). CrAss and *Bac* were normalized using PMMoV concentrations, then correlation tests (Pearson/Spearman) and multiple linear regressions were performed.

Results: Across 137 samples from R and U, COVID-19 concentrations showed a moderate positive relationship with PMMoV at both locations (r=0.53, R, r=0.43, U). In multivariate models, PMMoV remained a strong positive predictor (β +1.06 in R, β +0.26 in U), while PMMoV normalized markers were negatively linked to COVID-19. CrAss correlated with COVID-19 at R (ρ= 0.35) and U (ρ= 0.37), while *Bac* showed stronger negative correlations (r= 0.33 to 0.41, R; r= 0.45 to 0.46, U). Multivariate models supported these trends, with negative coefficients for crAss (β 0.16, R) and *Bac* (β 0.15, R, β 0.50, U). This indicated that higher relative fecal marker levels corresponded with lower COVID-19 concentrations.

Significance: Indicators can be beneficial in wastewater surveillance for COVID-19 with potential application in pathogen detection and monitoring, advancing food safety and public health efforts.

Award Application: Undergraduate Student Award

P2-015: LOW-VOLTAGE LOCALLY ENHANCED ELECTRIC FIELD ELECTROPORATION FOR AGRICULTURAL WATER DISINFECTION

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Irrigating water is a major contamination route for fresh produce with foodborne pathogens. Current disinfection practices rely heavily on chemical- and UV-based approaches to meet microbial standards; however, these approaches face limitations related to energy demand, byproduct formation, negative impacts on soil health, and practical on-farm implementation.

Purpose: This study aims to develop a locally enhanced electric field treatment (LEEFT)-based system as a low-energy, chemical-free alternative for agricultural water disinfection that addresses the conventional approaches' limitations.

Methods: A LEEFT-enabled, flow-through filtration system was developed by integrating silver nanoparticles-doped polyaniline (Ag/PANI) onto a porous sponge matrix. The resulting Ag/PANI nanosponge was fully characterized for its electrochemical and physicochemical properties. The antimicrobial performance of the developed system was evaluated against *Escherichia coli* O157:H7 and *Listeria innocua* as foodborne pathogen models.

Results: The Ag/PANI nanosponge generated localized electric field hotspots under low-applied voltage and reactive oxygen species that enhanced the membrane disruption in bacteria. The designed nanosponge exhibited superior disinfection performance, with >5 log

reduction (99.999%) of foodborne bacterial models under both static and dynamic water flow conditions at very low applied voltages of 10V and 15V, respectively. Effective disinfection was maintained at a water flow rate of 1.5 L/h without the formation of harmful byproducts, with a low energy consumption of <150 J/L.

Significance: These findings demonstrate that LEEFT-based Ag/PANI nanosponges offer a scalable, low-energy, chemical-free, and decentralized water treatment approach for reducing food safety risks associated with agricultural water. The interconnected 3D macroporous structure enables high flow flux while preventing clogging from suspended particles or bacterial debris.

Award Application: Developing Scientist Award

P2-016: PERSISTENCE AND GENOMIC DIVERSITY OF SALMONELLA NEWPORT IN WATERS COLLECTED FROM THE EASTERN SHORE OF VIRGINIA

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: *S.* Newport is a leading cause of salmonellosis in the U.S. and has been linked to multistate-outbreaks associated with vegetables. Although environmental persistence of *S*. Newport in the surface waters at the Eastern Shore of Virginia (ESVA) has been reported, comprehensive genomic analyses of lineage diversity, antimicrobial resistance, and plasmid content remain limited.

Purpose: This study leveraged comparative genomic approaches to assess the genetic diversity of (i) *S.* Newport isolated from surface waters in ESVA from different growing seasons, and (ii) compare with broader diversity of *S.* Newport isolated from geographically similar regions.

Methods: *S.* Newport genome assemblies from Virginia, Maryland, and Delaware (n = 464; clinical, human and environmental) were retrieved from NCBI Pathogen Detection and quality filtered (□300 contigs; N50 □50 kb). MLST types were assigned, and antimicrobial resistance genes and plasmid were identified using AMRFinderPlus and PlasmidFinder v2.1.6. For ST5 and ST118, using kSNP3 reference genomes were selected for SNP calling using

CFSAN hqSNP pipeline, core SNP parsimony trees were visualized in iTOL.

Results: Ten MLST sequence types were identified, with ST5 and ST118 predominating. SNP analysis showed minimal genetic variation (1–5 SNPs) among pond water isolates across multiple years, with closely related isolates detected at the same locations over time and at different locations across years, indicating long-term persistence and spatial dissemination. Antimicrobial resistance genes were most frequent in ST45—primarily *tet*(A), *sul*2, *flo*R, *aph*(3")-Ib, and *aph*(6)-Id—although overall AMR prevalence was low.

Significance: These findings indicate that *S.* Newport persists in ESVA surface waters over multiple years and spreads across locations and time, with serovar distributions consistent with previous reports, underscoring implications for agricultural water safety.

Award Application: Developing Scientist Award

P2-017: PREVALENCE AND ABUNDANCE OF ANTIMICROBIAL RESISTANCE GENES IN GLOBAL FRESHWATER RESOURCES

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Presentation type/Format: Technical

Category: Water

Abstract:

Introduction: Antimicrobial resistance is a growing global concern due to its impacts on ecosystems and human health, with freshwater environments serving as major exposure pathways. The global prevalence and abundance of antimicrobial resistance genes (ARGs) in freshwater systems remain poorly characterized.

Purpose: Quantify global freshwater ARG prevalence and abundance and identify environmental drivers of their distribution.

Methods: PubMed, Scopus, Web of Science, and AGRICOLA were systematically searched

using a CoCoPop strategy.After deduplication, eligible studies were identified through BERT-assisted auto-screening followed by two-reviewer manual assessment. Gene-level abundance (copies/mL), prevalence (%), and associated metadata were extracted. Random-effects meta-analysis and mixed-effects meta-regression were performed in R(metafor, lme4), and AICc used for model selection.

Results: Among 78 ARGs, *sul1* and *intI1* were most frequently reported. The pooled mean ARG concentration was 1.65 $\log_{10}$ copies/mL (95% CI:1.52-1.78), equivalent to ~44 copies/mL, with extreme heterogeneity (I^2 100%). Water-source subgroup analyses showed the highest concentrations in surface waters (95% CI:2.59-2.88 $\log_{10}$;~542 copies/mL), exceeding groundwater (95% CI: -0.40 to 0.12 $\log_{10}$; ~0.73 copies/mL) and city/tap water (95% CI: -0.51 to -0.06 $\log_{10}$;~0.52 copies/mL). Land-use subgrouping indicated elevated levels in agricultural-livestock settings (~63,100 copies/mL) versus urban/residential (~229 copies/mL) and rural/natural sites (~214 copies/mL). A mixed-effects model (fixed: drug class, water source, land use; random: study, season, country) explained substantial variance (R^2=0.72),with water source as the dominant predictor.

Significance: This study establishes global, quantitative benchmarks for drug-class-specific ARGs in freshwater sources. The results directly inform exposure assessment and risk-based frameworks for evaluating human health risks associated with ARGs.

Award Application: Developing Scientist Award

P2-018: SPATIOTEMPORAL DYNAMICS OF MULTIDRUG-RESISTANT (MDR) ENTEROBACTERALES: SEASONALITY AND WATER PHYSICOCHEMICAL PROPERTIES DRIVE HIGH THIRD-GENERATION CEPHALOSPORIN RESISTANCE IN CENTRAL CHILE

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Presentation type/Format: Poster

Category: Water

Abstract:

Introduction: Antimicrobial resistance (AMR) is a critical global health threat, with surface waters acting as key pathway for the dispersal of MDR Enterobacterales.

Purpose: We characterized AMR *Escherichia coli*, *Klebsiella* and *Enterobacter* in central Chilean surface waters and identified risk factors for MDR detection.

Methods: Water samples (1L; N= 480) were collected quarterly (Nov 2023–Aug 2025) from 60 sites in Maipo and Mapocho watersheds. Samples were filtered and cultured on MacConkey agar plates: one unsupplemented and two supplemented with ciprofloxacin or ceftazidime (2 µg/mL each). Isolates were identified via MALDITOF MS, and AMR profiles determined by disk diffusion per CLSI guidelines. Random forest (RF) and Generalized Linear Mixed Models (GLMM) identify environmental factors associated with detection of MDR bacteria**.**

Results: Of 1,648 isolates, 765 were profiled. *E. coli* (n=696) dominated, with 77.2% MDR, 6.8% extensively-drug resistant (XDR), and 0.4% pan-drug resistant (PDR). *Klebsiella (n*=60*)* exhibited 95% MDR and 38.3% XDR phenotypes, while all *Enterobacter* (n=9) were MDR. High resistance to 3rd-generation cephalosporins (421/765) was observed, while carbapenem resistance remained low (61/765). According to RF, the factors most strongly associated with MDR detection were seasonality and water physicochemical properties. GLMM confirmed, higher environmental temperatures increased MDR odds (OR: 1.13; $p < 0.001$), while higher salinity (OR: 0.74 per 100 ppm; $p < 0.001$) and summer (OR: 0.26 vs. Winter; $p = 0.004$) acted as protective factors. In contrast, presence of human settlements showed no association.

Significance: Integrating environmental monitoring with RF revealed temporal dynamics and watershed characteristics driving MDR. These underscore the need to move beyond static monitoring frameworks toward seasonally informed monitoring strategies that can proactively track irrigation-water contamination and reduce downstream AMR contamination risks.

Award Application: Developing Scientist Award

P2-019: AGGREGATION BEHAVIOR OF TULANE VIRUS, A HUMAN NOROVIRUS SURROGATE, UNDER VARIOUS IONIC CONDITIONS: IMPLICATIONS FOR THERMAL TOLERANCE

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus is a leading cause of foodborne illness, but its behavior and thermal resistance in aggregated forms remain unclear. Tulane virus, a suitable surrogate for human norovirus, also has unknown aggregation behavior under varying ionic conditions.

Purpose: To examine how ionic strength, pH, and aggregation affect Tulane virus properties and thermal stability.

Methods: Tulane virus was propagated in LLC-MK2 cells and purified via iodixanol density gradient ultracentrifugation. Virus stocks were sonicated, filtered to reduce aggregation, and suspended in Tris-EDTA buffer. Particles were characterized by dynamic light scattering and

transmission electron microscopy. Zeta potential and isoelectric points were measured across pH 3.3–10 and $CaCl_2$ concentrations (1, 10, and 100 mM). Aggregation effects on viral titer were assessed via plaque assay after 5-hour exposure under different ionic and pH conditions. Thermal stability of aggregated and dispersed virus suspensions was evaluated at 60°C for 2.5 min. All experiments used three replicates, except for the heat treatment plaque assay that were conducted with four replicates.

Results: Tulane virus was primarily monodispersed in Tris-EDTA buffer (pH 7.2), with diameters of 39–41 nm and intact morphology. Zeta potential measurements revealed an isoelectric point of 4.5–6.3, with near-zero surface charge promoting aggregation. Higher $CaCl_2$ concentrations induced larger aggregates (hydrodynamic diameter up to ~2,917 nm at 100 mM $CaCl_2$, pH 6.3). Plaque assays showed aggregation reduced viral titers under specific pH and ionic conditions ($p<0.05$). Aggregated and dispersed particles did not indicate any difference in thermal tolerance when treated for up to 2.5 min at 60°C ($p>0.05$).

Significance: Tulane virus readily aggregates under ionic conditions typical of food and environmental systems, providing insights into adsorption and stability of human norovirus surrogates.

Award Application: Developing Scientist Award

P2-020: ANTIVIRAL ACTIVITY OF NATURAL COMPOUND-BASED SANITIZERS AGAINST MURINE NOROVIRUS IN ALUMINUM SURFACE MODEL

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus, a major cause of foodborne gastroenteritis worldwide, resists conventional disinfectants. Developing effective and safe antiviral sanitizers is crucial.

Purpose: Identify natural compounds with antiviral activity against norovirus and validate their efficacy in surface models.

Methods: Molecular docking simulations were conducted using the PyRx platform. Protein–ligand interaction analyses were visualized with Discovery Studio Visualizer, while ADMET profiling was carried out for compounds targeting the conserved ILE52–ASN58 region of the VP1 protein. Favorable compounds were assessed for cytotoxicity using cell viability assays.

Selected compounds were incorporated into alcohol-based sanitizer formulations based on the WHO standard. Antiviral efficacy was evaluated against MNV using ASTM E1053 surface protocols and quantified by $TCID_{50}$.

Results: Molecular docking analysis showed carvacrol, resveratrol, ECG, EGCG, and Oolonghomobisflavan B (*Camellia sinensis* extract) strongly interacting with the VP1 conserved region. Cytotoxicity assays confirmed suitability for topical application. In aluminum surface assays, carvacrol showed the highest antiviral activity with a reduction of 2.0–3.26 log_{10} at 1 h and 3.26–3.32 log_{10} at 24 h. *C. sinensis* extract showed intermediate activity with a reduction of 1.58–3.0 log_{10} at 1 h and 2.18–3.12 log_{10} at 24 h. Resveratrol showed a reduction of 1.67–1.92 log_{10} at 1 h and 2.18–2.6 log_{10} at 24 h, and ECG showed a reduction of 1.6–1.82 log_{10} at 1 h and 2.1–2.45 log_{10} at 24 h, while EGCG showed limited efficacy (0.32–2.18 log_{10} at 1 h and 1.03–2.8 log_{10} at 24 h).

Significance: Molecular docking is an effective approach for identifying natural compounds with antiviral potential, supporting their application in sanitizers for norovirus control.

Award Application: Developing Scientist Award

P2-021: APPLICATION OF DIVERSE METAL-BASED DEEP EUTECTIC SOLVENT FORMULATIONS FOR NOROVIRUS CONCENTRATION AND IMPROVED DETECTION

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human noroviruses are among the most prevalent foodborne viruses worldwide, and their low abundance in food and environmental samples necessitates effective virus concentration prior to detection. Deep eutectic solvents (DESs) are low-cost and environmentally friendly reagents with tunable charge properties, making them promising candidates for virus concentration. While early-generation DESs have shown potential for virus concentration, metal-tuned DESs remain unexplored.

Purpose: This study aimed to evaluate novel norovirus concentration strategies using different formulations of DESs and hydrophobic magnetic deep eutectic solvents(hmDESs).

Methods: Murine norovirus (MNV) was diluted in PBS and mixed with cobalt- or dysprosium-based DESs, with distilled water used as a control. Two cobalt-based and two dysprosium-based DES formulations were prepared using trioctylphosphine oxide (TOPO) at concentrations of 0.025 or 0.1 (n = 4). Additionally, two hmDESs were synthesized using

TOPO, metal chlorides ($CoCl_2$ or $DyCl_3$), and fatty acids (n = 2). Virus–DES complexes were magnetically separated, washed to remove unbound virus, and eluted using modified Luria Broth. Virus recovery was quantified by RT-qPCR.

Results: Cobalt-based DESs showed capture efficiencies of 12.7% and 16.2% and recovery efficiencies of 4.5% and 5.4% for the 0.025 and 0.1 Co-complex formulations, respectively. Dysprosium-based DESs achieved higher capture efficiencies, with the 0.025 Dy-complex reaching 35.7% capture and 6.1% recovery, while the 0.1 Dy-complex showed 20.5% capture and 7.4% recovery. HmDESs containing $CoCl_2$ achieved 26.0% capture and 5.8% recovery, while hmDESs containing $DyCl_3$ achieved 31.8% capture and 4.4% recovery.

Significance: These results demonstrate the potential of metal-based DES systems as cost-effective and environmentally friendly tools for norovirus concentration, supporting their application in food safety monitoring and viral surveillance.

Award Application: Developing Scientist Award

P2-022: WITHDRAWN

WITHDRAWN

P2-023: EFFICACY OF CURCUMIN AND QUERCETIN AS NATURAL PHOTOSENSITIZERS FOR PHOTOINACTIVATION OF THE NOROVIRUS SURROGATE MS2 IN PARSLEY

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Category: Viruses and Parasites

Abstract:

Introduction: Viruses can contaminate fresh herbs through contaminated water during both preharvest and postharvest stages. Photoinactivation (PDI) with blue light diodes mediated by natural photosensitizers is an alternative technology for inactivating viruses in foods. Little is known about the effectiveness of different phenolics on inactivation of viruses on parsley.

Purpose: This study assessed the effect of curcumin- and quercetin-mediated photodynamic inactivation on the norovirus surrogate bacteriophage MS2 on fresh parsley leaves.

Methods: Samples (3 g) of parsley (*Petroselinum crispum*), previously sanitized (chlorine solution; 100 ppm, 15 min), were inoculated with 100 µl of MS2 (~8 log PFU/g). The leaves were immersed in curcumin or quercetin solution (75 µM) for 2 min and exposed to PDI treatment (LED at 430 to 470 nm wavelength and 33.6 mW/cm^2 of light irradiance) for 6, 8, or 10 min. The viral titer was determined before and after PDI treatments using the double agar plate technique with *Escherichia coli* C3000 as the host. Control treatments without light exposure or photosensitizers were also evaluated. All experiments were carried out in triplicate. Statistics considered $p<0.05$.

Results: There was no difference in MS2 inactivation between phenolics after 6-min PDI-treatments. Curcumin- and quercetin-mediated PDI resulted in reductions of approximately 1.2 and 1.3 log PFU/g on parsley leaves after 8 min, respectively although differences were not significant. Treatment with quercetin promoted higher inactivation of MS2 on parsley leaves compared to curcumin after 10 min of exposure, reducing ~2.5 log PFU/g of MS2 vs in 2.0 log PFU/g in curcumin-mediated PDI.

Significance: Results show moderate MS2 inactivation, with quercetin showing superior performance among the two evaluated photosensitizers. Findings suggest the tested phenolics as promising photosensitizers for photodynamic inactivation on fresh herbs.

P2-024: GREEN TEA ALGINATE EDIBLE COATING REDUCES FOODBORNE PATHOGEN CONCENTRATION ON WHITE MUSHROOM (AGARICUS BISPORUS)

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: White mushrooms (*Agaricus bisporus*) are widely consumed around the globe. Mushrooms have been involved in *Salmonella* and *Listeria* linked recalls and outbreaks. Edible coatings with natural extracts have emerged as a promising strategy to improve safety of fresh mushrooms during storage.

Purpose: The antimicrobial activity of an edible alginate coating with green tea extract (GTE; *Camellia sinensis*) was assessed against *Salmonella* and *Listeria* on *A. bisporus*.

Methods: The coating solution was composed of 1.5% alginate (w/v), 0.75% glycerol (v/v), 0.04% sunflower oil (v/v), 0.05% Tween 80 (v/v), sterile distilled water and GTE (w/v) at the minimum inhibitory concentration (0.125 mg/g). Mushrooms (20 g) were dipped into *L. monocytogenes* and *S.* Typhimurium suspension (5 log CFU/g) before and after coating. Samples were stored at 4°C and 95% relative humidity. Pathogens were enumerated at 0, 1,

2, 4, 6 and 8 days (detection limit: 1.5 log CFU/g). Non-contaminated coated samples were used as control. The coating was characterized by using a non-contact 3D optical profiler. Statistics considered $p<0.05$.

Results: *L. monocytogenes* and *S*. Typhimurium counts increased by 1.5 log CFU/g over the 8 days of storage in non-coated mushrooms. In mushrooms coated after contamination, *L. monocytogenes* counts decreased by 1.39 log CFU/g after 1 day of storage, and no viable cells (< 1.5 LOD) were detected after 2 days. *S*. Typhimurium counts reduced 1 log CFU/g after 8 days of storage. In mushrooms coated before contamination, both pathogens reduced 2 log CFU/g over the storage. Weight loss was lower in coated (3.5%) vs. uncoated mushrooms (6.35%) on day 8 ($p < 0.05$). Coated mushrooms were measurably smoother (surface and roughness parameter) than non-coated mushrooms during storage ($p < 0.05$).

Significance: The environmentally friendly GTE coating reduced *L. monocytogenes* and *S. Typhimurium* concentration on mushrooms during storage.

P2-025: ENTERIC VIRUS PERSISTENCE IN THE COLON: EFFECTS OF AN INTACT AND ANTIBIOTIC-PERTURBED MICROBIOTA

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Each year, over one in eight Canadians experience foodborne illness, mainly due to human norovirus (HuNoV) and hepatitis A virus (HAV). However,the behavior of these viruses during gastrointestinal transit remains poorly understood.

Purpose: This study evaluated the persistence of HuNoV, murine norovirus (MNV-1), and HAV in colonic fermentation under stable or ciprofloxacin-perturbed microbiota.

Methods: Feces from four donors were grown in a PolyFermS inoculum reactor using Macfarlane medium. Anaerobic 25 mL batch fermentations (10% v/v microbiota) were inoculated with viruses (MNV-1: 7.3 log PFU or HAV: 6.7 log PFU or HuNoV: 6.9 log genome copies) and incubated at 37°C, with or without ciprofloxacin (1 mg/mL). Samples at 0, 6, and 12 hours were analyzed for viral integrity (PMAxx RT-qPCR) and infectivity (plaque assay, MNV-1, HAV). Microbiota effects were assessed via short-chain fatty acid analysis and 16S

rRNA gene sequencing. Experiments were performed in triplicate and analysed using two-way ANOVA with Tukey's test (p □ 0.05).

Results: Viruses persisted throughout colonic fermentations. HuNoV RNA remained stable after 12h-fermentation (5.287 ± 0.187 to 5.150 ± 0.053). Plaque assays showed that MNV-1 (5.596 ± 0.264 to 5.186 ± 0.146 log PFU/mL) and HAV (4.701 ± 0.041 to 4.739 ± 0.040 log PFU/mL) stayed infectious. Similar stability was observed under antibiotics exposure. Ciprofloxacin significantly altered fermentation outputs, decreasing acetate (42.903 ± 4.985 µM versus 14.843 ± 1.041 µM with the antibiotic treatment) and butyrate (14.237 ± 3.941µM versus 3.857 ± 1.095 µM) concentrations and shifting microbiota composition. Viral presence did not significantly affect short chain fatty acids profiles or microbial community metrics (p □ 0.05).

Significance: These findings refine dose-response estimates relevant to fecal–oral transmission and provide insight into enteric virus survival in the human colon under varying microbiota conditions.

Award Application: Developing Scientist Award

P2-026: EVALUATING ZEBRAFISH EMBRYO AND LARVA MODELS FOR HUMAN NOROVIRUS GII.4 AND GII.17: INFECTIVITY THRESHOLDS AND SERIAL PASSAGING PERFORMANCE

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human noroviruses (HuNoV) are major causes of gastroenteritis globally, yet research is limited by the lack of robust replication systems.

Purpose: This study compared zebrafish embryo and larva protocols for HuNoV replication, focusing on GII.17 infectivity and GII.4 replication dynamics.

Methods: Zebrafish embryos (3 h post-fertilization) and larvae (3 days post-fertilization) (n = 10 per condition) were microinjected with 3 nL of HuNoV-contaminated stool suspensions. Embryos were incubated at 29.5°C for 72 h, and larvae at 32°C for 48 h. Viral RNA was quantified by RT-qPCR at 1 h post-injection and after incubation. For GII.17, serial 10-fold dilutions (up to 1:1000) were tested to determine the minimal infectious dose. Serial passaging was performed using recovered virus. Experiments were repeated four times; statistical analysis used two-way ANOVA ($\alpha < 0.05$).

Results: Both systems supported replication of GII.4 and GII.17, reaching ~6.8 log RNA copies/zebrafish in embryos and ~6.5 log in larvae. For GII.17, undiluted and 1:10 diluted samples replicated successfully in both systems (up to 1.71 ×10^7 RNA copies/embryos), while 1:100 dilution infected only embryos; 1:1000 failed in both. Serial passaging of GII.4 revealed replication after the first passage in embryos but persisted through two passages in larvae, indicating greater robustness of the larval system.

Significance: Zebrafish embryos require a lower infectious dose and offer a faster workflow, while larvae enable extended passaging. Both models provide reliable tools for HuNoV infectivity studies, supporting advancements in food safety and public health.

P2-027: EVALUATION OF PRE-TREATMENT AND DEHYDRATION EFFICACY FOR MS2 INACTIVATION IN MICROGREENS

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: Microgreens are a popular fresh produce commodity, and producers are interested in creating value-added products through drying methods.

Purpose: Prior research determined that thermal dehydration and freeze-drying of microgreens yield insufficient virus reduction. Thus, the efficacy of combining sanitization treatments with drying technologies for virus reduction on microgreens was evaluated.

Methods: Microgreens (sunflower, radish, broccoli) were inoculated with 6 $\log_{10}$ plaque forming units (PFU) of MS2 bacteriophage and subjected to a full-factorial design involving three pre-treatments (peracetic acid + hydrogen peroxide (PAA), plasma-activated water (PAW), deionized (DI) water) and three drying methods (freeze-drying, thermal dehydration, no drying). Experiments were conducted in duplicate biological replicates with triplicate technical samples (n=6). Virus concentration was quantified via plaque assay. Statistical differences were analyzed using R via two-way ANOVA followed by Tukey's HSD post-hoc test (p<0.05).

Results: The combination of PAA application and thermal dehydration achieved the highest virus reduction. Specifically, this method yielded reductions of 3.8, 3.1, and 3.2 log PFU/mL in sunflower, broccoli, and radish microgreens, respectively. These results reflect a statistically

significant interaction between the pre-treatment and drying variables ($P < 0.05$).

Significance: These findings demonstrate that while drying alone is inadequate for viral pathogen control, combining PAA pre-treatment with specific drying protocols significantly enhances viral inactivation on microgreens, offering a validated processing strategy to improve value-added produce safety.

Award Application: Developing Scientist Award

P2-028: EVALUATION OF STANDARD 50 ML CENTRIFUGE TUBES TO REPLACE CONICAL BOTTLES IN THE CONCENTRATION STEP OF THE FDA BAM CHAPTER 19C

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: *Cyclospora cayetanensis* is a coccidian parasite that causes diarrheal illness and is a worldwide public health concern due to its association with the consumption of contaminated fresh produce and water.

Purpose: Expand the array of supplies validated in the current *FDA Bacteriological Analytical Manua*l (BAM) Chapter 19c method to include use of 50mL tubes as an alternative for conical bottles.

Methods: Five (5) high level (200 oocysts) and twelve (12) fractional level (6 oocysts) samples were spiked with *Cyclospora cayetanensis* in 10L of agricultural water. The BAM Chp. 19c protocol was followed in its entirety except for the concentration of the backflush, which was separated into eleven (11), 50mL tubes for centrifugation.

Results: The use of 50mL tubes at the concentration step in place of conical bottles provided similar recovery rates in comparison to the validated BAM method. All high-level samples had Cts of 34 or lower. Six (6) fractional samples out of twelve (12) were positive (50%), which is within the guidelines provided by the Microbiological Methods Validation Subcommittee

(MMVS). Unseeded control samples were tested and results came back negative. Samples are deemed positive with Ct values of 38 and below. The use of 50mL tubes did not affect any other steps in the BAM protocol.

Significance: The current BAM method stipulates the use of specialized supplies, which presents a logistical constraint for laboratories that do not possess this specific inventory. This extension validates the adoption of standardized, widely available laboratory consumables, thereby ensuring that the protocol is accessible to a broader range of analytical facilities.

P2-029: FELINE CALICIVIRUS AND TULANE VIRUS INACTIVATION ON SURFACES BY ULTRAVIOLET LIGHT EMITTING DIODE SYSTEMS

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Category: Viruses and Parasites

Abstract:

Introduction: Ultraviolet-light emitting diode systems (255 and 279 nm UV-C LED) are being researched for virus decontamination on surfaces. Human noroviruses (HuNoVs) are known to be transmitted via cross-contamination through contaminated food-contact surfaces. However, cultivable surrogates including feline calicivirus (FCV-F9) and Tulane virus (TV) are used during inactivation studies, due to the difficulties associated with current HuNoV cultivation systems.

Purpose: The objective of this study was to determine the UV-C LED doses for the inactivation of FCV-F9 and TV on polypropylene surface (a model food-contact surface).

Methods: Surface-spread and air-dried FCV or TV (50 µL of ~6.5-7 log PFU/mL) on sterile propylene discs were treated with 255-nm (dose up to 14.5 mJ/cm^2) or 279-nm UV-C LED (dose up to 13.5 mJ/cm^2). Data of recovered infectious viruses using plaque assays from triplicate trials were statistically analyzed using mixed model analysis of variance with Tukey's adjustment (p□0.05). The linear model was used to calculate the dose (D_{10} value) to decrease the infectivity of FCV or TV by one-log.

Results: Linear model 255-nm UV-C LED D_{10} value for FCV and TV was 3.25±0.24 and 8.18±0.76 mJ/cm^2, respectively, while 279-nm UV-C LED D_{10} was 6.54±1.10 and 6.71±0.92 mJ/

cm^2, respectively, on polypropylene surfaces.

Significance: These data show that 255-nm UV-C LED systems have better ability to inactivate FCV (but not TV) on polypropylene surfaces than 279-nm UV-C LED. Both UV-C systems showed similar doses for the inactivation of TV (p□0.05), though, the dose was higher for TV than for FCV with the 255 nm UV-C system (p□0.05). These doses using the conservative TV surrogate are useful to design optimal dose delivery systems for HuNoV inactivation on surfaces.

Award Application: Developing Scientist Award

P2-030: HEPATITIS A VIRUS DETECTION USING A NOVEL CRISPR-CAS13A AND RNASE-PRETREATMENT-BASED METHOD

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Hepatitis A virus (HAV) is transmitted through fresh and frozen berries. Food industry needs methods capable of distinguishing between infectious and non-infectious HAV to prevent losses associated with food recalls.

Purpose: Develop a method to detect potentially infectious HAV using CRISPR-Cas13a coupled with RNase pre-treatment.

Methods: For CRISPR-Cas13a, two HAV targets were designed and tested: capsid and polymerase genes. A two-step CRISPR detection was performed, consisting of RT-RPA amplification at 39°C/20 min, followed by a T7-CRISPR-Cas13a reaction at 37°C/60 min. For the RNase pre-treatment, we first evaluated it by RT-qPCR. 20 U of RNase ONE were used in 140 µl of inactivated HAV (100°C/15 min) and incubated at 37°C/90 min, then HAV RNA was detected and quantified. In addition, a $TCID_{50}$ assay was performed to confirm HAV viral inactivation by 100°C/15 min.

Results: Upon testing, both CRISPR-Cas13a targets showed specific detection of HAV. The method detected a positive sample within 36 min of 50 RFU (relative fluorescence units). The non-template control remained below 1 RFU. As control, we assessed the ability of RNase to degrade HAV. The RNase pre-treatment eliminated extracted HAV RNA, between 5.76 to 0.33 log gc/ml. When non and RNase pre-treatment was applied to the tissue-culture derived HAV, no significant difference ($p > 0.05$) was detected. This suggests there was minimal free RNA in tissue culture. RNase pre-treatment assay was able to detect HAV RNA as low as 10^3 gc/ml. As an additional control, when the RNase pre-treatment was applied to heat inactivated HAV (80^{o}C), across doses, we observed a range between 1.32 to 0.09 log_{10}gc/ml reduction. Heat inactivated HAV was assayed by $TCID_{50}$, across all doses (10^7 to 10^1 $TCID_{50}$/ml), we observed complete inactivation suggesting the absence of infectious HAV.

Significance: A novel method for assessing a proxy for infectious HAV, an intact HAV capsid, has been developed.

Award Application: Developing Scientist Award

P2-031: NOVEL CRISPR-CAS13A-BASED RNA DETECTION ASSAY FOR HEPATITIS A VIRUS: INTER-LABORATORY VALIDATION IN AGRICULTURAL WATER AND FROZEN STRAWBERRIES

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Recalls and outbreaks associated with hepatitis A virus (HAV) contamination of frozen berries have resulted in an increased interest in testing for enteric viruses in products and environmental samples. However, current detection methods for viruses remain expensive and time-consuming.

Purpose: The aim of this study was to do an inter-laboratory validation of a CRISPR-Cas13a assay for RNA detection of HAV in agricultural settings compared to the ISO 15216-2:2019 RT-qPCR method.

Methods: Strawberries (25 g/sample) were inoculated with HAV at either 0, 10^2, 10^3, 10^4 or 10^6 genome copies (g.c.)/sample then frozen at -80°C overnight, while artificial ground water (AGW; 500 mL/sample) was inoculated at the same concentrations on the day of the experiment. Following the ISO method, HAV was recovered and extracted. HAV RNA was then detected either by RT-qPCR or by a two-step isotherm CRISPR-Cas13a system combining recombinase polymerase amplification and detection by Cas13a. All experiments were replicated three times.

Results: In both AGW and frozen strawberry samples, recovery of HAV was affected by virus concentration (P□0.0013, Chi-Square test); however, no significant difference in HAV amplification was seen between the RT-qPCR and CRISPR-Cas13a methods (P>0.05). At 10^2 g.c./sample of HAV in AGW, 1/3 samples were positive with the CRISPR-Cas13a method, while no sample was positive with RT-qPCR detection. At 10^3 g.c./sample, both detection methods were equivalent (1/3 positive). For frozen strawberries, all three samples were positive with the CRISPR-Cas13a method at 10^2 g.c./sample compared to 2/3 samples with RT-qPCR detection.

Significance: This study validates the effectiveness of a novel CRISPR-Cas13a detection method in the detection workflow of enteric viruses in frozen berries and agricultural water. This method yields equivalent results to the standard RT-qPCR method and has the potential to be further optimized for user-friendly field applications.

P2-032: LIQUID NITROGEN PRETREATMENT AS A STRATEGY TO REDUCE LISTERIA MONOCYTOGENES ON FROZEN BERRIES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Many recalls associated with frozen berries contaminated with *Listeria monocytogenes* have taken place in the U.S. recently, leaving questions unanswered on how to reduce the presence of this pathogenic microorganism on food items during production.

Purpose: To evaluate the survival of *L. monocytogenes* on contaminated berries after liquid nitrogen pretreatment, which can be used before individual quick freezing process of produce.

Methods: Strawberries, raspberries, and blueberries (10g/sample) were inoculated with *Listeria monocytogenes* at 3 log CFU/g to mimic typical contamination levels on produce, and 0 CFU/g (uninoculated). After 30min drying time, samples were submerged in liquid nitrogen for 5s or 25s. *L. monocytogenes* was recovered from all samples in enrichment broth. Samples were directly plated onto modified oxford agar (LOD: 1.4 log CFU/g). They were enriched for 24h and plated if the count was under the LOD. Each experiment was replicated 3 times.

Results: A significant reduction (P<0.0398, Student t-test) of *L. monocytogenes* level was

observed on all types of berries when treated samples (5s and 25s) were compared to the control group, except for strawberries treated during 25s. When submerged for 5s, blueberries showed a reduction of 0.6±0.2 log CFU/g, like what was seen for raspberries (0.7±0.5 log CFU/g), and strawberries (1.1±0.4 log CFU/g); no difference was seen between berry types (P>0.05, ANOVA). In the 25s treatment, only blueberries had a higher reduction of 1.5±0.3 log CFU/g than the 5s treatment (P<0.0327, Student t-test). Only one strawberry and one blueberry sample were below the LOD for direct plating, but remained positive once enriched.

Significance: Although no sample showed full inactivation, this study shows that pretreatment used to enhance the organoleptic qualities of frozen berries can also help reduce the bacterial load of *L. monocytogenes* onto the end product, which could lead to less recalls and foodborne illnesses.

P2-033: OPTIMIZATION OF TWO-STAGE CULTURE CONDITIONS FOR HIGH-TITER PRODUCTION OF A LISTERIA MONOCYTOGENES-SPECIFIC PHAGE

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Presentation type/Format: Technical

Category: Viruses and Parasites

Abstract:

Introduction: The efficient propagation of *Listeria monocytogenes* (LM)-specific phages remains challenging due to the host's long generation time and low energy production efficiency.

Purpose: This study aimed to optimize the propagation conditions and characterize the biological properties of the LM-specific phage vB_LmoP_KFSLM4.

Methods: Two-stage culture (TSC) was compared with plate wash method (PWM) for selection of the best propagation method. After the selection of TSC method, the host and LM phage were cultured in PYNM medium (peptone-yeast extract-NaCl-MOPS) added with glucose or glycerol at various concentrations of 1, 3, 5, 7, and 9 g/L for the reduction of the host's generation time. The phage was isolated and purified prior to further characterization. The morphology of phage vB_LmoP_KFSLM4 was examined by TEM. Its whole-genome sequencing was performed using the Illumina NextSeq 2000 platform. The host range analysis and stability of the phage were evaluated.

Results: Among two propagation methods, TSC method produced higher phage titer of 9.62±0.03 log PFU/mL, which was significantly higher than that obtained with PWM ($p < 0.05$). The generation time of LM was significantly reduced from 3.27±0.18 h to 1.82±0.07 h in PYNM added with 3 g/L glucose ($p < 0.05$). The phage vB_LmoP_KFSLM4 consisted of a non-contractile tail (163.59±12.53 nm) and an icosahedral head (70.97±6.54 nm). The phage genome consisted of 37,745 bp with 65 ORFs, and was classified as a member of the genus *Psavirus*. The phage exhibited narrow specificity, infecting only 14 LM strains among 51 foodborne pathogens. The phage was stable following storage at a pH range of 4 to 11 and a temperature range of -80°C to 50°C.

Significance: This study provides an effective propagation condition and method for LM-specific phages by improving the intrinsic property of LM as a host.

P2-034: PERSISTENCE OF HUMAN NOROVIRUS AND ITS SURROGATES IN VOMITUS DEPOSITED ON POLYESTER

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus (HuNoV) is a leading cause of viral gastroenteritis worldwide. Although transmission occurs primarily via the fecal–oral route, exposure to vomitus is also recognized as an important pathway. Polyester, the most widely used textile fiber, is common in healthcare and food-preparation settings as clothing, rags, and aprons, making it a relevant surface for viral persistence studies.

Purpose: This study aimed to evaluate the persistence of HuNoV and its surrogates (murine norovirus-1 (MNV-1), MS2 phage, and Tulane virus) in artificial vomitus deposited on polyester.

Methods: Artificial vomitus was prepared using the INFOGEST 2.0 static in vitro digestion protocol. Viruses were individually inoculated into the vomitus at a final concentration of 10^6 PFU/mL in a final volume of 1.0 mL. Samples (30 µL per coupon) were deposited onto polyester coupons (1 cm^2) and allowed to dry for 1 h. Coupons were stored at room temperature for 0, 2, 4, or 6 days, after which viruses were recovered in 900 µL of EBSS buffer. PBS 1X controls and samples in solution were included to assess recovery losses. Viral

titers were determined using RT-qPCR and plaque assays. All experiments were performed in triplicate.

Results: RT-qPCR analyses demonstrated the persistence of HuNoV, MS2, and MNV-1 RNA in artificial vomitus, PBS, and on polyester coupons throughout the study period. Infectivity by plaque assay revealed that MNV-1 remained viable for 2 days in both artificial vomitus and PBS solution. MS2 showed significantly greater stability, remaining infectious for up to 4 days in artificial vomitus and 2 days in PBS. A significant effect of the matrix was observed after 2 days (2-way ANOVA, $p < 0.0001$).

Significance: These findings provide new insights into the role of vomitus and polyester surfaces in the environmental persistence of HuNoV and its surrogates, highlighting the importance of effective cleaning and disinfection practices following vomiting incidents.

Award Application: Developing Scientist Award

P2-035: PERSISTENCE OF VIRUSES ON FOOD CONTACT SURFACES RELEVANT TO PRODUCE PACKINGHOUSES

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Virus persistence on nonconventional food contact surfaces (FCS) within produce packinghouses can result in cross-contamination of fresh produce.

Purpose: This study aims to investigate the survival of viral pathogens and their surrogates on common produce packinghouse FCS.

Methods: The persistence of Aichivirus (AiV) A and Tulane virus (TuV) (a human norovirus surrogate) was evaluated on stainless steel, rubber, foam, and wood. A cocktail (5 log plaque forming units [PFU]) of AiV and TuV was spot-inoculated onto the FCS, allowed to dry, and incubated at 15^{o}C, 21^{o}C, or 27^{o}C under clean and dirty conditions at 50% relative humidity. Samples were quantified at different time points using the plaque assay technique.

Results: D-values were computed to determine the required time (h) for 90% virus reduction. Under clean conditions, D-values were significantly impacted by virus type and temperature (P<0.05). AiV persisted longer than TuV overall, with mean D-values of 68.33 (95% CI: 52.44-86.9) and 44.2 (36.56-51.3), respectively. At 15°C, viruses persisted the longest with D-

value of 54.36 (45.07-64.37), followed by 21°C and 27°C with 53.46 (41.54-65.58) and 31.33 (21.9-41.34) D-values, respectively. While FCS did not significantly impact D-values, stainless steel and rubber had the highest D-values of 45.7 (36.59-55.07) and 45.33 (37.06-54.21), respectively. Available data under dirty conditions suggests similar trends. However, a higher D-value was observed for clean surfaces (50.23 [43.5-57.51]) compared to dirty surfaces (42.07 [26.71-58.98]).

Significance: These findings show that viral pathogens can remain infectious for several days on produce packinghouse surfaces, particularly at lower temperatures, highlighting the need for effective cleaning and sanitation measures to mitigate pathogen spread.

Award Application: Developing Scientist Award

P2-036: SEQUENCING AND CHARACTERIZATION OF NOROVIRUS IN IQF-IMPORTED OYSTERS ASSOCIATED WITH A 2025 OUTBREAK

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Norovirus is the leading cause of viral foodborne illnesses and has been implicated in outbreaks associated with shellfish. Shellfish pose a higher public health risk as they can concentrate viruses from contaminated waters.

Purpose: The objective of this analysis was to sequence and characterize norovirus associated with an outbreak from imported, IQF oysters on the half shell.

Methods: As described in FDA BAM Chapter 26 Section A3, Concentration and Extraction of Enteric Viruses from Molluscan Shellfish, RNA was extracted from three subsamples of oyster concentrates and RT-qPCR was utilized for detection. Conventional RT-PCR was utilized to amplify norovirus GI and GII followed by gel electrophoresis. Big-dye terminator sequencing of the capsid and polymerase regions of the norovirus genome were used for characterization. Nucleotide sequences were aligned using BioEdit and NCBI BLASTn.

Results: Norovirus was detected in oysters implicated in an outbreak in 2025. Norovirus GI was detected at 1.3 MPN genomic copies/gram, 95% CI [0.18, 9.3], sequencing was

unsuccessful. Norovirus GII was detected at 12 MPN genomic copies/gram 95% CI [5.3, 28] and resulting amplicon demonstrated that the strains from the implicated product were as follows; sub 1 was characterized as GII.P7, sub 2 was characterized as GII.P17, and sub 3 was characterized as GII.17[P17].

Significance: Eating contaminated, uncooked molluscan shellfish can pose a health risk which can lead to deadly illnesses caused by enteric viruses such as norovirus. These findings demonstrated an epidemiological link between clinical illnesses and a food sample. In addition, this analysis helped to mitigate public health risk associated with the consumption of this product.

P2-037: SYNERGISTIC ANTIVIRAL PROPERTIES OF CARVACROL FORMULATIONS WITH SURFACTANTS AGAINST MS2 BACTERIOPHAGE, A HUMAN NOROVIRUS SURROGATE

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human norovirus is the leading cause of gastrointestinal foodborne illness and is transmitted via contact with infected individuals, contaminated surfaces, and foods. Due to its non-enveloped structure, chemical inactivation of norovirus is challenging. Carvacrol, a naturally extracted phenolic compound, has demonstrated antimicrobial properties but yielded limited antiviral efficacy for sanitizing formulations in past work due to its poor aqueous solubility and potentially limited contact with virus particles. Incorporating surfactants may enhance antiviral efficacy by improving delivery systems with emulsions.

Purpose: To empirically study the synergistic antiviral efficacy of carvacrol-surfactant combinations against MS2 bacteriophage, a human norovirus surrogate.

Methods: Oil-in-water emulsions were prepared using carvacrol at various concentrations combined with either cationic (cetyltrimethylammonium bromide, CTAB) or anionic (sodium dodecyl sulfate, SDS) surfactants in nuclease-free water. Controls included no surfactant, no carvacrol, and neutralization controls. MS2 suspensions were exposed to carvacrol emulsions with different contact times (5–60 min), followed by plaque assay. Measurements were made

in triplicate and analyzed using Two-way ANOVA with Tukey's post hoc test ($p<0.05$).

Results: 1% carvacrol displayed minimal viral reduction (<1 log). In contrast, cationic carvacrol formulation yielded a 1.74 ± 0.36 log reduction at 5 min and a 4.02 ± 0.43 log reduction at 60 min. Anionic carvacrol formulation displayed the highest viral reductions of 4.46 ± 0.18 and 5.89 ± 0.07 log reduction at 5 and 60 min contact time, respectively.

Significance: Antiviral efficacy of carvacrol-surfactant formulations highly depended on surfactant and concentration, with some significant synergistic enhancement in viral inactivation observed. These findings highlight the importance of formulation design for rapid and effective viral inactivation.

Award Application: Developing Scientist Award

P2-038: VALIDATION OF PEG PRECIPITATION FOR OPTIMIZING VIRAL CONCENTRATION EFFICIENCY IN FRESH VEGETABLES

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Efficient viral concentration is a critical step in ensuring the sensitivity of foodborne virus detection in fresh produce. While traditional protocols often employ extended Polyethylene Glycol (PEG) precipitation periods to maximize recovery, these lengthy procedures can delay urgent public health responses. There is a requirement to scientifically determine the minimum time necessary for effective viral recovery to enhance laboratory throughput and response speed.

Purpose: This study investigated the correlation between PEG precipitation duration and recovery efficiency to establish a streamlined protocol that balances analytical rigor with time efficiency.

Methods: Concentration efficiency was systematically evaluated using representative vegetable and fruit matrices, including lettuce, pre-cut salad mix, cherry tomatoes, and grapes. Three PEG incubation time conditions (1 hour, 5 hours, and 16 hours) were

compared. The performance of each condition was validated by calculating recovery percentages and determining the LOD for each matrix.

Results: Comparative analysis revealed that shortening the PEG precipitation time to 1 hour did not compromise detection sensitivity. In all tested matrices, the 1-hour protocol achieved an LOD of 1–2 log gc/g, showing results equivalent to those of the 5-hour and 16-hour conditions. Recovery rates remained stable within a valid and robust range (20–52% at 1 log gc/g), demonstrating that the majority of viral particles are effectively concentrated within the first hour of incubation.

Significance: This study provides empirical evidence that a 1-hour PEG precipitation period is sufficient for high-sensitivity detection of foodborne viruses in vegetables. These findings support a more rapid and agile diagnostic framework, facilitating faster response times during foodborne viral outbreak investigations.

P2-039: VERIFICATION AND PROFICIENCY TESTING OF BAM CHAPTER 19B FOR DETECTION OF CYCLOSPORA CAYETANENSIS IN SPINACH

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: The FDA developed and validated a real-time PCR method targeting the *Cox3* gene (Mit1C qPCR) to detect *Cyclospora cayetanensis* in fresh produce which is published in FDA BAM Chapter 19b for regulatory outbreak investigations and surveillance.

Purpose: To evaluate laboratory performance and implementation of FDA BAM Chapter 19b for detection of *C. cayetanensis* in spinach by using proficiency testing (PT) samples.

Methods: Commercially available spinach was verified as free of *C. cayetanensis* by FDA BAM Chapter 19b. The PT exercise comprised eight blinded spinach samples: seven inoculated with 100 oocysts per test portion (average Ct 34.09±0.63) and one uncontaminated sample sent to 24 laboratories. The study design supports both PT according to ISO 17043 and in-house method verification according to ISO 16140-3 utilizing μKPI tools for microbiological method performance indicators.

Results: Participants reported results as detect (Ct <38) or non-detect. Sixteen of the 24 laboratories correctly identified all spinach samples. The identical (blinded) replicates were analyzed, yielding a mean detection rate of 81.5% for inoculated samples. Within each set of seven contaminated replicates/laboratory false negative rates varied and ranged from 12.5% to 25%. One false positive was reported. Substantial variation in Ct values was observed within and between replicates, as well as within and between laboratories.

Significance: The BAM chapter 19b includes washing of produce, DNA extraction and dual real-time PCR. The PT revealed that several laboratories did not achieve the required detection sensitivity for *C. cayetanensis*. These results underscore the need for targeted training when a new method is introduced and to ensure that the optimized workflows are followed.

P2-040: VIRUCIDAL ACTIVITY OF COPPER AND COPPER–ZINC ALLOYS AGAINST TULANE VIRUS

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Human noroviruses, a major cause of acute gastroenteritis, have a low-infectious dose that facilitates transmission via person-to-person contact and contaminated surfaces.

Purpose: Copper has demonstrated strong antiviral activity against murine norovirus (MNV-1) and human norovirus VLPs. However, its rapid surface discoloration limits practical use, prompting exploration of copper alloys.

Methods: We assessed the virucidal properties of copper and copper-based alloys against Tulane virus (TuV), a well-established surrogate for human noroviruses. Environmental surfaces included 1×1-inch coupons of pure copper, pure zinc, and four copper alloys: Brass 260 (70% Cu, 30% Zn), Brass 280 (60% Cu, and 40% Zn), nickel silver (60–70% Cu, 10–25% Ni, and 5–20% Zn), and commercial bronze (90% Cu, 10% Zn). Stainless steel coupons were used as a control. Each coupon was inoculated with 50 µL of TuV and sampled at 0, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h post-drying, using n=4 samples from two independent experiments per condition. Infectivity was quantified by $TCID_{50}$ assay, and TuV RNA was measured by real-time

RT-PCR.

Results: Commercial bronze exhibited the fastest inactivation, achieving a 2.1-log reduction in infectivity after 1 h of drying, followed by Brass 260 at 2 h. Pure copper, Brass 280, and pure zinc required □4 h to achieve >2-log reduction, whereas TuV showed a 2.1-log reduction on stainless steel after 6 h. After 1 hr, a decrease of 3 to 6 Ct values of viral RNA from commercial bronze and Brass 260 was observed, whereas eluates from other alloys showed no change in Ct values up to 24 h of drying. Copper-zinc alloys, particularly commercial bronze and Brass 260, exhibited superior antiviral activity compared to pure copper or zinc, indicating a synergistic interaction between Cu and Zn in the inactivation of TuV.

Significance: Our findings highlight copper–zinc alloys as effective antiviral surfaces against human norovirus, outperforming pure Cu and Zn.

P2-041: A COMPARISON OF CAFFEINE IN COFFEE, ENERGY DRINKS, AND ENERGY SHOTS

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Given the recent rise in health-related incidents involving products like energy drinks and shots, we wanted to compare caffeine levels in various beverages and provide serving recommendations.

Purpose: We compared the caffeine content in coffee and energy drinks and estimated the maximum daily servings for each beverage when compared to the daily recommended limits from the American Medical Association (AMA).

Methods: Two-three samples of 16 ground coffees (n = 48) brewed in the laboratory, 4 brewed coffees from coffee shops (n = 11), 19 energy drinks (n = 57), and 6 energy shots (n = 18) were prepared and analyzed for caffeine. Caffeine was extracted using hot water (□90°C) with added magnesium oxide, followed by filtration and analysis by HPLC with UV detection. Caffeine results were compared to nutritional label values where available.

Results: Caffeine was measurable in all energy drinks/shots and coffees tested, including decaffeinated coffees and averaged from 181– 645 ppm (energy drinks), 1967–4926 ppm (energy shots), 291–468 ppm (coffee shops), 30–770 ppm (ground coffees). One energy drink

tested at greater than 20% of the label value (44%). Flavored (585 ppm) and espresso coffees (770 ppm) averaged higher caffeine concentrations than traditional roasted coffee (457 ppm). One serving of 11 out of 26 energy drinks would exceed the AMA daily recommended limit for pregnant women (200 mg), while 25 of 26 would exceed the limit for teenagers (100 mg).

Significance: Consumers may not be aware of being overstimulated from caffeine and other stimulants found in coffee and energy drinks/shots. This is especially important for high-risk groups like teenagers and pregnant women.

P2-042: ANTIBACTERIAL ACTIVITY OF FRESH AND PROCESSED RED MUSCADINE JUICE AND THE ROLE OF THEIR POLAR COMPOUNDS ON ESCHERICHIA COLI O157:H7

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: *Escherichia coli* O157:H7 is a major foodborne pathogen associated with outbreaks linked to fruit juices and other minimally processed beverages, highlighting the need for effective natural antimicrobial interventions.

Purpose: The objectives of this study were to evaluate the antimicrobial activity of fresh red muscadine juice (FRMJ) and processed red muscadine juice (PRMJ) against *E. coli* O157:H7 and to identify the compounds responsible for this activity.

Methods: Polar and phenolic compounds in FRMJ and PRMJ were characterized using high-performance liquid chromatography. Antimicrobial effects of FRMJ, PRMJ, their polar and polyphenol fractions, and selected synthetic organic acids, with or without sugars, were evaluated against *E. coli* O157:H7.

Results: Both FRMJ and PRMJ significantly reduced (P □ 0.05) a 5-log CFU/mL cocktail of *E. coli* O157:H7 within 4 h at 37°C. Polar fractions containing malic, tartaric, and tannic acids exhibited the strongest antimicrobial activity (P □ 0.05). Among the individual synthetic acids tested, tannic acid demonstrated the greatest inhibitory effect against *E. coli* O157:H7.

Overall, FRMJ, PRMJ, and their polar compounds showed strong antimicrobial activity, indicating that red muscadine juice contains naturally occurring antibacterial compounds effective against *E. coli* O157:H7.

Significance: Red muscadine juice and its polar compounds may be used as natural, clean-label antimicrobial ingredients to improve the microbiological safety of nonalcoholic beverages.

P2-043: ANTIMICROBIAL EFFECTS OF ROASTING AND BREWING ON BACILLUS CEREUS, CLOSTRIDIUM BOTULINUM, AND LISTERIA MONOCYTOGENES IN READY-TO-DRINK COFFEE MADE WITH ARABICA BEANS

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Despite the growing popularity of ready-to-drink (RTD) coffee products, the effects of roast level and extraction conditions on antimicrobial activity against foodborne pathogens are not well understood, complicating risk assessments.

Purpose: To evaluate the antimicrobial effects of light and dark roasted, cold-/hot-brewed Arabica coffee on *Listeria monocytogenes*, *Bacillus cereus,* and *Clostridium botulinum.*

Methods: Light and dark roasted Arabica coffee were brewed using cold brew (4°C, 16h) and hot brew (92°C, 4min) methods at a 1:15 coffee-water ratio and adjusted to pH 6.0 and 1.5°Brix. Coffee extracts were inoculated with acid-adapted *L. monocytogenes* and stored at 10°C and 20°C for 20 days, or *B. cereus* and *C. botulinum* stored at 27°C for 6 months. Microbial populations were enumerated in triplicate on Modified Oxford with Tryptic Soy agar overlay or Mannitol Yolk Polymyxin agar. Botulinum neurotoxin (BoNT) production was assessed using the mouse bioassay. Uninoculated samples were monitored for pH and background microflora. Two trial repetitions were analyzed using linear mixed-effects modeling.

Results: Greater inactivation of *L. monocytogenes* was observed at 20°C than 10°C. Roast level was the most significant factor affecting *L. monocytogenes* reduction (p<0.05), with dark roast coffee showing greater inactivation than light roast coffee extracts (up to 2.26 log CFU/mL). Brew temperature was not significant. All roast and brew types inhibited BoNT production through 6 months. Dark roast, cold brew coffee reduced *B. cereus* populations to below detection (<1.3 log CFU/mL) by 1 month, while light roast, hot brew coffee showed sporadic survival through 5 months. All treatments reduced *B. cereus* populations below detection by 6 months.

Significance: Intrinsic RTD coffee properties, particularly roast level, contribute to pathogen inhibition and prevention of botulinum toxin production, supporting reduced food safety risk under the conditions evaluated.

P2-044: BIOMASS-DERIVED MULTIPURPOSE PHOTOLUMINESCENT CARBON QUANTUM DOTS FOR PRESERVATION AND SMART QUALITY MONITORING OF JUICE

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Mitigating microbial contamination in fruit-based beverages commonly relies on chemical preservation and high-pressure processing, raising concerns related to health risks, microbial resistance, and nutrient degradation, which calls for a transition into non-thermal alternatives such as photoluminescence (PL) based techniques.

Purpose: Carbon quantum dots (CQDs) were synthesized via one-step hydrothermal process from anthocyanin (An) extracted from residues of *Hibiscus sabdariffa.* For biopreservation, apple juice samples (5 mL) were divided into 4 treatment groups: fresh juice only or juice+AnCQDs (negative controls), juice+yeast cells (positive control), and juice+yeast cells+AnCQDs (test). Simultaneously, PL of the smart AnCQDs-CS film strips (SFSs) was measured upon immersion into positive control samples at zero time, 2, 4, 6 and 8 days days.

Results: AnCQDs exhibited pH responsiveness especially in low pHs, antioxidant (48.20%), antimicrobial activity, and negligible (<4.0%) inhibition to normal cell lines, inferring their safety upon consumption. Increasing temperature from 30 to 70°C resulted in progressive quenching of the dots' PL intensity with no shift in emission maxima, indicating their thermal stability as reliable temperature-sensing ingredients. AnCQDs addition (0.40 mg/mL) to juice

significantly ($P < 0.05$) inhibited *Rhodotorula mucilaginosa* RKDS25125 by 4.0 logs after 6 days of storage at 7°C, without compromising browning degree/color/total soluble solids of juice. The SFSs delivered immediate (1–2 minutes) and distinct PL response after excitation at 365 nm that correlated with spoilage progression of apple juice.

Significance: The dual applicability of AnCQDs in juice/polymeric structures as low cost, non-toxic multifunctional biopreservatives, and as pH/temperature sensors in juice quality assessment, can enable manufacturers to receive precise information about juice freshness when specified storage conditions are not maintained.

P2-045: COMPARATIVE STUDY ON ENUMERATION MEDIA FOR ALICYCLOBACILLUS SPP. IN APPLE JUICE

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: The accurate detection and enumeration of *Alicyclobacillus* (ACB) is important to control spoilage incidents in apple juices. Current methods for the enumeration of ACB emphasize the use of *Bacillus acidoterrestris* (BAT) agar. Despite this emphasis, there is no study that comprehensively evaluates the performance of BAT as compared to other popular nutrient media types with ACB isolated from different sources.

Purpose: Compare enumeration results using BAT agar to acidified potato dextrose (APD) agar and yeast starch glucose (YSG) agar following existing standard IFU 12 method.

Methods: 48 guaiacol-producing strains of *A. suci, A. acidophilus,* and *A. acidoterrestris* were activated in BAT, YSG, and APD broths from frozen working stock. During activation, the

optical density (OD) at 600 nm was measured over 3 days to monitor growth. One mL of activated inoculum was mixed with 10 g of autoclaved apple juice, and 89 mL of the respective nutrient broth. The bags were heat-shocked at 80°C for 10 minutes and cooled before 2 mL of the mixture was enumerated using pour plating onto three different types of agars. The plates were incubated at 45°C for 5 days before enumeration. Three biological replicates were completed for all experiments. A Censored Bayesian mixed-effects analysis was used to draw conclusions.

Results: Meaningful differences between APD broth activation performance and BAT and YSG were noticed, with APD consistently underperforming by a log. However, no meaningful differences were found between BAT, YSG, and APD as enumeration media, with general enumeration differences within 0.2 log following the standard IFU 12 method.

Significance: In a practical setting, BAT, YSG, or APD agar can be used to reliably enumerate ACB in apple juice, allowing for greater flexibility in the detection of the spoilage bacteria, especially for smaller processors who may not have access to BAT.

Award Application: Undergraduate Student Award

P2-046: DO PHYSICOCHEMICAL PROPERTIES OF HIGH ACID GREEN JUICE AFFECT HPP PATHOGEN INACTIVATION EFFICACY?

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Presentation type/Format: Technical

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: HPP is a preferred method of pathogen control in high acid juices due to its ability to retain nutrients and product palatability. The effect of physicochemical properties such as °brix and pH have not been assessed with respect to their impact on HPP pathogen inactivation efficacy.

Purpose: Assess the effect of pressure, dwell time, °brix and pH on HPP inactivation of *E. coli* O157:H7 in green juice.

Methods: Freshly prepared green juices with combinations of unadjusted (4.06pH, 6.4°brix) and adjusted pH (+0.1,+0.2,+0.3; 0.1M NaoH) and °brix (+10,+15,+20; sucrose) properties were spiked with 5 strain *E. coli* O157:H7 cocktail. Juice preparations were assigned to the following treatments: untreated, 500 or 550 MPa for 60 or 120s. Samples were plated on TSA and SMAC, pre- and post-HPP and during post-treatment storage. Log reduction values from *E. coli* counts were analyzed using GLM with post-hoc pairwise comparisons (emmeans, Tukey

HSD, R 4.4.0).

Results: Adjustment to juice pH and ºbrix had no effect on microbial inactivation. Mean log reduction values of combinations of pH and °brix on TSA and SMAC were 5.79±0.04 and 7.87±0.02, respectively. HPP treatment and time post-treatment were significant factors influencing inactivation of the general bacterial population (TSA) and *E. coli* (SMAC). HPP at 550 MPa increased log reductions significantly compared to those at 500 MPa on TSA, regardless of dwell time (6.16±0.08 vs. 5.42±0.11 log CFU/mL reductions). Inactivation of *E. coli* at 500 MPa/60s was significantly reduced compared to the other 3 HPP treatments. However, within 2 days post-treatment all HPP-treated samples averaged 7.94±0.05 log CFU/mL reductions and HPP-treatment was insignificant.

Significance: HPP at 500 and 550 MPa for 60 or 120 s did not significantly differ in their ability to achieve *E. coli* O157:H7 inactivation indicating an opportunity to decrease pressure and dwell times in industry standard HPP treatments for pathogen inactivation.

P2-047: EFFECTS OF COMBINATORIAL HURDLES ON A NON-ALCOHOLIC BEER MATRIX CHALLENGED WITH SALMONELLA SEROVAR JAVIANA, ESCHERICHIA COLI, LISTERIA MONOCYTOGENES, PSEUDOMONAS AERUGINOSA, AND BACILLUS CEREUS

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Consumers' interest in decreasing alcohol consumption is leading alcoholic beverage producers to begin producing non-alcoholic versions of their products.

Purpose: This study explores if remaining hurdles; pH (5.0, 4.2), carbonation (0, 0.75, and 1.5 volumes), and antimicrobial compounds (none, 10ppm hop acids, and 100ppm potassium sorbate), are sufficient to control food pathogens; *Salmonella* Javiana, *E. coli* O157:H7, *L. monocytogenes* 4b, *P. aeruginosa*, and *B. cereus* in non-alcoholic beer.

Methods: A factorial combination of these hurdles produced 18 unique treatments, along with 2 leading alternative treatments (kettle souring and chitosan addition), to be challenged. A bacterial cocktail of all 5 pathogens (10^8 CFU/ml) was inoculated into 12oz bottles of non-alcoholic beer. Samples were stored at ambient conditions and tested in

triplicate at days 1, 7, 14, 28, and 60, by spread plating on selective and differential media. Negative controls were plated at days 1 and 7 to confirm lack of growth. ANOVA with post-hoc Tukey was used to determine significant population changes.

Results: Results show that pH is a critical factor, both on its own and in enhancing the effect of other hurdles. Carbonation at 1.5 volumes is effective in controlling enteric bacteria, but at reduced levels may support their growth and survival. Hop acids reduced the population of gram-positive bacteria. Several treatments achieved reduction to below the limit of detection (<10 CFU/ml) representing 4-log reduction. However, high pH and lack of other hurdles was shown to support the growth and survival of pathogens.

Significance: These results provide context on how recipe design decisions impact the intrinsic hurdles in non-alcoholic beverages and can help process authorities and producers navigate beverage risk and further processing requirements. This work also provides novel findings on the effect of moderate to low CO_2 levels in non-alcoholic beer stability.

Award Application: Developing Scientist Award

P2-048: EVALUATION OF GENE-UP® PRO LYCO FOR DETECTING SPOILAGE ORGANISMS, OENOCOCCUS OENI, LEVILACTOBACILLUS BREVIS AND ZYGOSACCHAROMYCES PARABAILII, IN KETCHUP

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Common causes of ketchup package bloating and gassy spoilage include acid tolerant organisms such as *Lactobacillus* and certain yeasts. Not only do these organisms create bloating, but they can also cause a sour taste and dull color.

Purpose: To evaluate the rapid molecular detection of key spoilage organisms in reformulated ketchups (high-fructose corn syrup [HF] and low-sodium [LS]) using a multiplex real-time PCR assay.

Methods: 25 g samples were inoculated at low (~1.5–2 CFU/sample) or high (~6–8 CFU/

sample) contamination levels, enriched for 72 h at 30°C in YM or MRS broth, and tested on the GENE-UP PRO Lyco assay with parallel plating on YM or (acidified) MRS. Uninoculated controls were included.

Results: For samples spiked with *Levilactobacillus brevis*, low-level detection was 16/20 in HF ketchup and 13/20 in LS ketchup; both matrices yielded 5/5 positives at high level and 0/5 in controls. Presumptive negatives confirmed negative on plate. In HF ketchup spiked with *Zygosaccharomyces parabailii*, low-level detection was 7/20 by PCR versus 11/20 on YM agar; several PCR negatives showed late rising curves, indicating need for algorithm adjustment. All 5/5 high-level samples were positive; 0/5 controls were negative. In LS ketchup spiked with *Oenococcus oeni*, GU detected 16/20 low-level and 5/5 high-level samples; all 5 controls resulted negative. Mixed HF samples co-inoculated with yeast and lactobacilli showed concurrent detection of both targets at 125–250 µL assay inputs.

Significance: GU PRO Lyco assay reliably detected target spoilers in ketchup at ~6–8 CFU/25 g across formulations, with matrix-dependent sensitivity at very low inocula. Further testing will be done using an updated assay algorithm. Mixed-enrichment workflows supported simultaneous detection of yeast and lactic acid bacteria without loss of sensitivity.

P2-049: STRENGTHENING PRODUCE SAFETY WITH STEC PATHOGENICITY TESTING

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: According to WHO, approximately 35–45% of STEC infections globally are linked to produce row crops such as sprouts, carrots, leafy greens or onions. EHEC is a highly pathogenic subset of STEC carrying both the *stx* and *eae* genes. Detection of these genes is key to reducing risk in the food supply.

Purpose: The GENE-UP® Pathogenic *E. coli* (PEC) assay is an AOAC-approved PCR method based on the detection of pathogenicity genes highly correlated with the co-localization of the *stx* and *eae* genes within the same organism. The purpose of this study was to demonstrate the efficacy of PEC for screening raw diced onion for the presence of pathogenic *E. coli.*

Methods: Thirty 375-gram samples of raw diced onion were enriched with 3375 mL pre-warmed BPW. Enrichments were contaminated with *E. coli* O157:H7 (ATCC 35150) at 3 levels per AOAC testing methods: N=20 low level, N=5 high level and N=5 uninoculated as a control. Enrichments were incubated at 42°C prior to PEC analysis at the 10- and 24-hour time points. PEC presumptives were confirmed directly via PCR for *stx* and *eae*, *E. coli* O157:H7 and *E. coli* Top 6 serogroups. Enrichments were also tested post-immunoconcentration with VIDAS-UP *E. coli* Serogroups (ESPT) and the resulting DNA used to repeat the 3 PCR assays referenced above. PEC presumptives were culturally confirmed as recommended in BAM 4A with IDs confirmed via VITEK2 GN.

Results: PEC presumptive screen results were as follows: 17/20 at the low level, 5/5 at the high level and 0/5 uninoculated controls. Results were identical at both time-points whether assayed directly or post-VIDAS immunoconcentration. PEC presumptives were 100% in agreement with BAM cultural methods and latex. All IDs were 100% in agreement with VITEK GN.

Significance: These data indicate that the GENE-UP Pathogenic *E. coli* assay (PEC) assay is a suitable method for screening 375g raw diced onion for the presence of pathogenic *E. coli* while reducing the number of unconfirmed positives.

P2-050: EVALUATION OF NATURAL PRESERVATIVE EFFICACY AGAINST YEAST AND MOLD IN A MODEL BEVERAGE SYSTEM AT PH 3.8

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Microbial spoilage is a critical challenge for acidic beverage products, and the effectiveness of natural preservatives against yeast and mold in acidic systems (pH 3.8) can vary by formulation.

Purpose: To evaluate the efficacy of five natural preservatives to a conventional preservative in inhibiting the growth of yeast and mold in a model beverage system at pH 3.8.

Methods: A model beverage system was adjusted to pH 3.8 using citric acid and then formulated with five natural preservatives at their recommended concentrations: mushroom extract (0.2%), cultured dextrose (0.3%), rosemary plus cultured dextrose (0.3%), rowanberry extract (0.25%), and a multi-extract blend (0.1%). The conventional preservative potassium sorbate (0.1%) was included as control along with an unpreserved broth control. Five replicate samples of each variable were inoculated with a multi-strain cocktail yeast or mold. Samples were incubated for 120 hours at 25°C. Microbial growth was monitored by

measuring optical density (OD_{600}) at defined time intervals.

Results: The rowanberry extract, multi-extract blend and potassium sorbate completely inhibited the growth of yeast and mold throughout the 120-hour incubation period, with OD_{600} values remaining close to zero. Compared to the broth control, cultured dextrose required significantly more time to reach maximum OD_{600} ($p < 0.05$), while mushroom extract and rosemary plus cultured dextrose were not significantly different ($p > 0.05$). Growth of mold in the broth control and mushroom extract began between 24 and 48 hours. Rosemary plus cultured dextrose delayed mold growth until 48 hours, while cultured dextrose delayed the growth 72 hours.

Significance: Rowanberry extract and the multi-extract blend demonstrated complete the most efficacy to inhibition of yeast and mold in a model beverage system at pH 3.8. These findings support the potential use of select natural preservatives for clean-label beverage applications.

P2-051: GROWTH INHIBITION OF ALICYCLOBACILLUS ACIDOTERRESTRIS IN ACIDIC BEVERAGES BY SUCROSE FATTY ACID ESTERS

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Presentation type/Format: Technical

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Sucrose fatty acid ester (SE) is an emulsifier that has a wide range of functions such as emulsifying, solubilizing, dispersing, etc. Among SEs, those with specific fatty acid chains and degrees of esterification are known to specifically inhibit the growth of heat-resistant spore-forming bacteria. However, the inhibitory effect of SE on the growth of *Alicyclobacillus acidoterrestris* (AAT), which causes spoilage of acidic beverages, was unknown.

Purpose: To verify the inhibitory effect of SEs on the growth of AAT, we conducted a growth test of AAT in acidic beverages containing SE.

Methods: Three commercially available acidic beverages (pH and juice content ranged 3.2–3.8 and 10–100%) and two types of SE (sucrose palmitic acid ester "*P*-1670" and sucrose myristic acid ester "M-1695" (Mitsubishi Chemical Co.)) were used in this study (two replicates each). Autoclaved SE was aseptically added to beverages to give concentrations ranging from 0 to 1000 ppm. A spore suspension of AAT strain DSM 3923 was added to 10 ml of an acidic

beverage to a final concentration of 10^3 spores/ml, followed by storage at 45°C for 7 days. The growth of AAT in the acidic beverage was evaluated by measuring the viable cell count (YSG Agar, 45ºC for 48 hours) after storage. Test plots where the viable cell counts of both two replicates were 10^3 CFU/ml or less were judged to have inhibited AAT proliferation.

Results: After 7 days of storage at 45°C in the three SE-free beverages, the viable cell counts were 10^5 to 10^6 CFU/ml, confirming that AAT proliferated in these acidic beverages. The growth of AAT in beverages was inhibited by the addition of 10 to 900 ppm of SE, and the minimum bacteriostatic concentration tended to increase with increasing fruit juice concentration.

Significance: This study demonstrates that AAT proliferation can be inhibited by very low concentrations of emulsifiers, providing a new approach to control AAT-induced spoilage in acidic beverages.

P2-052: RISK ASSESSMENT OF SPOILAGE CAUSED BY THERMOPHILIC SPORE-FORMING BACTERIA DURING SUMMER STORAGE OF READY-TO-DRINK BEVERAGE IN WAREHOUSES AND POSSIBLE SPOILAGE PREVENTION USING SUCROSE FATTY ACID ESTERS

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Presentation type/Format: Technical

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: With the recent rise in summer temperatures, Ready-to-Drink (RTD) beverages stored in warehouses for long periods during the summer may be subject to deterioration due to the growth of thermophilic spore-formers that survive after heat pasteurization.

Purpose: In this study, we examined whether RTD beverages inoculated with *Geobacillus stearothermophilus*, a major thermophilic spore-former, would deteriorate under cyclic temperature conditions simulating those of a summer warehouse, and if so, whether the deterioration could be suppressed by adding the emulsifier, sucrose fatty acid esters (SE).

Methods: The milk coffee (7.8% sugar, 29% raw milk, heat pasteurized at F_0=30) containing SE “P-1670” (Mitsubishi Chemical Co.) prepared in our lab was used in this study. The spore suspension of *Geobacillus stearothermophilus* strain ATCC 7953 (10^2 spores/ml) was inoculated

into the milk coffee in small glass tubes (two replicates) and incubated at the cycle of 45°C (8 hours) - 30°C (16 hours) for five weeks. The bacterial growth was assessed by the decrease of pH (pH difference between inoculated and non-inoculated milk coffee).

Results: After five weeks incubation, the pH of the spore-inoculated SE-free milk coffee decreased by 0.34–0.48, showing that *G. stearothermophilus* has proliferated (the pH did not decrease when spores are not inoculated). In contrast, addition of 1500 ppm SE completely suppressed the pH decrease (ranged - 0.01 to 0.02). The SE concentrations between 0 ppm and 1500 ppm have not been tested.

Significance: Although the data is limited, this study shows the risk of heat-resistant bacteria that could survive heat pasteurization at around F_0=4 growing in beverages stored in warehouses during the summer season. It is believed that more comprehensive risk management will be possible in the future by increasing the number of bacterial species tested.

P2-053: ANTIMICROBIAL EFFICACY OF VINEGAR-FERMENTATE AND NATURAL FLAVOR SYSTEM AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Raw refrigerated pet foods present an elevated food safety risk from *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 due to high moisture content and near-neutral pH.

Purpose: This study aimed to evaluate the antimicrobial efficacy of a vinegar-fermentate (EverWild® 40L) and natural flavor system (EverWild® S100), alone and in combination, against foodborne pathogens relevant to raw pet food.

Methods: Vinegar-fermentate (1.00–3.50%), natural flavor (0.25–1.00%), and combination treatments were prepared in Brain Heart Infusion broth adjusted to pH 6.0, alongside a no-antimicrobial control. Samples were inoculated with *Salmonella* spp. (Typhimurium, Infantis, Enteritidis), *E. coli* O157:H7, and *L. monocytogenes* and incubated at 30°C. Growth kinetics were monitored using BioScreen-C Pro. Lag time and maximum growth rate (μmax; h^{-1}) were modeled using the Gompertz equation, and minimum inhibitory concentrations were

determined. Data were analyzed by one-way ANOVA (p □ 0.05).

Results: Control samples of *Salmonella* spp., *E. coli*, and *Listeria* exhibited rapid growth with short lag times (<0.2 days) and significantly higher μmax (up to 0.50 h^{-1}; p □ 0.05), confirming robust proliferation. Vinegar-ferment showed a concentration-dependent effect, with partial inhibition at 1.00–2.50% and significant growth suppression at 3.50%, reflected by reduced μmax (0.04–0.11 h^{-1}). Natural flavor demonstrated strong antimicrobial activity even at low concentrations (0.25–0.50%), achieving complete inhibition across all pathogens with lag times extended to □5 days. Combination treatments provided the most comprehensive control, specifically, EW 40L (3.50%) combined with EW S100 (0.20%) resulted in no detectable growth, indicating more efficacy.

Significance: Vinegar-ferment and Natural Flavor system offer an effective clean-label strategy for pathogen control in raw pet food systems. These findings support their potential application as clean-label preservation solutions.

P2-054: VALIDATION OF A CLEAN-LABEL ANTIMICROBIAL HURDLE FOR PATHOGEN CONTROL IN A LOW-PH CARBONATED-BEVERAGE BASE

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Low pH contributes to microbial stability in carbonated beverage bases but does not serve as a stand-alone lethality step against acid-adapted pathogens. Tunnel pasteurization is commonly used for in-package microbial control, yet additional clean-label antimicrobial hurdles may provide supportive pathogen control.

Purpose: This study aimed to evaluate the efficacy of a clean-label, natural flavor-based antimicrobial system (Flavoset® 5900L) for inactivation of *Salmonella* spp. and pathogenic *Escherichia coli* in a low-pH carbonated-beverage base (pH 3.5).

Methods: A beverage base formulation containing Flavoset® 5900L (1.20%) was compared with a non-antimicrobial control. Samples were inoculated with acid-adapted *Salmonella* Agona (ATCC BAA-707) and *E. coli* O26 (ATCC BAA-2196) to approximately 6 log CFU/mL and stored at 4°C and 25°C. Samples were plated for enumeration of surviving cells on days 0, 1, 2, 3, 7, and 14 using XLT-4 agar for *Salmonella* and Sorbitol MacConkey agar (SMAC) for *E. coli*.

Statistical differences were evaluated using one-way ANOVA.

Results: Post-inoculation levels on Day 0 averaged 5.53–6.03 log CFU/mL for *Salmonella* and 5.38–5.98 log CFU/mL for *E. coli* in acidified carbonated beverages. In untreated controls, both pathogens remained quantifiable throughout storage, with *Salmonella* declining to 2.88 log CFU/mL at 4°C and 0.85 log CFU/mL at 25°C by Day 14, while *E. coli* levels persisted at approximately 5.14–5.30 log CFU/mL. In contrast, Flavoset® 5900L (1.20%) achieved complete inactivation of both pathogens within 24 h at both temperatures, reducing counts below the detection limit (0.30 log CFU/mL) with no regrowth through Day 14 confirming the strong antimicrobial efficacy in acidified carbonated beverages.

Significance: These findings demonstrate that a natural flavor-based antimicrobial system provides rapid pathogen inactivation in low-pH beverage applications and supports a multi-hurdle food safety strategy beyond pH alone.

P2-055: ANTIMICROBIAL EFFICACY OF NATURAL FLAVOR AGAINST SPOILAGE MICROORGANISMS IN FRUIT-BASED CONCENTRATES

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Fruit-based concentrates with high nutrient content, water activity (a_w), and sugar concentration are highly susceptible to yeast and LAB contamination, posing challenges for beverage preservation. Clean-label antimicrobial solutions are increasingly sought to inhibit spoilage organisms and extend shelf life.

Purpose: The study evaluates the inhibitory effect of a natural flavor (Flavoset® 5900L) against yeast and LAB strains isolated from spoiled fruit-based concentrates.

Methods: Flavoset® 5900L treatments (0–1.69%) and no-antimicrobial control were prepared in potato dextrose broth (PDB) and de Man, Rogosa and Sharpe (MRS) broth for yeast and lactic acid bacteria (LAB), respectively. All broths were adjusted to pH 4.00 to represent a worst-case spoilage scenario. Samples were aliquoted into 100-well honeycomb plates in duplicates and inoculated with yeasts and LAB strains. Growth curves were generated using

the Bioscreen-C Pro at 30°C. Lag time (days) and maximum growth rate (μ_max; h^{-1}) were modeled using a Gompertz function. Minimum inhibitory concentration (MIC) was defined as the lowest concentration with no observable growth. Data were analyzed using one-way ANOVA (p□0.05).

Results: Flavoset® 5900L completely inhibited yeast and lactic acid bacteria (LAB) growth at concentrations □0.51%. Control samples exhibited rapid growth, with maximum specific growth rates (μmax) of 0.12 h^{-1} for yeast and 0.23 h^{-1} for LAB, and short lag phases of 0.19 and 0.66 days, respectively. In contrast, natural flavor-treated samples showed complete growth inhibition (p□0.05). No growth was observed for LAB across all tested concentrations, while yeast isolates exhibited concentration-dependent inhibition.

Significance: The findings confirm the potential of natural flavor as an effective clean-label antimicrobial solution for fruit-based beverage systems, emphasizing the importance of targeted preservation strategies for acidic environments to ensure microbial spoilage.

P2-056: IDENTIFICATION AND ISOLATION OF DOMINANT SPOILAGE MICROBES IN CONVENTIONAL DARK LEAFY GREEN VEGETABLE JUICE DURING REFRIGERATION STORAGE

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: The market for dark leafy green vegetable juices (DLGVJs) has expanded rapidly in recent years. However, concerns related to microbial safety and spoilage-driven food loss continue to constrain the full growth potential of this product category. To better protect the safety and quality of DLGVJs, a comprehensive understanding of the microbial populations contributing to the spoilage of DLGVJs is very much needed.

Purpose: The purposes of this study were to characterize the microbial populations in conventional DLGVJs and identify and isolate dominant spoilage microorganisms from them.

Methods: Fresh DLGVJs prepared with conventional kale, chard, and collard greens were stored at 4°C for 21 days. The pH, aerobic plate counts (APC), and fungal populations were measured on Days 0, 4, 7, 14, and 21. For culture-independent analysis, 16S rRNA and ITS sequencing were performed to characterize the microbial communities. On Day 21, dominant spoilage microorganisms were isolated, and whole-genome sequencing was used

to characterize these isolates.

Results: Results showed a gradual pH decrease and increasingly saturated juice color across all DLGVJs. APC showed an overall decrease while fungal populations exhibited an initial increase followed by a decline in conventional DLGVJs. High-throughput sequencing identified *Pseudomonas*, *Leuconostoc*, and *Periweissella* as dominant spoilage-associated bacteria, and *Sporobolomyces*, *Alternaria*, and *Symmetrospora* as predominant fungi. Although conventional and organic DLGVJs shared several microbial taxa, notable differences in community composition were observed. The dominant lactic acid bacteria isolated were *Enterococcus faecium* and *Leuconostoc mesenteroides*.

Significance: These findings advance our understanding of microbial ecology in DLGVJs and provide a scientific basis for developing targeted interventions to ensure microbial quality and safety, supporting the growth of the DLGVJ market.

Award Application: Developing Scientist Award

P2-057: INVESTIGATION OF THE FERMENTATION PROCESS TO REDUCE FURANOCOUMARINS AND ANALYZE FUNCTIONAL COMPOUNDS IN POMELO (CITRUS GRANDIS OSBECK) VINEGAR

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: This study was conducted in response to recent market fluctuations, overproduction, and insufficient processing utilization of *Citrus grandis* (Wendan pomelo) in Taiwan, as well as the potential interaction risks posed by furanocoumarins enriched in the flavedo and albedo layers, which may interfere with drug metabolism.

Purpose: The objective of this study was to establish a fermentation process for Wendan pomelo vinegar that effectively reduces furanocoumarin content while maintaining product quality.

Methods: Four formulations comprising different fruit-part combinations (flavedo + albedo + segment membrane + flesh; albedo + membrane + flesh; membrane + flesh; and pure flesh) were subjected to a three-week fermentation and maturation process. Throughout fermentation, organic acid profiles, total phenolic content, and antioxidant activity were systematically monitored. In addition, the concentrations of 6′,7′-dihydroxybergamottin

(DHB) and bergamottin were quantified by HPLC, and in vitro safety was assessed using Hep G2 cell MTT assays, AChE inhibition, and CYP3A4 enzyme activity.

Results: All formulations reached an acidity exceeding 5% within three weeks, meeting the requirements for fruit vinegar production. Although formulations containing outer peel exhibited higher phenolic contents and antioxidant capacities, they also retained the highest DHB concentrations and showed significant CYP3A4 inhibition. In contrast, the pure-flesh formulation exhibited no detectable DHB or bergamottin after the second week of fermentation, maintained 80–90% Hep G2 cell viability at 4.5 ppm, and showed no significant inhibition of AChE or CYP3A4, indicating a markedly reduced drug-interaction risk.

Significance: The resulting product exhibits high safety, stability, and compatibility with drug metabolism, demonstrating strong potential for the value-added utilization of Wendan pomelo and the development of low-interaction-risk functional vinegars.

P2-058: LOW-ENERGY PASTEURIZATION OF COCONUT WATER AND ALMOND MILK BASED ON A SYNERGISTIC COMBINATION OF MILD HEAT AND ETHYL LAUROYL ARGINATE (LAE)

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: To address the growing demand for safe and minimally processed, "fresh-like" foods, this study evaluates the synergistic efficacy of mild heat combined with Ethyl Lauroyl Arginate (LAE) as an alternative to conventional thermal processing.

Purpose: To investigate the synergistic antibacterial efficacy of LAE combined with mild heat treatment for the pasteurization of coconut water (CW) and almond milk (AM).

Methods: Commercial CW and AM were inoculated with *E. faecium* and treated at 55°C with LAE (25–100 ppm) for 15–120 s using capillary tubes in a constant-temperature water bath. Conditions achieving ~5-log reduction were selected for refrigerated storage studies. Thermal only trials (55–85°C) were conducted to identify a process achieving ~5-log reduction. Shelf life of the processed CW and AM under optimal conditions were analyzed weekly for 4 weeks based on total plate count and yeast/mold counts. The controls included thermally pasteurized and untreated samples. All experiments were conducted in triplicates.

Results: Thermal treatment alone at 55°C resulted in negligible inactivation of *E. faecium*. However, combining mild heat (55°C) with LAE demonstrated strong antimicrobial synergism.

LAE exhibited strong synergy with mild heat, achieving 4.89 ± 0.58 log CFU/mL reduction in CW (50 ppm LAE, 60 s at 55°C) and 5.03 ± 0.35 log CFU/mL reduction in AM (100 ppm LAE, 60 s at 55°C). During 4-week refrigerated storage, total plate count and yeast/mold levels in LAE+mild heat-treated samples remained significantly lower (P<0.05) than both the control and samples treated with conventional heat (75°C for 2 min).

Significance: This study demonstrates an effective, energy-efficient alternative to conventional thermal pasteurization for plant-based beverages. By achieving superior microbial safety at lower temperatures, LAE-assisted processing can mitigate quality degradation and satisfy consumer demand for safe, high-quality, and minimally processed products.

P2-059: MICROBIOLOGICAL AND PHYSICO-CHEMICAL DYNAMICS OF BLACK TEA KOMBUCHA FERMENTATION AND THE SURVIVAL OF SALMONELLA ENTERITIDIS

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Presentation type/Format: Technical

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Kombucha, a fermented tea beverage, has gained popularity for its perceived health benefits; however, improper fermentation conditions may allow the survival of foodborne pathogens, posing food safety concerns.

Purpose: This study evaluated the survival of *Salmonella* Enteritidis in black tea kombucha fermentation and characterized the associated physicochemical, microbial, and microbiome changes over a 14-day incubation.

Methods: Kombucha was prepared using black tea and a commercial symbiotic culture of bacteria and yeast (SCOBY) and fermented at 23 ± 1°C. *Salmonella* populations were enumerated on XLT4 agar, while indigenous microbial populations, including total yeasts and molds and lactic acid bacteria, were quantified. Physicochemical parameters, including pH, titratable acidity, and total phenolics were measured. Data were analyzed using two-way analysis of variance (p □ 0.05). Changes in the SCOBY microbial community during fermentation were characterized using Oxford Nanopore long-read sequencing of the full-length 16S rRNA gene and analyzed using the Nanopore Galaxy platform.

Results: An initial *Salmonella* population of 7.24 log CFU/mL declined, reaching 2.01 log CFU/mL by day 10 of fermentation. The reduction coincided with a decrease in pH to 3.38 and a corresponding increase in organic acid. In contrast, *Salmonella* populations increased within 24 h in unfermented base tea and remained stable. Total yeast and mold populations increased from 4.20 log CFU/mL to 5.62 log CFU/mL, while LAB increased from 4.95 log CFU/mL to 6.64 log CFU/mL and stabilized, corresponding with organic acid production. Total polyphenol content also increased, suggesting the breakdown of complex compounds during fermentation.

Significance: The results demonstrate that controlled kombucha fermentation creates microbial and physicochemical conditions that reduce *Salmonella* survival, highlighting its potential to enhance the microbial safety of fermented beverages.

Award Application: Developing Scientist Award

P2-060: NEUTRALIZING EFFECT OF NATURAL ANTIOXIDANTS PRESENT IN COFFEE ON THE PRODUCTION OF OCHRATOXIN A-INDUCED REACTIVE OXYGEN SPECIES (ROS)

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Coffee is susceptible to contamination by ochratoxigenic fungi.

Purpose: This study aimed to determine the neutralizing effect of antioxidants (AO) present in coffee on the production of ROS induced by ochratoxin A (OTA).

Methods: Viability assays with MTT-reduction method were performed for Vero cells exposed to AO (chlorogenic acid, caffeic acid, caffeine, trigonelline, green coffee bean extract [GCBE] and 2 roasted CBE) with or without OTA. Apoptosis and necrosis were determined by flow cytometry with an Annexin V/Propidium iodide cell death kit and the ROS production by CM-H_2DCFDA probe. Both analyses were performed for OTA alone and with GCBE (highest percentage of protection at viability assays). Three independent tests were conducted for all tests, in duplicate. Statistical differences were determined with an ANOVA test.

Results: Statistically significant differences were found between the viability percentages between OTA (250 μM) and those obtained with co-incubation of OTA and GCBE (125 and 250 μg/mL). Flow cytometer results showed that when exposed to OTA, 35.6±3.0% of the cells were in early apoptosis and 8.3±0.4% in late apoptosis/necrosis, while both cell death types were impaired when incubated with GCBE: 25.9±5.0% of the cells were in the early apoptosis and 2.8±1.1% in late apoptosis/necrosis and 22.8±3.0% in the early apoptosis and 2.9±1.1% in late apoptosis/necrosis, for 125μg/mL and 250μg/mL, respectively. OTA induced ROS production in 82.5±16.6% of the cells. Its production decreased when incubating the cells with 125μg/mL (33.9±18.7%) and 250μg/mL (28.4±7.1%) of GCBE.

Significance: Results demonstrate that OTA induced ROS production. Also, its apoptosis/necrosis induction on Vero cells was diminished by GCBE, explained by a synergism of antioxidant compounds present in coffee beans.

P2-061: OPTIMIZATION OF STERILIZATION CONDITIONS FOR TEA- OR MILK-BASED, READY-TO-DRINK COCKTAILS WITH LOW ALCOHOL CONTENTS

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Beverages containing tea or milk require high-temperature sterilization due to the microbiological spoilage risk posed by spore-forming bacteria, while the presence of bactericidal alcohol may alleviate sterilization conditions.

Purpose: This study investigated the optimal sterilization conditions for tea- or milk-based ready-to-drink (RTD) cocktails containing less than 5% alcohol, aiming to minimize flavor deterioration.

Methods: Spores were suspended in phosphate-buffered saline (PBS) or a model RTD cocktail (pH 7.0) containing alcohol at 0%, 1%, and 3% for *Bacillus coagulans*, and 0%, 3%, and 5% for *Thermoanaerobacterium aotearoense*. After sealing in cans (n=2, 150 mL each), samples underwent retort processing. Surviving spores were enumerated by culture methods, and Log Reduction Values (LRV) were calculated. To evaluate the effect of shortened thermal processing on sensory quality, model RTD cocktails were sterilized at 121.1℃ for varying durations and assessed by sensory panels.

Results: For the heat-resistant strain *B. coagulans*, thermal treatment at 121.1℃ for 10 min in PBS increased the LRV from 3.2 at 0% alcohol to 3.7 at 1%, and over 6.4 at 3%. Similar trends were observed in other *B. coagulans* and *T. aotearoense* strains, as well as in the model RTD cocktails. These findings indicate that alcohol concentrations less than 5% enhance thermal inactivation of spores, suggesting improved lethality and the potential to reduce required processing times. Sensory evaluation further demonstrated that shorter thermal processing significantly improved sensory quality, particularly fresh flavor characteristics, compared to fully sterilized counterparts.

Significance: This study presents an approach that balances consumer safety with desirable flavor quality in low-alcoholic RTD cocktails, including those containing tea or milk. This approach enables RTD cocktails to achieve a fresher flavor profile than non-alcoholic beverages.

P2-062: OPTIMIZATION OF TIME AND TEMPERATURE OF HOT WATER TREATMENT AS A KILL STEP TO INACTIVATE ENTEROCOCCUS FAECIUM FROM VARIOUS CITRUS FRUIT SURFACES

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Growing consumer preference for unpasteurized juice demands innovative and validated risk mitigation strategies in juice production. FDA juice HACCP regulation mandates a 5-log reduction in the pertinent pathogen as a critical control point. Treating whole fruit prior to juice extraction presents a potential kill step to reduce microbial contamination at the source and maintain quality.

Purpose: To evaluate the efficacy of hot water immersion in reducing *Enterococcus faecium* ATCC 8459, a validated surrogate for foodborne pathogens from contaminated lemons, limes, mandarins, and oranges.

Method: Citrus fruits were spot inoculated (100µL) with nalidixic acid-resistant *E. faecium* and subjected to hot water immersion at 80°C (0, 5, 10, 15, and 20 seconds). Each fruit was hand massaged in 100 mL Dey-Engley Neutralizing Broth (DE) and plated on tryptic soy agar (TSA) supplemented with nalidixic acid. Untreated inoculated fruits served as controls for each

commodity. Differences among treatments were assessed by ANOVA at $p<0.05$.

Result: Hot water treatment at 80°C for 5 second significantly reduced surface *Enterococcus faecium* (~8 log CFU/ cm^2) by 5.44 ± 0.11 log CFU/cm^2, 6.29±0.23 CFU/cm^2, 7.20±0.11 CFU/cm^2, and 6.85±0.08 CFU/cm^2 in lemon, lime, mandarin and orange respectively. At the 20 second time interval, counts further declined to 4.87±0.17 log CFU/cm^2 in lemon, 1.16±2.00 log CFU/ cm^2 in lime, 3.90±0.42 log CFU/cm^2 in mandarin, and 4.11± 0.19 log CFU/cm^2 in orange. Lime showed the greatest inactivation at 20 seconds, significantly lower than other fruits at the same exposure ($P<0.05$). Overall, longer immersion times yielded greater reductions, with significant differences across treatment durations within each fruit.

Significance: Hot water immersion is an effective, scalable intervention for reducing microbial loads on citrus surfaces, supporting its use as a non-chemical kill step to meet FDA 5-log reduction requirements in unpasteurized juices.

P2-063: RAPID AND RELIABLE DETECTION AND DIFFERENTIATION OF GUAIACOL- AND NON-GUAIACOL-PRODUCING ALICYCLOBACILLUS IN FRUCTOSE BY FOODPROOF® REAL-TIME PCR

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: *Alicyclobacillus* spp. are gram-positive, spore-forming, thermophilic, acidophilic bacteria known for their resistance to pasteurization and their ability to cause off-flavors in products by producing guaiacol. The differentiation between guaiacol- and non-guaiacol-producing bacteria is vital for the beverage industry, as guaiacol is one of the main reasons for product waste and customer dissatisfaction.

Purpose: The scope of this study was to differentiate between guaiacol-positive and guaiacol-negative *Alicyclobacillus* by real-time PCR in fructose samples, such as those used for soft-drink production.

Methods: Fifty grams of naturally and artificially contaminated fructose samples were dissolved in VE-Water and heat-treated for 10 minutes. Following this, the samples were diluted 1:10 in BAT broth and enriched at 45°C for 24 and 48 hours. DNA was extracted with the foodproof® StarPrep® Two Kit, *Alicyclobacillus* Protocol and analyzed with the foodproof *Alicyclobacillus* LyoKit® PCR assay. In parallel, the microbiological method IFU Microbiological Method No. 12:2019, as established by the International Fruit and Vegetable Juice Association, was used as a reference.

Results: The detection and differentiation between guaiacol-positive and guaiacol-negative *Alicyclobacillus* can be achieved after a 24-hour enrichment at 45°C, if using the PCR system for *Alicyclobacillus.*

Significance: The *Alicyclobacillus* PCR detection method from Hygiena saves seven days of time-to-results and requires less hands-on time in comparison to the reference IFU method, even with low levels of contaminants. By detecting potential guaiacol production in raw materials such as fructose, the assay effectively supports spoilage prevention in beverages, which is crucial for achieving cost savings and protecting brand reputation.

P2-064: SCREENING METHOD FOR WINE SAMPLES TO DISTINGUISH BETWEEN LIVING AND DEAD YEASTS USING THE ATP BIOLUMINESCENCE HYGIENA® INNOVATETM SYSTEM

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: The Innovate™ System is an ATP-based method designed for the rapid detection of microorganisms in a wide range of products. This method does not detect dead cells, as only living cells produce ATP. Therefore, the ATP detection system can be used in the wine production process to determine whether any living cells remain after the initial fermentation process that could lead to off-flavors or bottle explosions.

Purpose: The objective of this study was to differentiate live and dead yeasts in wine samples using an ATP-based method for the detection of low contamination rates.

Method: Different yeasts were artificially inoculated directly into 250 mL wine samples, which had been filtered using a PES filter. The filter paper was transferred, with the top side facing inwards, into a 10 mL tube containing 3.2 mL SDB. To detect low levels of contaminants, an out-of-pack 24–48-hour enrichment step at 28°C was required to ensure that sufficient ATP was present for detection. After 24, 48, and 72 hours, samples were taken for measurement using the Hygiena System for ATP detection; results were confirmed with plate-count analysis.

Results: Uninoculated wine samples, which were naturally contaminated with dead yeast cells, did not generate positive results when tested with the Innovate System, in alignment with the plate method. Samples, inoculated with living yeast cells, produced ATP and could be successfully detected with the ATP detection Kit after 24 hours of incubation with an inoculation concentration down to 1.5 CFU/250 mL sample.

Significance: The Innovate System, in combination with the RapiScreen Dairy Kit, is a method for distinguishing between living and dead yeast contamination in wine. Low-level contamination of living yeasts can be detected in as little as 24 hours.

P2-065: RAPID DETECTION OF MICROORGANISMS IN HUMAN MILK FORTIFIER USING HYGIENA'S ATP-BASED INNOVATE™ MICROBIAL SCREENING SYSTEM

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: Commercial sterility testing for microorganisms in infant nutrition is critically important since the product is consumed by a highly vulnerable population with immature immune systems. The presence of any microbial contamination in these products can pose serious health risks.

Purpose: This study was designed to evaluate and compare a rapid ATP-based screening method with a traditional culture-based method for the detection of risk organisms in a Human Milk Fortifier.

Methods: Six organisms of concern, including *Bacillus cereus* spores, *Clostridium sporogenes*, *Cronobacter sakazakii*, *E. coli*, *Salmonella enterica* and *Staphylococcus aureus,* were prepared to inoculate 5 mL sachets of sterile human milk fortifier. Organisms were added to triplicate sachets at a level of 1–10 CFU, incubated at 33°C and tested over a 10-day period. Sample aliquots (50 µL) were aseptically removed each day and tested with the RapiScreen™ Dairy

assay. At the same time, samples were plated for culture confirmation.

Results: Using an established threshold of 40 Relative Light Units (RLUs) to differentiate contaminated products, the ATP-based method detected all organisms and replicates after 24 hours of incubation, with the exception of *Clostridium*. For *Clostridium*, the RLU signal remained below the threshold throughout the 10-day incubation period, indicating no observable detection. Culture results were in complete agreement, demonstrating growth for the 5 detected organisms and no growth for *Clostridium*.

Significance: These results demonstrate that the Innovate™ System is sensitive and reliable for the detection of multiple microorganisms of concern in human milk fortifier, with performance comparable to traditional culture methods. The ability to identify contamination within 24 hours highlights the value of rapid ATP screening as an effective tool to enhance commercial sterility testing for infant nutrition.

P2-066: THE POTENTIAL USE OF GARLIC OIL AS A NATURAL ANTIMICROBIAL TO CONTROL SPOILAGE YEAST GROWTH IN REFRIGERATED PICKLE PRODUCTS

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Presentation type/Format: Poster

Category: Beverages and Acid/Acidified Foods

Abstract:

Introduction: There is demand among consumers for clean-labeled food products that have a simplified ingredient list which is at odds with common preservatives.

Purpose: Evaluate garlic oil as a natural antimicrobial alternative to potassium sorbate for the prevention of yeast spoilage in refrigerated pickle products.

Methods: Garlic oil products from two companies (A1, A2 and B1) with 0.04% to 0.000625% oil were screened with acetic acid (0–60 mM) in cucumber juice medium (CJ). Five known spoilage yeasts were tested, including *Pichia* and *Zygosaccharomyces* species. Data from 96 well microtiter plates included growth rate, lag time (LAG), maximum optical density (maxOD) as measured with a microtiter plate reader. Anaerobic growth experiments were performed with *Z. bailii* in 60 mM acetic acid CJ with potassium sorbate or 0.01 to 0.001% garlic oil (A2) to determine cell survival by plating on yeast-malt agar. Controls included media without garlic or cells. Data were analyzed using MATLAB and R Studio with (p<0.05) for statistical inference.

Results: Oil A2 showed the greatest inhibition, with a LAG of 10.82 ± 1.11 h, compared to oil

A1 and B1 with LAGs of 3.37 ± 0.64 h and 3.26 ± 0.70 h, respectively at 0.01%. For *P. membranifaciens*, the addition of 0.0025% garlic oil in CJ (60 mM acetic acid) significantly increased LAG from 13.50 ± 2.18 to >96 h and decreased maxOD from 0.25 ± 0.07 to 0.004 ± 0.004. LAG for *Z. bailii* changed significantly from 2.61 ± 0.52 to 18.06 ± 2.81 h with 0.01% oil addition. *Z. rouxii* LAG changed from 19.61 ± 3.29 to >96 h and maxOD from 0.19 ± 0.02 to 0.01 ± 0.01 OD with 0.00125% oil addition. *Z. bailii* had the same or lower final cell count (approx. 4.8 log CFU/mL) under anaerobic conditions for 11 days in 0.01% oil as treatments with 0.1% potassium sorbate.

Significance: The data offer evidence of the potential for selected garlic oil products to replace potassium sorbate for “clean-label” refrigerated pickle products by delaying yeast growth.

Award Application: Developing Scientist Award

P2-067: BAYESIAN HIERARCHICAL META-ANALYSIS OF VIRAL THERMAL INACTIVATION ACROSS FOOD MATRICES

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Thermal inactivation is widely used to reduce the foodborne viral risk. However, viral inactivation kinetics and associated thermal resistance parameters reported in the literature vary widely across viruses, food matrices, and experimental conditions, limiting the direct comparability and application in food safety assessment and management.

Purpose: We synthesized published viral thermal inactivation data on human norovirus, its surrogates, and hepatitis A virus to estimate thermal resistance parameters and food matrices (dairy, shellfish, and produce), and virus types effects.

Methods: Thermal inactivation data were compiled from 14 independent studies spanning food matrices and foodborne viruses, with infectivity assay and RT-qPCR measurements analyzed separately using a Bayesian hierarchical Bigelow model structure. Temperature, food, virus type, and interactions were modeled on the $\log_{10}(D)$, with D-value reported; random effects accounted for between-study variability. Left-censored observations were incorporated directly in the likelihood using a censoring specification.

Results: For infectivity data, thermal treatment reduced D-values, corresponding to z = 16.7°C (95% credible interval [CrI]: 16.7, 20). Food matrix strongly influenced thermal resistance, in which, relative to water/saline buffer, D-values were higher in shellfish (55-fold;

95% CrI: 9.5, 1585) and higher in dairy (2.3-fold; 95% CrI: 1.2–14.1). No significant impact was found across virus types. According to RT-qPCR data, thermal inactivation was weaker compared to infectivity data (z = 33.3°C; 95% CrI: 33.3, 33.3), and virus and matrices effects were generally uncertain, excepting for a lower D-value observed for Tulane virus (0.14-fold; 95% CrI: 0.04–0.8).

Significance: The meta-regression models provide pooled thermal resistance parameters for foodborne viruses across food matrices and assay types, supporting more precise predictions of viral thermal inactivation in foods.

P2-068: CHALLENGE TESTING TO DETERMINE GROWTH OF LISTERIA MONOCYTOGENES ON FRESH SPECIALTY MUSHROOMS (OYSTER, KING OYSTER AND BEECH) AT 4°C AND 10°C STORAGE TEMPERATURES

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Availability of specialty mushrooms has increased in recent years and a knowledge base is needed to support food safety risk management for these foods.

Purpose: To determine whether specialty mushroom types: oyster, king oyster and beech, can support the growth of *Listeria monocytogenes* (Lm) during refrigerated storage.

Methods: Challenge tests were conducted as per Health Canada guidelines by inoculation of 10 g portions of mushrooms with low levels (15–30 CFU/g) of Lm, followed by storage for up to 22 days at 4°C or 15 days at 10°C. Lm levels on replicate portions were evaluated every 3 days by suspension in diluent and plating on selective agar. n=132 portions stored at 4°C and n=132 at 10°C were tested over 3 trials for each mushroom variety: *Pleurotus ostreatus* (oyster), *Pleurotus eryngii* (king oyster), and *Hypsizygus marmoreus* (beech). Lm counts were graphed as mean log CFU/g vs. days of storage, with statistical difference (a=0.05) analyzed using ANOVA.

Results: *Listeria* species were not detected as background microbiota on the mushrooms. Lm levels did not significantly increase ($p>0.05$) on beech mushroom stored under refrigeration, with poor survival after Day 0: Lm undetected (<5 CFU/g) in 77% of white beech and 55% of brown beech at 10°C. Lm levels increased ($p<0.05$) on some (7 of 12) oyster and king oyster

mushroom lots at each storage temperature, reaching or exceeding 10,000 CFU/g by Day 9 at 10°C and Day 15 at 4°C. Other lots (5 of 12) of oyster and king oyster mushrooms did not support any growth of Lm *(p*>0.05) during the study. The influence of mushroom brand/ source on Lm growth requires investigation.

Significance: This study highlights existing knowledge gaps in the understanding of the food safety risks associated with specialty mushrooms.

P2-069: INTEGRATED MICROBIAL RISK AND SUSTAINABILITY ASSESSMENT OF DRIED APPLE PRODUCTION ON HUMAN HEALTH

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Multiple interventions including pre-treatment have been studied to enhance pathogen control during dried apple production. However, despite their effectiveness in microbial risk reduction, such interventions may also increase energy use and environmental footprint.

Purpose: The objective of this study was to quantify the impact of manufacturing dried apples on human health, measured as disability-adjusted life years (DALY), using Quantitative Microbial Risk Assessment (QMRA) and Life Cycle Assessment (LCA).

Methods: This study integrates QMRA with attributional LCA across the dried apple supply chain. The system boundary includes apple transportation from orchards to processing facilities, storage, washing with water and sanitizers, slicing, pre-treatment, drying, and

packaging, post-processing storage, and distribution to consumers. QMRA quantified *Salmonella* exposure and illness risk, while LCA assessed environmental impacts using the ReCiPe (H) endpoint 2016 method. Both impacts were expressed as DALYs.

Results: Integrated DALY combines health impacts quantified by QMRA and LCA and it represents the cumulative human health burden of manufacturing and consumption of dried apples. The climate impact was 1.769E-06 DALY/kg packaged dried apple. Heating accounts for 51% of climate contributions to human health impacts. The attributional LCA model estimated human health impacts in terms of ozone depletion, human carcinogenic toxicity and non-carcinogenic toxicity as 4.5139E-09, 4.1301E-07, and 2.1459E-07 DALY/kg bulk packaged dried apple, respectively. DALY for QMRA is currently being estimated. The integrated analysis will identify key trade-offs between microbial risk reduction of various interventions and associated environmental health impacts.

Significance: By quantifying both microbial risks (through QMRA) and environmental impacts (via LCA) into a unified metric, DALY, Industry can identify the most sustainable and safe microbial interventions.

Award Application: Developing Scientist Award

P2-070: CROSS-INSTITUTIONAL VALIDATION OF SALMONELLA INACTIVATION MODELS FOR APPLE DRYING

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: The performance and validity of pathogen inactivation models as process validation tools have not been well characterized, including for fruit drying processes.

Purpose: This study was designed to investigate cross-institutional validation of *Salmonella* inactivation models for apple drying.

Methods: Isothermal/iso-moisture studies were conducted at 50-90°C and 0.10-0.99 water activity (a_w) across two laboratories (IFSH, MSU) using Gala apple tissue inoculated with *Salmonella.* Inactivation models were developed using log-linear primary and modified Bigelow secondary models, and compared by parameter estimation and Welch's *t*-test (α = 0.05). Cross-validation was conducted by predicting *Salmonella* inactivation with parameters from the other institution, and model fit was assessed using RMSE and bias analysis at different test conditions. Bootstrapping (i = 1000) was performed to evaluate parameter overlaps between institutions, and a synthesis model was developed using combined data (n

= 1189) from the two laboratories.

Results: Modeling methods did not differ significantly between institutions ($P > 0.05$), yielding the same parameters and RMSE. Applying MSU parameters to IFSH data yielded an RMSE of 1.27 log CFU/g and a mean residual (±SE) in inactivation prediction of -0.31±0.05 log CFU/g ($P < 0.05$). Using IFSH parameters on the MSU data resulted in an RMSE of 1.67 log CFU/g and a mean residual of -0.99±0.06 log CFU/g ($P < 0.05$). Removing extrapolated conditions in the cross-validation reduced mean residuals to -0.04±0.05 (MSU) ($P > 0.05$) and -0.28±0.08 log CFU/g (IFSH) ($P < 0.05$), and lowered RMSE to 1.18 and 1.14 log CFU/g, respectively. The combined model resulted in parameters (z_T:15.37±0.17°C, z_{aw}:0.52±0.01) similar to the model involving a wide range of a_w (MSU) ($P > 0.05$).

Significance: Validating and characterizing model performance, especially under extrapolated conditions, is critical before applying inactivation models in real-world settings.

Award Application: Developing Scientist Award

P2-071: DEVELOPING A PREDICTIVE MODELING FRAMEWORK TO QUANTIFY THE EFFICACY OF PAA AGAINST SALMONELLA BASED ON DIFFERENT BIOLOGICAL ENVIRONMENTAL FACTORS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Peracetic acid (PAA) is a widely adopted antimicrobial in the food industry, but the underlaying factors affecting its efficacy in food processing applications are poorly understood.

Purpose: To develop a reference framework that quantifies the effects of environmental and biological factors on PAA efficacy against *Salmonella* by using a predictive modeling approach.

Methods: Efficacy of PAA was assessed by the minimum bactericidal concentration (MBC) in vitro. *Salmonella* was challenged with nine PAA concentrations (50-600 ppm) and subjected to either 4, 26, or 37°C for 24 h. Four serovars (Typhimurium, Enteritidis, Newport, and Kentucky) with three strains of each were evaluated across a spectrum of bacterial inocula (3-8 $\log_{10}$ CFU/mL). After completing three biological replicates per strain (n=544), Cumulative Linked Mixed Models were constructed to predict probability of inactivation, confidence intervals, and estimated marginal means (EMMs) based on serovar, inoculum level, and temperature. Statistical significance was established at $p<0.05$.

Results: Inoculum levels were the main predictor of PAA efficacy in vitro. Lower levels of PAA (50-80 ppm) predicted to inactivate *Salmonella* up to 3 $\log_{10}$ CFU/mL (*p success*=0.94). In contrast, higher PAA levels (300-600 ppm) were predicted to be required when inoculum concentrations reached 8 $\log_{10}$ CFU/mL (*p success*=0.95). The influence of temperature was effectively illustrated by the EMMs. For instance, at 4°C, EMM for MBC values were on average 78 ppm higher than 26 or 37°C. Once the interaction of inoculum level and temperature was accounted, serovar effect became evident. At 26°C, the EMMs of MBC for Typhimurium and Enteritidis were on average 62 ppm higher than Newport and Kentucky.

Significance: This in vitro approach shows the applicability of predictive modeling to capture and explain the variability of PAA efficacy. Such benchmarks could be beneficial designing targeted antimicrobial interventions.

Award Application: Developing Scientist Award

P2-072: DOES VIRULENCE PREDICT GASTROINTESTINAL SURVIVAL? LINKING GENOMIC AND MACHINE LEARNING-BASED SALMONELLA VIRULENCE CLASSIFICATIONS TO PATHOGEN BEHAVIOR ACROSS THE GASTROINTESTINAL TRACT

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Strain-level variability in *Salmonella enterica* virulence may influence survival during gastrointestinal tract (GIT); however, the lack of standardized virulence classification limits its application in risk assessment.

Purpose: Assess the relationship between virulence classifications and *S. enterica* behavior across the (GIT).

Methods: Forty-two *S. enterica* strains with available whole-genome sequencing data were classified into low, medium, or high virulence using four approaches: serovar, presence of 13 virulence genes, two machine learning (ML) models based on functional genes and the pangenome. Strains were evaluated using an in vitro GIT model including oral, gastric, and intestinal phases. Agreement among virulence classification methods was assessed using Cohen’s Kappa, and associations between virulence classification and pathogens behavior

across GIT was evaluated using linear mixed-effects models.

Results: Moderate agreement was observed between the 13-gene and ML functional gene classifications (κ=0.517). During GIT simulation, mean gastric reductions ranged from 2.43 to 3.98 log CFU, while mean intestinal recovery ranged from 0.26 to 1.67 log CFU. According to the mixed-effects model, only serovar- and ML functional gene–based classifications were significantly associated with strain behavior. Using serovar classification, low-virulence strains showed lower gastric survival than medium-virulence strains (P=0.03), but greater intestinal recovery compared to medium (P=0.002) and high- virulence strains (P=0.001). In contrast, the ML functional gene classification indicated lower gastric survival for high-virulence strains compared with medium (P=0.0070) and low-virulence strains (P=0.003).

Significance: Virulence-based strain classifications using serovar and ML functional influenced predictions of strain behavior; however, opposing trends among methods highlight the need for a standardized virulence classification.

Award Application: Undergraduate Student Award

P2-073: EVALUATION OF A CUSTOM GPT FOR CONSUMER FOOD SAFETY GUIDANCE

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Large language models are increasingly used to answer consumer food safety questions, but their alignment with authoritative regulatory guidance is variable. Purpose-built configurations may improve the reliability and consistency of AI-generated food safety advice.

Purpose: This study evaluated the performance of a custom GPT (“Food Guard”) designed to provide FDA- and USDA-aligned consumer food safety guidance, compared with a general-purpose language model.

Methods: Food Guard was developed using static ingestion of authoritative U.S. food safety references, including the FDA Food Code, *USDA Kitchen Companion*, *FDA Food Facts*, and ServSafe textbook, combined with system-level instructions emphasizing actionable guidance, agency attribution, and explicit source citation. Twenty test questions were generated across six categories: basic handling and storage, temperature and time control, cross-contamination, high-risk populations, recalls and outbreaks, and edge cases. Responses from Food Guard and a GPT-4.0 baseline (via API) were evaluated by Google Gemini 3.0 Pro using a structured rubric. The rubric scored accuracy, actionability, source alignment, risk communication, and tone (0–2 each), with accuracy and source alignment weighted twice, yielding a maximum score of 13.

Results: Food Guard achieved a higher mean total score (12.65 ± 1.53) than the baseline model (10.40 ± 1.11). Accuracy was comparable between models (Food Guard: 1.9 ± 0.44; baseline: 2.0 ± 0), while the largest difference was observed in source alignment, where Food Guard consistently achieved perfect scores (2.0 ± 0) compared with the baseline (1.15 ± 0.36). Improvements were also observed in actionability and risk communication.

Significance: This research identifies that the primary barrier to using AI for consumer food safety is a lack of traceability rather than factual inaccuracy, emphasizing the role of source alignment in building accountability for public health use.

P2-074: EVALUATION OF THE SURVIVAL OF SALMONELLA ENTERICA ON AVOCADO DURING SIMULATED GASTRIC CONDITIONS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Gastric acidity is a key barrier against *Salmonella enterica*, influenced by food characteristics.

Purpose: This study evaluated the survival of *Salmonella enterica* under simulated oral and gastric conditions (SGC) on avocado pulp.

Methods: Avocado was chosen as a matrix. One gram of the matrix was inoculated with 100 µL of *Salmonella enterica* ATCC 14028 (~9 log CFU/mL) and allowed to dry at room temperature for 1 h. A total of 45 samples were sequentially exposed to simulated salivary fluid and then to simulated gastric fluid for 2 h (37°C, 150 rpm), during which gastric conditions were mimicked by gradually reducing the pH to 2.0 using HCl. *Salmonella* concentrations were enumerated at each stage and at 30-min intervals during the gastric simulation and inactivation models were fitted using analyzed through GInaFiT Version 1.8.

Results: Gradual and low reductions of *S. enterica* were observed during the SGC. Reduction ranged from of 0.27-0.37 log CFU at 30 min (pH 4.5±0.2), 0.38-0.62 log CFU at 60 min (pH 4.0±0.2), 0.20-0.69 log CFU at 90 min (pH 3.5±0.2) , and 0.32-1.00 log CFU at 120 min (pH

3.0±0.3), and higher levels of variability were observed as the exposure time increased. Survival data were better described by the Weibull model (R^2=0.60) than by the log-linear model (R^2= 0.50). The log-lineal model inactivation rate (:max) was 0.01 min^{-1}, while Weibull parameters were δ = 885.60 min and *p*=0.33, indicating tailing behavior and low inactivation, respectively. Limited reductions were observed, likely due to the protective effect of the avocado matrix under acidic conditions.

Significance: Avocados' buffering capacity appears to mitigate gastric stress, enhancing *Salmonella* survival during SGC simulation.

Award Application: Undergraduate Student Award

P2-075: LEVERAGING AI FOR FOOD SAFETY: A SYSTEMATIC REVIEW

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: While some fields have made advances in using AI to enhance food safety, others are just beginning to leverage this technology to improve efficiency and outcomes.

Purpose: The purpose of this study was to determine: 1) which AI applications are currently being used in food safety-related fields, 2) what drives AI use in other fields, and 3) how insights from fields with advanced AI usage can inform emerging retail food safety applications.

Methods: This systematic review followed PRISMA, a rigorous set of guidelines, for reporting systematic reviews, to identify English language peer-reviewed articles published between the last five years from the Web of Science database. Keywords were: 'AI' OR 'Artificial Intelligence' AND 'Safety' AND 'Food'. The search yielded 295 articles, and after reviewing for relevance, 48 articles remained.

Results: Currently used AI applications across food safety-related fields integrate with Internet of Things (IoT), e-nose systems, blockchain, and machine learning to address safety in supply chain management, agriculture, and food processing. The machine learning algorithms employed included Support Vector, Artificial Neural Networks, and XGBoost. The factors that drive AI use vary by field. In supply chain, the need for secure traceable data drives blockchain and IoT adoption for real-time monitoring. In food processing, demand for

quality assurance and adulteration detection drives AI-powered e-nose systems and smart packaging. In agriculture, the complexity of crop monitoring, soil assessment, weather analysis, and disease classification drives AI decision support tools. Insights from fields with advanced AI usage can benefit retail food safety by using similar frameworks for real-time monitoring, adulteration detection, and predictive decision support.

Significance: Insights addressed how researchers integrate digital technologies with AI machine learning algorithms to guide smart food safety outcomes.

Award Application: Developing Scientist Award

P2-076: MICROBIAL SAFETY ASSESSMENT OF FRESH RICE NOODLES: EFFECTS OF STORAGE TEMPERATURE ON PATHOGEN GROWTH AND TOXIN PRODUCTION

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Fresh rice noodles pose a significant food safety risk due to their high moisture content, neutral pH, and minimal processing, all of which favor the growth of foodborne pathogens.

Purpose: This study aimed to evaluate the growth and enterotoxin production of major foodborne pathogens in fresh rice noodles under different storage temperatures.

Methods: Fresh rice noodles were prepared at a commercial production facility in Washington state. Upon receipt, samples were divided into two treatment groups: uninoculated and inoculated with 2–3 log CFU/g with target pathogens. Both treatment groups were incubated at 12, 18, 29, and 35°C for 72 h. Samples were analyzed at six time points during incubation for pathogen growth, enterotoxin production, and changes in pH and a_w. Three independent replications were conducted for each treatment, resulting in a total of 936 samples.

Results: In uninoculated samples, all five target pathogens were isolated from fresh rice noodles. At 12°C, pathogen populations remained below the detection limit (1.7 log CFU/g)

for 72 h, whereas growth entered the stationary phase after 24 h at 18°C and after 6 h at both 29 and 35°C. No *Bacillus cereus* or *Staphylococcus aureus* enterotoxins were detected in uninoculated samples at any storage temperature. In inoculated samples, a significant time-temperature interaction was observed for pathogen growth ($p < 0.05$), resulting in pathogen growth entering the stationary phase at 72 h at 12°C, 24 h at 18°C, and 6 h at 29 and 35°C, and pH and a_w changes consistent with log phase growth. High levels of *B. cereus* enterotoxin were detected at 35°C after 48 and 72 h, while *S. aureus* enterotoxin was detected at 18°C after 72 h, 29°C after 24 h, and 35°C after 12 h.

Significance: These findings provide new data on pathogen growth and enterotoxin formation in fresh rice noodles, informing risk assessment and temperature control recommendations.

Award Application: Developing Scientist Award

P2-077: ONE SIZE DOES NOT FIT ALL: SIMULATION OF PRE-HARVEST TESTING STRATEGIES FOR UNIFORM AND CLUSTERED PATHOGEN CONTAMINATION OF LEAFY GREENS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Flexible supply chain risk models (SCRMs) can determine the relative power of different sampling plans to help manage risk in different pre-harvest pathogen contamination patterns, ranging from uniform to highly clustered, allowing for a sampling plan to be optimized for more likely contamination patterns.

Purpose: This study evaluated the effect of different pre-harvest pathogen contamination patterns on recall and public health risks, and the effect of different pre-harvest sampling plans in managing those risks.

Methods: SCRM was revised to incorporate clustered pre-harvest contamination to evaluate

a leafy green supply chain, allowing evaluation of uniform (100% of the field at 1 CFU/lb), large cluster (10% at 10 CFU/lb), and small cluster (1% at 100 CFU/lb) contamination. The overall risk of a positive retail test (recall risk) and number of lots that produced a positive retail test probability of over 10% (public health risk) were calculated. Trade-offs between different pre-harvest sampling plan attributes (i.e., mass, number of grabs, and number of tests) and risk were also evaluated.

Results: The overall risk of a positive retail test decreased with decreasing cluster size (1 in 4,400; 6,000; and 8,400 for 100%, 10, and 1% cluster size), while high public health risk lots increased with decreasing cluster size (22, 31, and 33, respectively). Pre-harvest sampling plans with more tests better managed lower concentrations of uniform contamination (overall risk increased from 1 in 462,100 to 1 in 112,100 for 2 tests and 1 test respectively) and plans with more grabs better managed clusters (1 in 193,100 and 750,400 in 1% cluster size for 60 and 100 grabs, respectively).

Significance: SCRM was able to simulate several pre-harvest sampling plans and found that uniform pathogen contamination risk was better managed by plans using multiple tests (therefore more total mass), whereas clustered contamination risk was better managed by plans with more grabs.

Award Application: Developing Scientist Award

P2-078: PREDICTIVE MODEL FOR BACILLUS CEREUS GROWTH FROM SPORES IN A COMBINED RICE-BEEF-VEGETABLES PRODUCT: ONE-STEP KINETIC ANALYSIS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Consumption of starch-based multicomponent products such as cooked rice with beef and vegetables is mainly the cause of *Bacillus cereus* food poisoning outbreaks. The illness occurs due to consumption of food subjected to inadequate rate of chilling after cooking or due to consumption of temperature abused foods.

Purpose: This study was conducted to quantify *B. cereus* growth from spores in a combined cooked rice-beef-vegetables (RBV) product between 10 to 49°C and develop a predictive model.

Methods: A heat-shocked 4-strain cocktail of *B. cereus* spores was inoculated in the cooked product. Samples were stored at isothermal temperatures and enumerated periodically. The growth curves were analyzed using the USDA IPMP-Global Fit software's one-step kinetic analysis, with the Huang model the primary model in combination with three secondary models: Ratkowsky square-root, Huang square-root, and Cardinal parameter models.

Results: Growth from *B. cereus* spores was observed at temperatures from 13 to 46°C, but not at 10 and 49°C. While the maximum growth temperatures estimated by the Ratkowsky and Huang models were similar, i.e., 45.2 and 45.3°C, respectively, the Cardinal model estimated 50.2°C. The Huang square-root model accurately described the minimum growth temperature (9.32°C), in contrast to the Ratkowsky (6.79°C) and Cardinal models (7.35°C). However, all three models exhibited an accurate growth prediction (RMSE ~ 0.48) with a similar Akaike information criterion (AIC) between 13 and 46°C.

Significance: These models can assist food service establishments and regulatory agencies to predict *B. cereus* growth in a RBV combination product, estimate its risk and determine compliance with regulatory performance standards.

P2-079: QUANTITATIVE MICROBIAL RISK ASSESSMENT OF FROZEN CHEESE TEXAS TOAST USING BATTELLE'S PRIA™ MODEL

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Quantitative Microbial Risk Assessment (QMRA) tools, such as Battelle's PRIA™, support data-driven evaluations of formulation options, preventive controls, and consumer-handled food safety risks.

Purpose: This study applied QMRA modeling to assess the risk of foodborne illness from frozen cheese Texas toast under variable consumer adherence to label instructions, capturing how deviations from recommended preparation influence pathogen exposure.

Methods: Model inputs included prevalence and concentration data for *Salmonella* spp. and *Listeria monocytogenes* in selected ingredients, obtained from scientific literature. Formulation, processing, distribution, and consumer preparation parameters were derived from internal company data. Two consumer preparation scenarios were evaluated: (1) partial adherence to labeled cooking instructions (7 min; 7–8 min) and (2) full adherence to labeled cooking instructions (8–10 min). The QMRA simulated scenario-specific risks associated with ingredient contamination and consumer adherence to label instructions to estimate food safety risk from a public health perspective.

Results: The model estimated that, even under partial adherence to cooking instructions (7 min; 7–8 min), the predicted risk was 0 illnesses per million servings for both *Salmonella* spp.

and *L. monocytogenes*, representing a conservative worst-case scenario. Comparable results were observed under full adherence to the labeled cooking instructions.

Significance: The QMRA model showed that, even under conservative worst-case assumptions, labeled cooking instructions effectively minimize the risk of *Salmonella* spp. and *Listeria monocytogenes* in frozen cheese Texas toast. These findings demonstrate the value of QMRA as a science-based tool for quantifying consumer-level food safety risk, supporting safety-by-design through validation of pathogen lethality, and providing a scientific basis for the development of a frozen cheese Texas toast food safety plan.

P2-080: QUANTITATIVE MICROBIAL RISK ASSESSMENT OF READY-TO-EAT DRESSINGS AND DIPS USING BATTELLE'S PRIA™ MODEL

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Quantitative Microbial Risk Assessment (QMRA) tools such as Battelle's PRIA™ support data-driven decisions by enabling food manufacturers to evaluate formulation options, validate preventive controls, and assess consumer-handled food safety risks.

Purpose: This study utilized QMRA modeling to quantify predicted foodborne illness risk in two model dressing systems across varying pH levels to support risk-based product development and preventive control verification.

Methods: Two dressing formulations, Blue Cheese Dressing and Ranch Veggie Dip, were evaluated using QMRA simulation ensembles. Inputs included prevalence and concentration data for *Salmonella* spp., *Listeria monocytogenes*, and *Clostridium botulinum* in select ingredients based on scientific literature. Formulation, processing, and distribution parameters were based on internal company data. Multiple ensembles of simulations were generated to estimate the food safety risks across the supply chain to validate formulation and manufacturing preventive controls.

Results: When product pH remained within the specification (3.9 – 4.3), the model predicted

0 illnesses/million servings for all pathogens in both products. At an elevated pH of 4.7, predicted *L. monocytogenes* risk increased to ~11 illnesses/million servings for Blue Cheese Dressing, while risk for Ranch Veggie Dip remained 0 illnesses/million servings.

Significance: Findings demonstrate that maintaining product pH within validated specifications ensures negligible risk from key pathogens in high-acid dressings. However, upward pH deviations (>0.3 units) may substantially increase *L. monocytogenes* risk in certain formulations. This underscores the importance of robust pH control and verification programs at the factory level. It also highlights the need to consider additional intrinsic factors, such as water activity, salt content, and pathogen die-off, in product design, along with implementing effective manufacturing controls.

P2-081: RISK ASSESSMENT OF ZEARALENONE AND ITS MODIFIED FORMS UNDER DIFFERENT TOXICITY ASSUMPTIONS IN SOUTH KOREAN MARKET FOODS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Modified mycotoxins (MMs) formed through plant or animal metabolism are often assumed to be less toxic than their parent compounds; however, some metabolites exhibit comparable or higher biological activity. For zearalenone (ZEN), certain phase I metabolites show higher estrogenic potency than ZEN, and some phase II metabolites may be hydrolyzed back to ZEN during digestion. Nevertheless, modified forms are rarely considered in routine mycotoxin risk assessment.

Purpose: This study assessed dietary risk from ZEN and modified ZEN (mZEN) in South Korean market foods and evaluated how inclusion of mZEN alters risk estimates compared with ZEN-only assessment.

Methods: Exposure assessment and risk characterization were conducted using occurrence data of ZEN and mZEN across 26 food matrices marketed in South Korea. To address left-censored occurrence data, lower-bound (LB) and upper-bound (UB) approaches were applied. Two risk characterization scenarios were evaluated: (□) assuming equal toxicity for ZEN and mZEN, and (□) applying literature-derived relative potency factors (RPFs) for mZEN based on estrogenic potency.

Results: Under the ZEN-only assessment, the highest estimated intake corresponded to

1.27% of the tolerable daily intake (%TDI) under the most conservative scenario (UB, high consumption), indicating low risk. When ZEN and mZEN were combined assuming equal toxicity, the maximum %TDI increased to 29.06% but remained below 100%. In contrast, applying RPFs increased the estimated %TDI compared with the equal-toxicity assumption in the most conservative scenario. These findings indicate that modified forms can contribute to overall ZEN-related risk depending on toxicological weighting assumptions.

Significance: Incorporating mZEN using potency-adjusted approaches can markedly alter dietary risk estimates, supporting inclusion of modified forms alongside parent ZEN in future risk assessment frameworks.

P2-082: USING CROWDSOURCED DATA TO INVESTIGATE VENUE-SPECIFIC NOROVIRUS-LIKE ILLNESS REPORTING PATTERNS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Human norovirus is the leading cause of viral gastroenteritis, with about 25% of cases caused by foodborne transmission. While foodservice outbreaks are well-documented, less is known about how venue characteristics influence transmission risk. Crowdsourced data offers a novel approach to characterize venue-specific risk factors.

Purpose: To compare norovirus-like illness reporting patterns between high-density venues and grocery/convenience stores using crowdsourced data.

Methods: Self-reported illness data (N=44,133) were extracted from iwaspoisoned.com for the years 2019–2024 (inclusive). U.S.-based reports were categorized as "norovirus-proxy" when both vomiting and diarrhea were reported. Two cohorts were analyzed: (i) high-density venues (lodging, airlines, airports, amusement parks; n=2,437); and (ii) grocery and convenience stores (n=41,696). The Chi-square test was used to compare the percent of total reports by venue type that met the norovirus-proxy criterion.

Results: Norovirus-proxy reporting differed significantly by venue type ($\chi^2 = 144.54$, df = 1, $p < 0.0001$). Specifically, 48.2% of reports from high-density venues met the proxy definition

versus 36.1% from grocery/convenience stores—a 12.1 percentage point difference. This pattern persisted across all individual years and through the SARS-CoV-2 pandemic (all $p<0.05$), with annual differences ranging from 8.8 to 12.9 percentage points.

Significance: Elevated norovirus-proxy reporting in high density venues suggests environmental factors – close contact, shared surfaces, confined spaces – are also important to transmission risk. Given these findings, infection control strategies in food service should account for both food safety and venue design/sanitation characteristics. This work provides quantifiable insight into the importance of venue and population density in norovirus transmission as it relates to establishments that sell and serve food.

P2-083: A RISK-INFORMED DECISION SUPPORT TOOL FOR AGRICULTURAL IRRIGATION WATER MANAGEMENT USING QMRA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Fresh produce safety depends on irrigation water quality, yet microbial contamination remains a persistent challenge, creating uncertainty for growers in complying with regulatory standards.

Purpose: The objective of this study was to develop a user-friendly, scenario-based risk tool that integrates scientific data and Quantitative Microbial Risk Assessment (QMRA) to guide growers in making evidence-based decisions for agricultural irrigation water management under Food and Drug Administration Subpart E of the Produce Safety Rule.

Methods: A QMRA framework was applied to simulate contamination, die-off, water

treatment, and exposure across a range of production scenarios. Inputs included field persistence data and models for *E. coli* and *Salmonella* on crops such as lettuce, tomatoes, and tree fruit and stakeholder-informed irrigation and mitigations strategies. Pathogen die-off and inactivation was modeled using survival kinetics and literature-informed relationships, with computational techniques and Monte Carlo simulations (10,000 iterations per scenario) applied to estimate infection risk based on user-selected pre-harvest scenarios.

Results: Risk estimates varied by crop type, irrigation method, and management strategy. For example, leafy greens irrigated with surface water showed 3.4 log reductions in risk after waiting 1 day to harvest, whereas drip-irrigated tomatoes had 7 log reductions. Mitigation strategies, such as delaying harvests up to 4 days or applying targeted water treatment such as chlorine or peracetic acid (PAA) reduced estimated infection risk from 1 in 10,000 to below 1 in 100,000 per serving, depending on the scenario evaluated.

Significance: The tool provides growers with scientifically grounded, scenario-specific guidance to reduce microbial risk, prioritize interventions, and support grower compliance with Subpart E, making QMRA outputs accessible for practical decision-making at the field level.

P2-084: ADVANCING PHAGE-BASED INTERVENTIONS FOR CATTLE COLONIZED WITH E. COLI O157:H7

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 remains a persistent public-health and food-safety concern, particularly within beef production systems where pre-harvest shedding contributes to carcass contamination. Bacteriophages offer a promising targeted intervention.

Purpose: Develop an effective phage cocktail capable of reducing or eliminating STEC O157:H7 colonization in cattle.

Methods: The activity for over 300 phage was determined with the *E. coli* O157:H7 strain ZAP2615 in vivo using 4 different liquid media (LB, MEM, M9 and M9+bovine mucus), bacteria attached to bovine epithelial cells and ex vivo experiments performed with cattle feces from animals colonized with ZAP2615. Sixteen cattle were rectally inoculated with strain ZAP2615 with half the cattle challenged with 3 doses of a five-phage cocktail given at approximately 12 hours intervals. Feces were collected daily for enumeration and prevalence culturing.

Results: A five-phage combination demonstrated strong activity in vitro, including in bovine rectal mucus and on bacteria attached to epithelial cells. Ex vivo evaluation using fecal samples from colonized cattle confirmed activity of the five-phage combination. Despite clear activity in vitro and ex vivo, the phage cocktail did not yield a measurable reduction of STEC O157:H7 during a controlled cattle challenge. Both treatment and control groups showed parallel declines in bacterial shedding indicative of a likely failure of effective phage delivery to the rectal colonization site.

Significance: These findings emphasize that while phage selection and cocktail design were successful, delivery to the colonizing bacteria remains a critical barrier for effective in vivo intervention.

P2-085: AMR IN SMALL-SCALE LEAFY VEGETABLE PRODUCTION SYSTEMS: A GLOBAL PERSPECTIVE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Small-scale farming is expanding globally to enhance food security. Understanding factors driving the dissemination of antimicrobial resistance (AMR) in these food systems is increasingly relevant to food safety.

Purpose: To evaluate prevalence of AMR bacteria at small farms in the mid-Atlantic USA and in South Africa (SA) as a factor of farm management that can vary from local- to global-scales.

Methods: Leafy vegetable and soil samples were collected at seven urban farms around Washington, D.C. and three integrated swine-vegetable farms in SA, in which composts and manures were the primary soil amendments, respectively. *Escherichia coli* was monitored at all sites and general AMR bacteria were monitored at the compost-based farms as well. All isolates were screened for resistance to a panel of clinically relevant antibiotics (e.g., tetracycline, ampicillin, ciprofloxacin, streptomycin) via replica plating or broth microdilution

and Kirby-Bauer disc diffusion. Whole-genome sequencing (WGS) was conducted on select isolates to identify antimicrobial resistance genes (ARGs).

Results: AMR bacteria were prevalent in the pre-harvest environment. AMR *E. coli* was widespread in the integrated farms (38.9% samples; n=35/90 *E. coli* isolates with AMR), though less abundant at the compost-based farms (3.6% samples; n=1/10 *E. coli* isolates with AMR). Nevertheless, the latter contained diverse AMR bacteria spanning *Pseudomonas, Bacillus,* and *Serratia,* among other taxa (n=87 isolates). Among the *E. coli* and environmental AMR strains, *tetA, tetB, blaSRT, fosB, aph(3'')-Ib* and *aph(6)-Id* were common ARGs, in some cases associated with mobile genetic elements.

Significance: Our findings highlight the potential for manure and even treated soil amendments as reservoirs of AMR bacteria in small-scale agriculture. Establishing universal standards for risk assessments involving AMR is important for protecting food safety and public health.

P2-086: ANTIMICROBIAL EFFICACY OF PEROXYACETIC ACID (PAA) TREATMENT IN HARVESTED RAINWATER USED FOR IRRIGATION OF FRESH PRODUCE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Harvested rainwater is a potential reservoir of human foodborne pathogens and must be treated prior to application in fresh produce irrigation.

Purpose: To evaluate peroxyacetic acid (PAA) as a treatment for harvested rainwater at an urban and a peri-urban farm in Maryland.

Methods: First, the antimicrobial efficacy of 8 ppm PAA treatment on harvested rainwater (12 and 32°C) inoculated with ~7 log CFU/ml of an *E. coli* and *Salmonella* cocktail, respectively, was evaluated. Then, validation of 8 ppm of PAA treatment to effectively treat harvested rainwater prior to the irrigation period of a Brassica mix was evaluated over four independent growing trials (April–November). Following 5 mins of treatment, total plate counts (TPCs) were performed on rainwater samples to determine whether PAA affects the growth and survival of natural microbes over 7 days.

Results: 8 ppm PAA treatment for 5 minutes was significantly effective ($p < 0.05$) in reducing both *E. coli* and *Salmonella* cocktail populations to < 3 log CFU/mL at both temperatures (12 and 32°C), respectively, compared to the PBS control. No treatment resulted in > 7 log CFU/ml detected for both respective cocktail mixtures. Compared to untreated rainwater (day 0), no significant differences ($p > 0.05$) in the microbial population were observed following PAA treatment (day 0) during all growing trials. On day 7, the PAA concentration had completely

dissipated, however, no significant differences ($p > 0.05$) were observed in the microbial populations on day 7 for the PAA-treated samples indicating no significant growth over the period.

Significance: Harvesting rainwater offers an alternative source of irrigation water but must be treated to prevent cross-contamination of human foodborne pathogens to fresh produce. Farmers may consider treating rainwater with PAA since it is effective, easy to handle and produces non-toxic byproducts.

P2-087: ANTIMICROBIAL RESISTANCE AND GENOTYPIC DIVERSITY OF ESBL-PRODUCING ESCHERICHIA COLI IN BROILER AND LAYER FARM ENVIRONMENTS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The dissemination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in poultry production systems represents a critical concern for food safety and public health. This study compared antimicrobial resistance (AMR) profiles and the distribution of β-lactamase genotypes in ESBL-producing *E. coli* isolated from broiler and layer farm environments.

Methods: Environmental samples (feces and litter) were collected from eight poultry farms (four broiler and four layer farms). ESBL-producing *E. coli* were isolated using selective media and identified by standard microbiological methods. AMR was determined by the broth microdilution method. ESBL-associated genes (*bla*TEM, *bla*CTX-M, and *bla*SHV) and their alleles were characterized using PCR and sequence analysis.

Results: A total of 86 and 105 isolates were recovered from layer (n=36) and broiler (n=24) farms, respectively, with ESBL-producing *E. coli* accounting for 91.86% (79/86) and 75.24% (79/105). The phenotypic resistance profiles significantly varied depending on the production

system. Broiler-derived isolates (n=79) exhibited high resistance to quinolones, including nalidixic acid (66/79, 83.5%) and ciprofloxacin (53/79, 67.1%). In contrast, layer-derived isolates (n=79) showed higher resistance levels to cefotaxime (52/79, 65.8%), gentamicin (34/79, 43.0%), and trimethoprim/sulfamethoxazole (41/79, 51.9%). Both groups demonstrated similarly high resistance to ampicillin (63/79, 79.7% for broilers; 64/79, 81.0% for layers). Genotypically, layer isolates displayed broader TEM allelic diversity, whereas broiler isolates showed a pronounced predominance of CTX-M-1 group alleles, particularly *bla*CTX-M-55, with more concentrated genotypic profiles.

Significance: Differences in antimicrobial resistance and genotypic patterns between broiler and layer systems reflect distinct selection pressures and underscore the need for system-specific strategies to mitigate antimicrobial resistance.

Award Application: Developing Scientist Award

P2-088: ASSESSING THE ABILITY OF TREATED AGRICULTURAL WATER TO ELIMINATE ESTABLISHED E. COLI

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Currently, there exists a knowledge gap about the cumulative impact of agricultural water treatment on established pathogen persistence, decay, and die-off on plant tissue and soil during the growing season. With increasing regulatory and buyer pressure for growers to conduct agricultural water treatment to reduce the impact of human health pathogens on fresh produce production systems, additional information is needed.

Purpose: This project aimed to determine the impact of treated agricultural water on the survival of Shiga-toxigenic *E. coli* (STEC) and generic *E. coli* in agricultural soils and romaine

lettuce maintained under two Arizona growing conditions.

Methods: Soil microcosms and romaine lettuce heads, in triplicate, were inoculated with high (10^4 CFU/g) or low (10^2 CFU/g) STEC or generic *E. coli* strains. Microcosms and lettuce were exposed to treated irrigation water with 2 ppm or 4 ppm chlorine or 7 ppm or 10 ppm peracetic acid (PAA) and maintained in chambers simulated for Yuma or Maricopa, AZ conditions. Samples were cultured for viable microbes at 0, 12, 24, 48, 72, 168, 336 and 504 hours post-inoculation/treatment.

Results: Sanitizers eliminated low concentrations of STEC in Yuma soils after 168 hrs and after 72 hrs in Maricopa soils. At higher concentrations, STEC in soils were less effectively eliminated by PAA (504 hrs) than chlorine (168 hrs). On romaine lettuce, low levels of STEC were eliminated within 24 hrs, however, no sanitizer completely reduced bacterial populations. At high concentrations, pathogens were reduced after 168 hrs, but not eliminated. Overall, treated irrigation water reduced pathogens, but concentrated populations will not be completely eliminated if colonization occurs prior to exposure.

Significance: This study helps inform industry of effective strategies for mitigating the risk of foodborne pathogen contamination present in soils and plant tissues.

P2-089: ASSESSING THE RISK OF CONTAMINATION OF LEAFY GREENS AND LANDSCAPE USE IN CALIFORNIA FARMLAND – A PARTICIPATORY MODELING APPROACH

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Contamination of leafy greens with pathogenic *E. coli* has led to human outbreaks and is a well-known concern within strong agricultural regions such as the Salinas Valley in California.

Purpose: The objective of this study was to develop a framework for risk assessment to estimate the risk of contamination of leafy greens with pathogenic *E. coli* under different agricultural, climatic, and environmental scenarios.

Methods: We first conducted a literature review of risk factors for contamination of leafy greens. Then, we carried out expert opinion elicitation with stakeholders (researchers, industry representatives, regulators, and extension specialists) to populate parameters for which available data were deemed inexistant, insufficient, or contradictory. A framework for risk assessment integrating expert opinion, literature, and public data was then developed to estimate risk under different settings (e.g., contamination via wildlife, from grazing cattle, or

water runoff). This model was integrated into an interactive, open source, and user-friendly web-based interface developed in R, which allows the visualization of risk estimates and sensitivity analyses.

Results: Data from 18 experts and data retrieved from the literature and public databases were used in the model. For a scenario in a random location in January, where the user selected a medium initial probability of contamination (1 in 100 batches are contaminated), no wildlife deterrence methods, proximity to cattle located uphill (concentrated animal feeding operations with >5,000 head of cattle and grazing cattle), movement of cattle, no hobby farms or compost facilities nearby, public water supply use and subsurface drip irrigation, no history of flooding, and a sandy, alkaline soil, the final output is a medium probability of contamination of leafy greens with pathogenic *E. coli*.

Significance: The tool will help growers and other agents to proactively manage contamination risks in leafy greens.

P2-090: BEHAVIORS OF LISTERIA INNOCUA DURING CULTIVATION OF KING OYSTER MUSHROOMS (PLEUROTUS ERYNGI)

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Although demand for King oyster (*Pleurotus eryngii*) and other specialty mushrooms is increasing and *Listeria*-linked outbreaks have occurred, data on *Listeria* behavior during mushroom cultivation are limited.

Purpose: This study aimed to assess the behavior of *Listeria innocua* during King oyster cultivation and to characterize associated background microbiota.

Methods: King oyster mushrooms were cultivated in a monotub under controlled conditions (21–24°C, 95–99% RH) using pasteurized grain spawn and commercial growth substrates (GS). Rifampin-resistant *Listeria innocua* NRRL B-33162 (~7 log CFU/g) was introduced either during grain inoculation or spawn run. Two replicate experimental trials were performed. Colonized grains, grain–GS mixtures, and mushroom caps and bulbs (n=6) were sampled from inoculated and control groups, homogenized, and plated on selective agar for *L. innocua* enumeration and non-selective media for background microbiota.

Results: When *L. innocua* was introduced during grain inoculation, it persisted in colonized grains (6.89±0.23 log CFU/g) and bulbs (6.80±0.05 log CFU/g). Spawn-run inoculation

yielded *L. innocua* population in substrates (7.48±0.18 log CFU/g) and bulbs (5.97±0.23 log CFU/g). *L. innocua* counts in bulbs did not differ significantly between scenarios (P = 0.4876). Enrichment confirmed *L. innocua* in caps (3/6 positive) for both scenarios, indicating transfer to edible tissues. Total aerobic bacteria increased from <1 log CFU/g at the grain stage to 9.07±0.05 log CFU/g during the spawn run, with harvested mushrooms containing 5.76±1.28 log CFU/g (caps with stalks) and 8.18±0.58 log CFU/g (bulbs).

Significance: These findings show that early contamination enables *Listeria* to persist into edible tissues, highlighting contamination risks and the need for preventive controls.

Award Application: Developing Scientist Award

P2-091: BILE QUANTITY IN POULTRY LITTER-BASED AMENDMENTS CONTRIBUTES TO FOODBORNE PATHOGEN PERSISTENCE

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Poultry litter-based soil amendments have been recognized for their proclivity to influence bacterial survival in soil. Bile is a fecal biomarker found in biological soil amendments of animal origin (BSAAO) that can induce biofilm formation in certain foodborne pathogens. The quantity of bile in different types of poultry litter was determined, and the ability of the poultry litter extracts were evaluated for their ability to induce biofilms in *Salmonella* Newport and *Escherichia coli* O157:H7.

Purpose: Bile content was evaluated as an indicator for a poultry litter-based soil amendment's ability to induce biofilm formation in foodborne pathogens.

Methods: Fresh poultry bedding (FPB-before broiler flocks were introduced), used poultry bedding (UPB-after broiler flocks), and commercially available poultry pellets (PP) were assessed. One gram of each amendment was suspended in 10 ml of sterile deionized water and agitated at room temperature for 12 h. The extracts were filter-sterilized (0.2 µm). Bile

concentration of the extracts was measured using a fluorometric assay based on NADH reduction by 3-hydroxysteroid dehydrogenase in the presence of bile acids. *S*. Newport and *E. coli* O157:H7 (□5 log CFU/ml) were inoculated into the extracts (200 µl per well) and incubated at 37°C for 48 h. Biofilms formed were quantified using crystal violet staining.

Results: FPB, UPB and PP had significantly different bile concentrations 4.1, 62.0 and 144.4 µM respectively (p□0.05). FPB, UPB and PP resulted in a biofilm biomass (OD_{570}) of 0.21±0.02, 0.38±0.15, and 0.61±0.21 respectively in *S*. Newport. FPB, UPB and PP resulted in a biofilm biomass (OD_{570}) of 0.2±0.04, 0.38±0.26 and 0.31±0.14. Significantly higher biofilm biomass for both *S*. Newport and *E. coli* O157:H7 occurred in presence of PP than FPB (p□0.05).

Significance: Bile presence and concentration in poultry litter could be indicative of its risk to induce biofilm formation in *S.* Newport and *E. coli* O157:H7.

P2-092: BIOCONTROL OF LISTERIA MONOCYTOGENES AND SALMONELLA TYPHIMURIUM IN HYDROPONICS: ASSESSING THE EFFICACY OF PLANT GROWTH PROMOTING BIOINOCULANT CONSORTIA

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Hydroponic systems offer high yields, efficient resource use, and year-round production, but their nutrient-rich environments can also support foodborne pathogens. Plant growth-promoting rhizobacteria (PGPR) present a promising strategy, as they not only enhance plant health but may also help suppress human pathogens in soilless production systems.

Purpose: This study investigated the efficacy of PGPR bioinoculants against *L. monocytogenes* and *S.* Typhimurium in deep-water culture (DWC) and nutrient film technique (NFT) hydroponic systems throughout a basil production cycle.

Methods: Hydroponic systems were inoculated with one of three bioinoculants: a multibacillus-*Trichoderma harzianum* consortium (TG), *Bacillus subtilis* QST 713 (SN), and *Streptomyces lydicus* WYEC 108 (AC) at ~8 log CFU/ml, followed by pathogen inoculation (4-5 log CFU/mL) and tracking pathogen load in nutrient solution, roots, and leaves.

Results: Compared with the natural decline in the control, PGPR significantly reduced *Listeria* but not *Salmonella*, with effectiveness dependent on bioinoculant type, system, and production stage. TG was the most effective PGPR, with a 4.8–5.4 log reduction in NFT, corresponding to complete elimination of *Listeria* by day 28. In contrast, AC consistently sustained higher pathogen survival, often exceeding that of uninoculated controls.

Significance: PGPR bioinoculants can serve as a viable control against *L. monocytogenes* and *S.* Typhimurium in hydroponic systems, though their effectiveness depends on the pathogen, bioinoculant composition, system design, and growth stage. Integrating PGPR into hydroponics holds promise for enhancing microbial safety of fresh produce, but bioinoculant selection must be tailored to system and pathogen dynamics.

Award Application: Developing Scientist Award

P2-093: RISK FACTOR PROFILES AND FOOD SAFETY MANAGEMENT PRACTICES IN COMMERCIAL POTTED AND FRESH-CUT CEA HERB FACILITIES

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Controlled-environment agriculture (CEA) enables year-round, high-yield production but poses understudied food safety risks. Culinary herbs, widely grown in CEA, are in high demand and have been linked to foodborne illness outbreaks.

Purpose: This study characterized food safety risk profiles and management practices across operations that produce potted and fresh-cut herbs.

Methods: A multiple-case comparative study of six U.S. commercial herb operations was conducted incorporating four approaches including self-assessments, interviews, observations, and document review, guided by a harmonized tool adapted from established food safety assessment frameworks.

Results: Fresh-cut operations emphasized food safety alongside plant health and yield, evidenced from structured and formalized food safety management systems, while potted operations focused more on yield and plant health. Despite fewer contamination entry points, fresh-cut systems had higher risk scores (average of 5.17) due to harvest and postharvest handling factors, arising from tissue cutting, human contact, and suboptimal equipment sanitation practices. In contrast, potted systems had lower scores (average of 3.67) but more potential introduction points, with limited processing reducing overall risk, attributed to a lack of amplification points, as these operations have minimal processing and human handling of edible portions.

Significance: These findings indicate that food safety management in CEA herb production is shaped by operational complexity and market orientation, with product form and processing level as key risk determinants. There is thus a need for tailored produce safety guidance to address operation-specific risks and realities.

Award Application: Developing Scientist Award

P2-094: DIE-OFF OF E. COLI ON SPINACH GROWN IN INDOOR AND OUTDOOR DEEP-WATER CULTURE HYDROPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Hydroponics is a system of growing plants without soil, offering improved growth and environmental control through a diverse array of methods.

Purpose: The objective of this study was to assess the die-off rate of *E. col*i ATCC 25922 on spinach leaves (*Spinacia oleracea*) in indoor and outdoor Deep-Water Culture (DWC) hydroponic systems.

Methods: A total of six DWC hydroponic systems were built using 40-gallon heavy-duty storage totes. Spinach seedlings were transplanted into the systems and grown both outdoor and inside an environment-controlled greenhouse. On day 35, E. coli ATCC 25922 (3.8 log CFU/mL) was spot-inoculated onto the plant leaves. Spinach leaf samples were collected at 0, 24, 48, and 72 h and analyzed for *E. coli* die-off.

Results: The *E. coli* die-off was significant ($P < 0.05$) after 24 hours in the leaves of plants grown in both indoor and outdoor environments. The initial *E. coli* count was 3.8 log CFU/g, which decreased below the detectable limit after 24 h in outdoor samples and after 72 h in indoor samples. The indoor controlled environment had temperatures ranging from 23 to 25 °C, while the outdoor temperature ranged from 17 to 19°C during the die-off study. The overall reduction rate was higher in outdoor samples than in indoor samples.

Significance: This study demonstrates the microbial die-off on spinach leaves in both indoor and outdoor hydroponic systems, providing useful insights for the development of food safety risk management strategies.

Award Application: Developing Scientist Award

P2-095: EFFECT OF COMPOSTING ON THE MICROBIOME AND RESISTOME PROFILES OF DAIRY CATTLE MANURE WITH OR WITHOUT BIOCHAR AMENDMENT

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Composting is a commonly used animal manure management that may play a significant role in preharvest food safety by reducing produce contamination, and its effect may be modified by biochar amendment.

Purpose: We conducted a comprehensive analysis of the microbiome and resistome profiles of dairy manure to understand the effect of composting, with or without biochar.

Methods: A randomized dairy cattle manure composting trial with four biochar treatments (0%, 2%, 4%, and 10% [w/w]), each with five replicates, was conducted for six months from March to September 2023. Initial manure mixture (composed of sawdust and biochar) and final compost (n=20 each) samples were collected. DNA was extracted and profiled with shotgun metagenomic sequencing followed by bioinformatics analysis for the identification of bacterial taxa and antimicrobial resistance genes.

Results: Composting significantly ($P < 0.001$) reduced the mean sequence reads, with no

biochar effect (P=0.9592) based on negative binomial regression analysis. Composting shifted the microbiome profile: while the initial manure mixture was dominated by Firmicutes, the final compost was dominated by Proteobacteria. Similarly, a significant shift was observed in the resistome composition: tetracyclines, macrolides-lincosamides-streptogramins (MLS), aminoglycosides, multidrug resistance (MDR), and beta-lactams and glycopeptides, respectively were top five antimicrobial classes detected in the initial mixtures. On the other hand, MDR, glycopeptides, MLS and rifamycins, aminoglycosides, and phenicols, respectively represented top five antimicrobial classes detected from the final compost.

Significance: Composting although it shifted the microbiome and resistome populations and reduced their abundances, it did not eliminate them. Further studies are needed to optimize composting process for effective removal of microbes and their associated resistance elements.

P2-096: EFFECT OF SIMULATED LOUISIANA SUMMER PREHARVEST AND POSTHARVEST STORAGE CONDITIONS ON THE SURVIVAL OF SALMONELLA SPP. AND E. COLI O157:H7 ON CUCUMBERS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Fresh cucumbers are consumed raw and have been linked to foodborne outbreaks associated with *Salmonella* spp. and *E.coli* O157:H7. Environmental conditions encountered during preharvest and postharvest handling, storage, and distribution may significantly influence pathogen persistence on produce surfaces.

Purpose: This study evaluated the survival of three-strain cocktails of *Salmonella* spp. and *E. coli* O157:H7 on cucumber (*Cucumis sativus*) surfaces under simulated environmental stress conditions.

Methods: Defined surface areas (2 cm × 2 cm) were spot-inoculated with approximately 8 log CFU/mL of each pathogen cocktail, allowed to attach, excised, and recovered in phosphate-buffered saline. Inoculated samples were exposed to three environmental conditions: control (22°C, 65% RH), cold stress (4°C), and a diurnal temperature-humidity cycle designed to mimic Louisiana summer conditions. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test to determine significant differences ($p < 0.05$).

Results: A significant effect of time on pathogen survival was observed under diurnal conditions for both pathogens ($p < 0.0001$), with *Salmonella* spp. and *E. coli* O157:H7 populations declining by approximately 5.5 and 7.8 log CFU/cm^2, respectively, by 96 h. In contrast, pathogen populations under control and cold stress conditions exhibited comparatively minor reductions, with levels remaining above 6.2 log CFU/cm^2 for *Salmonella* spp. and above 6 log CFU/cm^2 for *E. coli* O157:H7 throughout the study period.

Significance: These findings demonstrate that realistic diurnal temperature and humidity fluctuations characteristic of Louisiana summer conditions substantially reduce pathogen persistence on cucumber surfaces. In contrast, constant ambient and refrigerated conditions allow prolonged survival. This underscores the importance of environmental variability in produce safety risk assessments.

Award Application: Developing Scientist Award

P2-097: EFFECTS OF PLASMA-ACTIVATED WATER TREATMENT ON MICROBIAL SURVIVAL AND CILANTRO GROWTH IN DEEP WATER CULTURE (DWC) HYDROPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Plasma-activated water (PAW) has gained popularity in hydroponic production due to its dual role as a nitrogen source with antimicrobial properties, which potentially enhances plant growth while mitigating food safety risks.

Purpose: This study investigated the effectiveness of air-based PAW treatments on the microbial survival and the growth of Cilantro (*Coriandrum sativum)* in Deep Water Culture (DWC) Hydroponic systems.

Methods: Plasma-activated water with a nitrate level of 100 ppm was produced using a PlasmaLeap100 direct bubble reactor. Cilantro (*Coriandrum sativum*) was grown for 16 days using PAW, pH-adjusted PAW, a combination of PAW and Nutrient solution (NS), and NS only. The systems were inoculated with *E. coli* ATCC 25922 and *Listeria innocua* ATCC 33090 in the second week of treatment. The treatment samples and the cilantro plants (roots and leaves)

were collected, and the microbial population and plant growth were determined.

Results: The *Listeria innocua* and *E. coli* populations decreased significantly ($P < 0.05$) after 72 hours in all treatment systems, with the highest reduction of 5.6 log CFU/mL in pH-unadjusted systems. *Listeria* appeared to be more susceptible to the treatments compared to *E. coli*. The initial hydrogen peroxide concentration in the PAW samples ranged from 3 to 10 ppm, which depleted below the detectable limit after 24 hours, while the nitrate concentration remained stable for 3 days of treatment. The root samples of the unadjusted PAW-treated samples had significantly lower populations *of E. coli* and *Listeria* compared to other samples. The combination of PAW and NS yielded the highest plant growth, with an average plant height of 8.9 cm and 5 leaves. However, plant growth was significantly lower in pH-unadjusted treatments.

Significance: These findings suggest that adjusting the concentration and pH of PAW is an effective strategy for controlling the growth of foodborne pathogens while ensuring the plant's growth.

Award Application: Undergraduate Student Award

P2-098: EVALUATING SOLAR HEATING TECHNOLOGY FOR ACCELERATED MICROBIAL REDUCTION IN RAW MANURE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Raw manure, rich in nutrients, improves soil health but raises concerns due to pathogen risks. EPA-approved treatments typically require weeks to meet standards.

Purpose: The study evaluated whether solar heating accelerates *E. coli* reduction in raw manure.

Methods: Laboratory trials heated manure inoculated with generic *E. coli* and *E. coli* O157:H7 (~8 log CFU/g) at 50, 55, 60, & 65°C for 3-5 h to compare reductions and assess *E. coli* as a surrogate. On-farm trials used a low-cost solar heater to treat inoculated manure (9.2-10.55 log CFU/mL *E. coli)* for up to 8 h. Temperatures (24.6-68.4°C) were tracked with thermocouples. Constrained quadratic and linear regressions related log reduction to time.

Results: At 50˚C & 55˚C, *E. coli* O157:H7 declined gradually from 8.05-8.23 log CFU/g to 6.43-5.42 log CFU/g by 5h. At 60°C & 65°C, the reduction were larger (3.61-3.56 log CFU/g) within the same time interval. Generic *E. coli* showed the same pattern, with a significant reduction in counts (4.19-3.13 log CFU/g) at 60°C & 65°C as compared to 50˚C &

55˚C (6.45-6.47 log CFU/g). In the on-farm study, longer treatment time led to higher microbial reductions, with every additional hour of treatment associated with an additional 0.5 log reduction but with diminishing returns (the time×temperature interaction was small and not significant), and reductions leveled off by ~8 hours (observed mean 2.60 log CFU/g; modeled ~2.2–2.5 log 99% reduction). A quadratic time model explained 82% of the variability and reduced error by ~6% versus a constrained linear model, indicating that time, more than temperature, drives reductions under field conditions. Moisture dropped from ~50.8-57.4% to 0-8.3% within 8 h, which may have influenced survival.

Significance: Solar heating shows potential for reducing microbial contamination in raw manure. However, minimizing moisture loss and extending treatment duration may be necessary to achieve higher reductions and meet PFRP standards.

P2-099: HYDROCLIMATIC DRYNESS, STREAMFLOW ANOMALIES, AND RECURRENT LEAFY GREEN OUTBREAKS IN THE SOUTHERN SALINAS VALLEY, CALIFORNIA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Recurrent leafy green-associated Shiga toxin-producing *Escherichia coli* outbreaks have been linked to the southern Salinas Valley in California, but the hydroclimatic context is unclear.

Purpose: To quantify hydroclimatic dryness “south of King City” and compare outbreak with non-outbreak years.

Methods: Discharge data (1990–2024) from two USGS gages (San Lorenzo Creek below Bitterwater Creek; Salinas River near Bradley) were summarized by hydrologic year into seasonal (Oct–Dec, Jan–Mar, Apr–Jun, Jul–Sep) mean flows and converted to standardized anomalies (q_z); a flow dryness index was defined as $-q_z$ (higher = drier). Monthly PRISM precipitation and mean temperature over a farm–rangeland area were used to estimate potential evapotranspiration (Thornthwaite) and calculate a 6-month Standardized Precipitation–Evapotranspiration Index (SPEI-6) from P−PET. Winter (Oct–Mar) flow dryness and SPEI-6 were compared between outbreak (2019–2020) and other years using rank statistics, correlation, and logistic regression.

Results: Across 34 hydrologic years, winter flow dryness in San Lorenzo Creek was strongly negatively correlated with winter SPEI-6, indicating that drier weather anomalies generally coincided with lower flows. Outbreak years 2019 and 2020 occurred in the drier half of the winter flow dryness distribution despite contrasting winter SPEI-6 values (near normal versus moderately dry), and models suggested higher outbreak odds under locally drier winter flow conditions.

Significance: Anomalously low winter and early spring flows, even without extreme basin-scale drought, may increase vulnerability of leafy green production areas to contamination by potentially reducing dilution and altering transport and persistence of fecal pollution from surrounding lands.

P2-100: IDENTIFYING INFLUENTIAL FACTORS OF E. COLI O157:H7 TRANSMISSION FROM ANIMAL OPERATIONS TO PRODUCE FIELDS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Contamination of produce, especially leafy greens, by *Escherichia coli* O157:H7, poses a significant risk to food safety; however, the transmission pathways facilitating this contamination are not fully understood.

Purpose: This two-year study from 2025 to 2026 seeks to identify weather conditions, such as rain and wind, that contribute to the transmission of *E. coli* O157:H7 from a cattle feedlot to the surrounding area to generate a predictive risk model for produce growers.

Methods: At a cattle feedlot with 6,000 head of cattle, sample collection plots (n=24) were placed at varying distances (500m–1,500m) around the feedlot. Each plot consisted of two sets of soil, water, air, and surrogate plant surfaces, and are sampled every two weeks from August to September in 2025 (n=4) and will be sampled from May to September in 2026 (n=10). Monthly feedlot soil samples were taken to identify the presence of *E. coli* O157:H7 and determine the strains present. Additional collection of weather data was utilized at the furthest North, South, East, and West sampling sites to develop a model between pathogen presence and weather factors.

Results: A total of four separate sampling events resulted in 96 plots collected, where 3.13% (3/96) were positive for *E. coli* O157:H7. Each positive sample was identified only from air sampling during two different two-week sampling events. Positive samples were identified at the closest sampling sites to the feedlot (500m). Preliminary analysis identified six significant precipitation events and four extreme heat events during the 2025 sampling period.

Significance: In the first year of this two-year study, few positive isolates suggest inconsistent transmission, possibly due to late-season sampling, reduced prevalence of *E. coli* O157:H7 in the feedlot. A second, longer sampling season spanning May to September in the following year will permit the development of a predictive risk-assessment model.

P2-101: INACTIVATION OF SALMONELLA TYPHIMURIUM IN POULTRY LITTER WITH PAPER BIOCHAR

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: We previously demonstrated biochar-inactivation of either *Salmonella* or *E. coli* in both cultivable soil and dairy manure compost. One of the most effective biochars utilized was highly alkaline paper biochar. *Salmonella* continues to be a problematic human pathogen in poultry meat, thus various means have been used to reduce salmonellae in poultry litter.

Purpose: A study was conducted to determine if alkaline paper biochar (PB) could inactivate *Salmonella* Typhimurium in poultry litter (PL).

Methods: Poultry litter (PL), used to rear one commercial broiler flock, was acquired from an area poultry processor, autoclaved, and adjusted to 49.8% moisture and 7.99 pH. Paper biochar (PB) was pyrolyzed anoxically, in-house, at 700°C for 1 h, combined with PL in bottles at 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% (wt.:wt.), tumbled on a bottle roller for 4 h, inoculated with ca. 8 log CFU/g *Salmonella* Typhimurium, and tumbled for another 2 h. Samples were stored at 21°C and analyzed weekly by diluting 1:10 in DE neutralizing broth (adjusted with HCl to reduce the PB/PL+DE broth mixture to pH 7.5), spiral plating 0.1 ml (total) + spread plating (1.0 ml total) on chromogenic, differential/non-selective TSA agar, and incubating at 37°C overnight before enumerating colonies.

Results: The PL-only, positive control samples supported 5.27-6.93 log of *Salmonella* from 0-3 weeks. The minimum PB concentrations required to significantly reduce *Salmonella* over time ($p<0.05$) were as follows: week 0-2.0% (-5.2 log), week 1-1.0% (-3.77 log), week 2-0.5% (-5.84 log), and week 3-0.5% (-6.12 log). At the end of three weeks, *Salmonella* populations were 6.62 log in the PL-only, positive control samples, 0.50 log in the 0.5% PB samples, and no *Salmonella* was detected by spread or spiral plating (limit of detection=10 CFU/g of PB/PL) when PL was amended with 1.0% PB.

Significance: These results may provide guidance on amending poultry litter with paper biochar for inactivating *Salmonella*.

P2-102: INFLUENCE OF ENVIRONMENTAL CONDITIONS AND IRRIGATION PRACTICES ON BACTERIAL INDICATORS IN PREHARVEST AGRICULTURAL WATER

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Contamination of fresh produce through preharvest agricultural water remains a food safety concern, as surface water sources are vulnerable to environmental and agricultural contamination.

Purpose: This study evaluated the presence of bacterial indicator organisms in surface-sourced preharvest agricultural water and the influence of environmental conditions and irrigation practices on water quality.

Methods: Over two growing seasons (Nov 2023–Apr 2024; Nov 2024–Apr 2025) preharvest agricultural water was collected at the Desert Research and Extension Center in Holtville, CA. Water was sourced from a surface canal and pumped via a closed system for irrigation. In Year 1, samples were collected from the end of the distribution system supplying fields irrigated by drip or overhead sprinkler. In Year 2, water samples either treated with a

peracetic acid-based sanitizer (8 ppm) or untreated, were collected from drip-irrigated systems. Samples were collected every 2–3 weeks and analyzed for total coliforms and generic *Escherichia coli* using the Colilert-2000® enzyme-substrate method (Standard Method 9223B). Meteorological data was obtained from a weather station located in the research facility.

Results: In Year 1, 176 water samples were analyzed. Detectable levels of generic *E. coli* were observed in 94 samples (53%), and total coliforms were detected in all samples. In Year 2, 36 water samples were analyzed, with detectable *E. coli* levels observed in 5 samples (14%) and total coliforms in 35 samples (97%). No significant differences were observed between untreated and treated water. Higher microbial indicator levels were observed during periods associated with precipitation events, particularly in samples collected from overhead irrigation during Year 1.

Significance: These results emphasize the influence of environmental conditions, particularly precipitation and associated runoff, on preharvest water microbial quality.

P2-103: INOCULATED L. MONOCYTOGENES, SALMONELLA, AND EHEC CONCENTRATIONS SIGNIFICANTLY DECLINED IN CONVENTIONAL AND ORGANIC-CONTROLLED ENVIRONMENTAL AGRICULTURE POND WATER OVER FIVE DAYS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Water can function as a reservoir of foodborne pathogens in water-based controlled environmental agriculture (CEA), which has become a common method of growing fresh produce.

Purpose: To understand the potential survival of foodborne pathogens in CEA pond water and to characterize its bacterial community composition.

Methods: Two conventional and two organic commercial CEA ponds were sampled by collecting three samples per pond (n=12), each sample corresponds to an area within the pond (i.e., seeding, middle, and harvest side). Water from all three pond areas was then combined to create a composite sample (n=4). Each composite sample was subdivided into three subsamples and inoculated at approximately 5×10^4 CFU/mL with a cocktail of either (i) *Listeria monocytogenes*, (ii) *Salmonella,* or (iii) enterohemorrhagic *Escherichia coli* (EHEC). These

subsamples were enumerated after inoculation, and on days 1, 2, and 5. The original 12 samples also underwent 16S rRNA amplicon sequencing.

Results: The concentration of the inoculated bacteria in all composite pond water samples significantly decreased ($p<0.05$) between day 0 and 5, as determined using a mixed effects model. A significant interaction ($p<0.05$) was observed between pond type (organic or conventional) and enumeration day for *Salmonella* and EHEC, which might indicate that pond type impacts the die-off of these organisms. Select taxa were significantly more abundant in either conventional (e.g., *Hyphomicrobium*) or organic (e.g., *Prosthecobacter*) CEA ponds.

Significance: These data indicate that the concentration of foodborne pathogens may decline over time in conventional and organic CEA pond water, dependent on background microflora, suggesting that foodborne pathogen die-off could possibly be validated as a control strategy.

Award Application: Developing Scientist Award

P2-104: LONGITUDINAL ASSESSMENT OF ESCHERICHIA COLI TVS353 CONTROL IN CERTIFIED ORGANIC SOILS USING MUSTARD GREEN COVER CROP BIOFUMIGANTS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Biofumigant cover crops such as mustard (*Brassica juncea)* are commonly used to suppress the growth of plant pathogens due to antimicrobial compounds like glucosinolates and isothiocyanates. However, their impact on foodborne pathogens is unclear.

Purpose: This 2nd-year study replicates prior research by longitudinally evaluating the impact of mustard green biofumigation, with and without plastic mulch, on the short-term survival of *E. coli* TVS353 in organic soils.

Methods: Pacific Gold (PG) mustard was grown as a cover crop in organic plots. Eight plots were planted with PG, and four plots remained as positive controls (n=12). After flowering, 1 x 6 m plots were inoculated with 2L of a rifampicin-resistant *E. coli* culture (8 log CFU/mL).

Plots were mowed and tilled, and four mustard plots were covered with plastic mulch. Three soil samples (10g) were collected from each plot on days 0, 1, 5, 9, 14, and 21, diluted in 90mL BPW, serially diluted, and plated in duplicate onto tryptic soy agar containing 80ppm rifampicin; plates were incubated for 24 h at 37°C for enumeration.

Results: *E. coli* populations on Day 0 ranged from 6.06 log CFU/g to 6.65 log CFU/g. By Day 5, populations increased in mustard plots (1.32 log CFU/g) and plastic mulch plots (0.48 log CFU/g) while decreasing in the positive control (0.98 log CFU/g). Over the 21-day sampling period, *E. coli* populations fluctuated across all treatments, but declined to similar levels (4.89–5.28 log CFU/g) by Day 21. Two-way ANOVA revealed a significant difference in the influence of time and treatment on *E. coli* survival ($p < 0.05$); however, mustard green biofumigation, with or without plastic mulch, did not consistently result in reductions in *E. coli* compared to the control plots throughout the study.

Significance: The use of mustard greens as a biofumigant does not appear to be an effective method of controlling *E. coli* in organic agricultural soils.

Award Application: Developing Scientist Award

P2-105: PERSISTENCE AND TRANSFER OF LISTERIA TO LEAFY GREENS IN HYDROPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Hydroponic systems enable decentralized vegetable production in private and public indoor spaces; however, crops grown in high-traffic environments may increase food safety risks, with *Listeria* posing concern due to its environmental persistence and high mortality rate.

Purpose: This study investigates the persistence and transfer of *Listeria* in indoor hydroponic lettuce production, focusing on two contamination pathways: human contact and nutrient solutions.

Methods: Four hydroponic systems were established: leaf inoculation via human touch, nutrient solution inoculation, combined inoculation, and a negative control. Based on genomic and ecological analyses of *Listeria* species, *L. marthii* was selected as a biosafety surrogate for *L. monocytogenes*. Triplicate lettuce samples and one nutrient solution sample were collected at 0 h, 5 h, 1, 2, 3, 7, 14, and 21 d post-inoculation from each system. *Listeria*

was detected and quantified using a combination of direct plating and enrichment methods. Co-occurring non-*Listeria* colonies observed on selective media, MOX agar, were characterized by Sanger sequencing of the 16S rRNA gene.

Results: *L. marthii* introduced on lettuce via human touch declined to 90 CFU/g by 7 d and was undetectable by 14 d. Inoculation of the nutrient solution did not result in cross-contamination of lettuce; however, transfer from inoculated lettuce to the nutrient solution was detected 5 h post-inoculation. In inoculated solutions, *L. marthii* increased to 100 CFU/mL by 2 d and declined to undetectable levels by 7 d. The bacterium co-occurring with *L. marthii* was identified as *Microbacterium*, which shared similar growth conditions and may influence the persistence of *Listeria*.

Significance: These findings suggest that human activities can sustain *Listeria* on crops for weeks and drive cross-contamination in indoor leafy green production, highlighting the need to limit human touchpoints in decentralized food production to enhance food safety.

Award Application: Undergraduate Student Award

P2-106: PERSISTENCE OF ESCHERICHIA COLI O157:H7, SALMONELLA ENTERICA, AND CRYPTOSPORIDIUM PARVUM IN FIVE SOIL TYPES

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: *Escherichia coli, Salmonella*, and *Cryptosporidium parvum* cause gastrointestinal illness in humans and have been associated with the consumption of contaminated fresh produce.

Purpose: This study aimed to evaluate the persistence of six serovars of *E. coli*, five serovars of *Salmonella*, and the survival and viability of a *Cryptosporidium parvum* oocysts in five different types of soil over 60 days, under controlled conditions.

Methods: Five different soil types were placed in pots (174.62 cm^3 in volume) and maintained an environmental chamber with day/night cycles and temperatures set to Puebla, MX. Soils were watered with purified water every four days or as needed throughout the experiment. Inoculation levels for bacteria were 7.0 $\log_{10}$ CFU/g, and *C. parvum* 10^5 oocysts/ml. Samples were collected at seven different time points (0, 1, 7, 14, 21, 28, and 60 days). Detection limit was 1 $\log_{10}$ CFU/25g.

Results: For statistical analysis, ANOVA and post hoc ($p < 0.05$) were conducted. *E. coli* persisted past day 28, with populations of approximately 4.21 $\log_{10}$ CFU/g across all soil types (NC1, NC2, DEM1, DEM2, and CER). In contrast, *Salmonella* persisted past day 28 only in three soil types (NC2, DEM2, and CER); in the remaining two soils (NC1 and DEM1), cells were not detected by plate counting. For *C. parvum*, oocyst numbers decreased over time; however, when detected, viability was not affected, similar oocyst counts were observed in the other soil types (NC1, NC2, DEM1, and DEM2). In CER soil, no oocysts were observed after day 28.

Significance: Results from this study indicate that over time, soil type influences pathogen persistence in soil and the risk of contamination of the edible parts of fresh produce near the ground.

P2-107: PLASMA-ACTIVATED NANOBUBBLE WATER REDUCED PATHOGEN SURROGATES AND INFLUENCED THE MICROBIAL COMMUNITY OF ROMAINE LETTUCE GROWN IN CONTROLLED ENVIRONMENT HYDROPONIC SYSTEMS

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Presentation type/Format: Technical

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Plasma-activated nanobubble water (PNBW) can reduce pathogens and improve water quality, but its use in controlled environment agriculture (CEA) has not been fully studied.

Purpose: To evaluate the effectiveness of PNBW on the reduction of pathogen surrogates and influence on microbial community in CEA.

Methods: Romaine lettuce was grown in Deep Water Culture (DWC) in a controlled growth chamber at 22°C, 60% relative humidity, growth light (150W, 14 h/24 h) and nutrient solution (pH 6.0 ± 0.2). Surrogate strains, *Escherichia coli* TVS353 and *Listeria innocua*, were mixed as initial inocula (5 log CFU/mL). Nanobubble water-only (NBW) and PNBW was performed for 20 min each day for 5 consecutive days after inoculation. Pulsed cold atmospheric plasma (180V, 90μs pulse, 1kHz rep frequency, 60kHz res frequency) was delivered through nanobubble (air flow rate 5.0 SCFH). Six DWCs with experimental duplicates were performed. *E. coli* and *L. innocua* in DWCs were quantified by selective media. Romaine lettuce was harvested on day 5 and plant health was evaluated. One-way ANOVA with Tukey's HSD test

was used (α = 0.05). Nutrient solution was collected (n = 36) for 16S rDNA amplicon metagenomics.

Results: Initial inocula in control DWCs were 5.00±0.03 log CFU/mL for *E. coli* and 4.79±0.04 log CFU/mL for *L. innocua*. On day 1, *E. coli* and *L. innocua* in NBW were 4.79±0.16 log CFU/mL and 4.48±0.34 log CFU/mL. For PNBW, both strains were below limit of detection (<1 CFU/mL) from day 1 to day 5. Both strains were declining over time in control and NBW. No significant difference was observed for plant health parameters between control, NBW, and PNBW (p > 0.05). Based on 16S, total bacterial levels in all samples decreased over time, and the composition of dominant bacteria shifted noticeably after repeated PNBW.

Significance: This study demonstrated the benefits of PNBW in CEA, showing a significant reduction in pathogen surrogates with no adverse impact on plant health.

P2-108: RECOVERY OF SALMONELLA FROM ARTIFICIALLY INOCULATED SURFACE WATER USING DEAD-END ULTRAFILTRATION METHODS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Dead-End Ultrafiltration (DEUF) is used in regulatory and research applications to concentrate and recover pathogenic bacteria, such as *Salmonella,* from agricultural water. Characterizing pathogen survivability within DEUF filters is essential for accurately detecting viable *Salmonella* in agricultural water.

Purpose: To determine the limit of detection (LOD) of *Salmonella* captured by the DEUF technique using two different pre-enrichment volumes.

Methods: Nipro ultrafilters (n=30) were used to filter 90L of surface water. The filters were then inoculated with *Salmonella* Gaminara GFP (CFSAN024441) at a low (n=20, 27 CFU) and high levels (n=5, 275 CFU), and 5 filters served as negative controls. Filters were stored at 4°C for 4 days to simulate transport and then backflushed following the FDA BAM method. Eluate volumes (100mL or 300mL) were pre-enriched at a 1:1 ratio and processed using the same modified FDA BAM method. LOD was calculated based on the Spearman-Kärber method and

results were compared.

Results: Selective plating for the 300mL enrichment showed a 65% (13/20) recovery rate, whereas the 100mL showed a 35% (7/20) recovery rate. All high-level inoculated filters were positive, and all control filters remained negative. The estimated LOD_{50} for the methods were 42 CFU per 100mL (95% CI: 1.8–969.1) and 18 CFU per 300mL (95% CI: 1.3–241.5) respectively.

Significance: Increasing pre-enrichment volume improves *Salmonella* recovery from DEUF filter eluate, particularly at low contamination levels. This study supports optimization of DEUF-based detection workflows to recover *Salmonella* from agricultural water samples collected during regulatory and research activities.

Award Application: Developing Scientist Award

P2-109: RISK ASSESSMENT OF >1,000 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O157:H7 STRAINS ISOLATED IN A MAJOR AGRICULTURAL REGION IN CENTRAL CALIFORNIA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC), one of the main bacterial causal agents of foodborne illness outbreaks associated with leafy greens, resides in ruminants and spreads into the environment by fecal shedding.

Purpose: To develop implementable field contamination control strategies, comparative genomics were applied to assess pathogenicity and outbreak risk profiles of STEC isolated in Salinas Valley from 1994–2016 and to identify “high-risk” strains and their environmental reservoirs.

Methods: Samples of soil, livestock and wildlife feces, produce, water and watershed sediment were collected. A total of 4,726 STEC isolates were selected for whole genome sequencing using Illumina platforms. Core genome multilocus sequence typing (cgMLST) was determined by cgMLSTFinder and run locally on a super computer at UC Berkeley using the database downloaded from Center for Genomic Epidemiology. The SNP cluster was determined using NCBI Pathogen Detection.

Results: A total of 1,352 isolates were confirmed to be O157:H7 by in silico typing. The majority of O157 strains were isolated from bovine (45%) and water (40%), followed by wildlife, soil, sediment, and produce. The cgMLST analysis revealed 140 cgSTs with the top 20 representing 57% of the total O157 strains. Pathogen Detection analysis placed the O157 strains into 74 SNP clusters. Nearly 60% were assigned to the top five SNP clusters that carried 186, 174, 172, 134, and 112 environmental O157 strains, respectively. SNP cluster PDS000181369 was the largest one that contained 4,802 matched strains at the time of analysis, including those linked to the 2006 spinach-associated outbreak and the 2018 Romaine lettuce-associated outbreak in the U.S.

Significance: Our study characterized the population dynamics of STEC in the Salinas Valley from 2006 to 2016 and identified environmental sources of isolates closely related to several epidemiologically important strains, including REPEXH01 and REPEXH02.

P2-110: SEED SANITIZATION AS A PREHARVEST INTERVENTION TO REDUCE FOODBORNE PATHOGEN CONTAMINATION AND INTERNALIZATION IN FRESH PRODUCE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Recent foodborne illness outbreaks associated with lettuce, cantaloupe, and cucumbers have raised concerns regarding seed-mediated contamination and subsequent internalization of foodborne pathogens into fresh produce.

Purpose: Evaluate the efficacy of commercially available sanitizers in reducing foodborne pathogens on seeds, assess their impact on seed germination, and determine their role in limiting pathogen internalization into plants.

Methods: Lettuce, cantaloupe, and cucumber seeds were artificially inoculated with *Salmonella* Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes* at approximately 7–8 log CFU/g. Inoculated seeds were treated with chlorine-based or peracetic acid sanitizers at multiple concentrations. Treated and untreated control seeds were plated on selective media and incubated under pathogen-specific conditions to enumerate surviving populations. Log reductions were calculated relative to untreated controls. To evaluate germination, treated

seeds were incubated under optimal conditions using MS agar, and germination percentages were recorded. Pathogen internalization was assessed using both in vitro agar-based plant growth systems and greenhouse experiments with sterile potting media. Plant tissues were analyzed for pathogen presence using selective enrichment and plating.

Results: Sanitizer treatments significantly reduced pathogen populations on seeds compared with untreated controls ($p < 0.05$), with reductions ranging from approximately 2 to 7 log CFU, depending on sanitizer type and concentration. Foodborne pathogens were not detected in plant tissues grown from sanitized seeds. Internalization was observed in plants grown from untreated seeds. Sanitizer application did not significantly affect seed germination rates for lettuce, cantaloupe, or cucumber ($p > 0.05$).

Significance: Seed sanitization effectively reduced foodborne pathogen loads and prevented pathogen internalization without compromising seed viability.

P2-111: SHOTGUN METAGENOMICS REVEALS DIFFERENTIAL CECAL MICROBIOME RESPONSES TO PROPIONIBACTERIUM FREUDENREICHII AND SALMONELLA VACCINATION IN NON-CHALLENGED BROILER CHICKENS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The cecal microbiome plays a crucial role in shaping immunity against pathogens.

Purpose: The effects of a dairy-derived probiotic, *Propionibacterium freudenreichii* (PF), and a *Salmonella* vaccine (VC) on the cecal microbiome of healthy chickens remain unknown.

Methods: Cecal contents from 32 six-week-old broiler chickens in 4 groups (n=4 birds/group × 2 experiments) were collected for DNA extraction. Groups were: Negative Control (NC; no supplementation), PF (with PF), VC (vaccination), and PV (PF+VC). Birds in PF and PV were given 7 $\log_{10}$ CFU/mL PF via drinking water from day 1 throughout the study. Birds in VC and PV were vaccinated with an *S.* Typhimurium vaccine. Shotgun metagenomic sequencing was performed on the NovaSeq X+ platform. The sequences were analyzed using the fastp-AMR++ pipeline, with Kraken2 for taxonomic analysis and MEGARes for the resistome database,

respectively. Further analysis was performed in R using the phyloseq, microbiome, and vegan packages. The statistical significance in α- and β- diversities was determined using the pairwise Wilcoxon test and PERMANOVA, respectively (*P*.adj <0.05). Differential abundance of species and s AMR genes between groups was determined using ANCOMBC-2 (q-value < 0.05, $|\log_2 fc| > 1$).

Results: A significant difference in β diversity was observed between the groups. Compared to NC, the abundance of *Weissella paramesenteroides* and *Lactococcus raffinolactis* was higher in PF. The abundance of *Lactobacillus johnsonii* and *Limosilactobacillus oris* was higher in PV. The abundance of *Ligilactobacillus animalis* was lower in PF, VC, and PV. AMR genes of classes tetracyclines, aminoglycosides, and MLS were present in all groups. *tet*W, *tet*O, *tet*40, *ant*6, aph3-dprime were the most abundant AMR genes.

Significance: The study indicates that the PF and the *Salmonella* vaccine improve microbiome health in broiler chickens by increasing the abundance of mutually dependent beneficial bacteria, improving food safety.

P2-112: STAGE-SPECIFIC EFFICACY OF CINNAMOMUM VERUM AND SYZYGIUM AROMATICUM ESSENTIAL OILS AGAINST HAEMONCHUS CONTORTUS EGGS AND LARVAE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Gastrointestinal nematodes in livestock compromise animal health and necessitate the use of synthetic drugs, which can result in chemical residues in the food chain. Effective pre-harvest food safety control requires inhibiting the parasite in both its infective and pre-infective stages, thereby preventing environmental contamination.

Purpose: Selected from a preliminary screening of botanical extracts, this study evaluated the ovicidal and larvicidal activities of cinnamon (*Cinnamomum verum*) and clove (*Syzygium aromaticum*) essential oils to determine their potential as a natural, multi-stage biological control strategy compatible with food safety standards.

Methods: Egg hatch assays (EHA) and larval mortality assays (LMA) were performed using *H. contortus* isolates. The oils were tested at concentrations ranging from 0.04 to 50 mg/mL. Probit analysis (SAS 9.0) was used to determine the lethal concentrations (LC50) and 95% confidence intervals.

Results: Both oils demonstrated significant antiparasitic activity against each biological stage ($P < 0.05$). In the LMA, *Cinnamomum verum* was the most effective, achieving an LC50 of 0.03 mg/mL (95% CI: 0.02–0.03). In contrast, *Syzygium aromaticum* demonstrated superior ovicidal efficacy in the EHA, achieving an LC50 of 0.008 mg/mL (95% CI: 0.005–0.010) and inhibiting hatching at trace concentrations.

Significance: This study highlights the stage-specific efficacy of *C. verum* and *S. aromaticum*. By targeting distinct developmental stages, these oils offer a comprehensive control strategy. This natural approach supports sustainable pre-harvest interventions, reducing dependence on synthetic anthelmintics and mitigating the risk of chemical residues in animal-derived foods.

P2-113: SURVIVAL AND PERSISTENCE OF ESCHERICHIA COLI AND LISTERIA INNOCUA IN CO-PLANTING DWC AND NFT HYDROPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Hydroponics is a soilless farming system that vegetable growers have widely adopted, as it is believed to be free of microbial contamination. However, microbial pathogens may thrive in these systems due to constant moisture, nutrient availability, and the presence of organic matter from root exudates.

Purpose: This study examined the persistence of *E. coli* ATCC 25922 and *Listeria innocua* ATCC 33090 in co-planting Deep Water Culture (DWC) and Nutrient Film Technique (NFT) hydroponic systems.

Methods: *E. coli* ATCC 25922 (5 log CFU/mL) and *Listeria innocua* (4 log CFU/mL) were inoculated into lettuce (*Lactuca sativa*), sweet mint (*Mentha sauvis*), and sweet basil (*Ocimum basilicum*) co-planting hydroponics systems (n=12), consisting of six DWC and six NFT systems. Nutrient solutions from both systems were sampled and assayed for microbial survival, along with changes in pH for 7-day periods. The root samples were collected to

assess differences in microbial community structure among the treatments. *E. coli* and *Listeria* levels were determined using the Compact Dry method. Tukey's honest significant test was used to determine a significant difference ($P<0.05$).

Results: *E. coli* and *Listeria* reduction was significant ($p<0.05$) after 3 days of post-inoculation in all the co-planting treatments in both DWC and NFT systems**.** *E. coli* levels in DWC decreased from 5.0 log CFU/mL to as low as 0.65 log CFU/mL on day 3. The change in *E. coli* population was variable between the treatments, with the highest persistence of 2.15 log CFU/mL and the lowest persistence of 0.1 log CFU/mL on day 7 post-inoculation. The lowest and highest persistence of *L. innocua* were below the detectable limit and 1 log CFU/mL, respectively. The reduction rate of *E. coli* and *Listeria* was significantly higher in NFT compared to DWC systems.

Significance: This study highlights the potential benefits of companion planting strategies in reducing food safety risks in hydroponic systems.

Award Application: Developing Scientist Award

P2-114: SURVIVAL OF ESCHERICHIA COLI IN INOCULATED COW MANURE-AMENDED SOIL AND ITS TRANSFER TO GROWING STRAWBERRIES: A FIELD STUDY

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Application of livestock manure can improve soil fertility, but improper use, such as raw or undertreated manure, may introduce foodborne pathogens to fresh produce.

Purpose: This study evaluated the persistence of rifampin-resistant *E. coli* in cow manure-amended soil and the microbiological quality of strawberries cultivated in the same soil.

Methods: Strawberry bare-root plants were grown in three soil treatments: soil (T1), soil + composted cow manure (T2; soil:manure = 2:1, w/w), and soil + composted cow manure + rifampin-resistant *E. coli* (T3; strains TVS 353, 354, 355; 8.22 log CFU/g; soil:manure = 2:1, w/w). Nine soil samples from T3 were collected at weeks 0, 1, 5, 8, 12, 16, 20, and 24 after planting. Samples were homogenized in twice their volume of 0.1% peptone water for 1 min, serially diluted, and 100 µL plated in duplicate on tryptic soy agar with rifampin (50 µg/mL). Two strawberries per sampling bag were harvested for enumeration of indicator organisms and detection of foodborne pathogens using FDA BAM methods. Three replicates were included per treatment. Data were analyzed by ANOVA followed by Tukey’s HSD test (α = 0.05).

Results: Aerobic Plate Count (3.86–3.93 log CFU/g), Total Coliform (3.34–4.00 log CFU/g), and yeast/mold (8.57–8.69 log CFU/g) in strawberries did not differ among treatments ($P > 0.05$). No strawberry samples tested positive for rifampin-resistant *E. coli*, generic *E. coli*, *Salmonella*, *Listeria monocytogenes*, or *E. coli* O157:H7. Rifampin-resistant *E. coli* in soil declined from 8.22 log CFU/g at week 0 to 1.51 log CFU/g by week 24, with a plateau from weeks 5–12 ($P > 0.05$). By week 24, the total reduction of 6.71 log CFU/g was significant compared to all other time points ($P < 0.05$).

Significance: This work provides a microbial baseline for understanding how improper manure application affects strawberry quality and soil.

P2-115: SURVIVAL OF SPRAY-APPLIED PATHOGEN SURROGATE ESCHERICHIA COLI ON LEMON FRUIT IN FIELD

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Produce-growing operations are required to conduct Agricultural Water Assessments to comply with the FDA FSMA Produce Safety Rule Subpart E. When food safety hazards associated with pre-harvest agricultural water are identified, growers may need to implement mitigation measures, including increasing the interval between final water application and harvest.

Purpose: To evaluate the survival of green fluorescent protein-tagged *E. coli* TVS353 on lemon fruit following contaminated spray application in Arizona.

Methods: Lemon trees were directly sprayed for 3 sec with *E. coli* contaminated water (~4 log CFU/mL) and sampled (N=115) up to 24h in October. Post-harvest, fruits were washed with 20mL of 1X phosphate-buffered saline (PBS), serially diluted, and spread plated onto tryptic soy agar with rifampicin (TSA-R), then incubated at 37 ± 2°C for 24h. Remaining wash and whole fruits were enriched with 200mL of tryptic soy broth with rifampicin (TSB-R), and after

incubation (24h, 37 ± 2°C), streaked onto TSA-R. To lower the limit of detection, additional fruits were washed with 100mL of 1X PBS and analyzed using the IDEXX Colilert protocol. Detection of *E. coli* was confirmed by fluorescence under UV light. All analyses were conducted in RStudio (V.4.2.3).

Results: Immediately after spray application (0h), mean *E. coli* concentrations on lemons were 3.23 ± 0.79 log CFU/fruit. By 1h, all samples were below the limit of detection via spread plating (<20 CFU/fruit) and IDEXX processing (<1 CFU/fruit) and remained non-detectable at subsequent timepoints (2-, 3-, and 24-h). Following enrichment, 19% (8/42) were positive at 1- and 2-h; however, no samples were positive at 3- and 24-h, 0% (0/33).

Significance: On lemon fruits, *E. coli* populations declined rapidly following contaminated spray application, becoming non-detectable by 3h. Compared to other commodities grown in Arizona (e.g., romaine lettuce), bacterial survival is strongly influenced by crop-specific factors.

Award Application: Developing Scientist Award

P2-116: SURVIVAL OF ESCHERICHIA COLI OUTBREAK STRAINS ON GREENHOUSE-CULTIVATED TOMATO, SPINACH, AND ROMAINE LETTUCE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: When food safety hazards are identified in the FDA FSMA Produce Safety Rule Agricultural Water Assessments (AGWAs), mitigation measures may be necessary, including extending the final water application and harvest interval; however, pathogen survival data on produce remains limited.

Purpose: To evaluate the survival of *E. coli* TVS353, O157:H7 REPEXH01, and O157:H7 TW14359 inoculated onto tomato fruits, spinach leaves, and romaine lettuce.

Methods: All commodities were cultivated in pots under greenhouse conditions in Arizona and spot-inoculated individually with each *E. coli* strain per fruit or leaf. Tomato fruits (N = 51), spinach leaves (N = 122), and romaine leaves (N = 60) were collected at 0, 1, 2, 3, 5, and 7 d post-inoculation, with an additional sampling of leafy greens at 10 d. For each sampling event, one tomato, two spinach leaves, or one romaine leaf was collected per sample bag. Leafy greens were homogenized using a stomacher, while tomatoes were washed with

buffered peptone water. Resulting suspensions were serially diluted in phosphate-buffered saline and enumerated on eosin methylene blue agar using the spread plating method. All statistical analyses were performed in RStudio (V.4.2.3).

Results: Bacterial strain did not significantly affect *E. coli* survival across commodities ($p > 0.60$). By 1 d, >3 log CFU/produce mean reduction was achieved on tomato and romaine, and by 2 d on spinach. At the final sampling time point (7 or 10 d), mean reductions were 4.95 ± 1.07, 4.39 ± 0.61, and 4.39 ± 0.43 on tomato fruits, spinach leaves, and romaine leaves, respectively. Reductions over time were significantly greater on tomatoes than on spinach or romaine lettuce ($p < 0.01$).

Significance: Although bacterial populations remained detectable at the final time point, significant reductions were observed within the first two days post-inoculation, providing scientifically valid pathogen die-off data to inform Produce Safety Rule decision-making.

Award Application: Developing Scientist Award

P2-117: SURVIVAL OF SALMONELLA ENTERICA, LISTERIA MONOCYTOGENES, AND GENERIC ESCHERICHIA COLI IN NUTRIENT SOLUTIONS USED IN HYDROPONIC SYSTEMS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Nutrient solutions (NS) are widely used in indoor agriculture to support crop growth, but the impact of recirculating NS on the microbial safety of fresh produce remains largely uninvestigated.

Purpose: This study aimed to investigate the behavior of common foodborne pathogens—*Salmonella enterica* (Sal) and *Listeria monocytogenes* (LM)—as well as generic *Escherichia coli* (EC) in fresh and used NS.

Methods: Freshly prepared NS or used NS collected from two commercial hydroponic facilities (F1 and F2) were inoculated with a three-strain rifampin (rif)-resistant Sal cocktail at high (~7 log CFU/mL) or low (~4 log CFU/mL) levels, a three-strain rif-resistant LM cocktail, or a single EC strain at high levels. Inoculated NS was aliquoted into 2-mL enclosed tubes and stored at 25°C for 21 days. Samples were collected on days 0, 1, 3, 5, 7, 14, and 21 for

microbial analysis. Surviving microorganisms were enumerated on trypticase soy agar supplemented with 50 µg/mL rifampin (TSAR) or selective media (Xylose Lysine Tergitol-4 agar for Sal, Modified Oxford agar for LM, and MacConkey agar for EC) supplemented with rifampin. Two biological trials were conducted, with three replicates per sampling point (n = 6). Data were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparison test ($\alpha = 0.05$).

Results: The electrical conductivity of used NS was 1.83 mS/cm for F1 and 2.50 mS/cm for F2, and total aerobic counts were 3.26 and 3.20 log CFU/mL for F1 and F2, respectively. Sal and EC in fresh NS showed <1-log CFU/mL reductions, whereas both decreased by ~4–5 logs in used NS over 21 days, regardless of NS source. LM decreased by <1 log in fresh F1 NS and ~3 logs in used F1 NS, and by ~3 logs in fresh F2 NS and ~6 logs in used F2 NS. Low-level Sal trials showed similar decline trends to high-level trials.

Significance: This study reported the behavior of foodborne pathogens and EC in fresh and used NS, with better microbial survival observed in fresh NS.

P2-118: SURVIVAL OF SALMONELLA SPP. IN SOILS AMENDED WITH PECAN SHELLS AND POULTRY MANURE

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Poultry manure is often used as a soil amendment but can promote pathogen survival, increasing contamination risk. Pecan shell is a waste product with antimicrobial properties with potential for use as a soil amendment.

Purpose: Compare *Salmonella* die-off the die-off of *Salmonella* in inoculated ground pecan shells and poultry manure, and then to evaluate its survival in soils amended with pecan shells, poultry manure, or a mixture of both pecan shell and poultry manure, compared with unamended soil.

Methods: Ground pecan shell and poultry manure were inoculated (~9 log CFU/mL) with a five strain rifampicin-resistant *Salmonella* cocktail. Samples were enumerated at days 0, 3, 7, 10, 14, 21, and 28. Soil treatments (unamended, pecan shell amended, poultry manure amended, and mixed) were inoculated with *Salmonella* and enumerated at days 0,1, 3, 7, 10, 14, 21, 28, 42, and 56. All trials were performed in triplicate with 3 biological replicates (n=9). Data was evaluated using GLM and Tukey HSD (p□0.05). Die-off models were generated using GInaFit.

Results: Die-off was more rapid in poultry manure, showing significant reductions at each time point. Populations were significantly different between both substrates with higher reduction in manure compared to shell by day 14 (5.28 and 1.70 log CFU/g) onwards. Manure and shell data best fit to a Weibull model with shape parameter (*p)* values of 1.63 ± 0.12 and 0.39 ± 0.02. In the soil treatments, initial die-off was higher in shell amended soil, however by day 56 the most significant reduction was in unamended soil, followed by shell, mixed, and manure amended soils respectively (5.09, 3.94, 2.85, and 2.81 log CFU/g). Soil amendment data was best fit by a biphasic model.

Significance: Pecan shell as a soil amendment may reduce the ability of *Salmonella* to survive in soil compared to poultry manure; however, shell may also provide a protective environment for pathogens when unincorporated into other substrates.

P2-119: EFFECTIVENESS OF HOUSEHOLD WASHING PROCEDURES WITH SANITIZERS FOR REDUCING SALMONELLA ENTERICA SEROVAR NEWPORT ON FRESH PRODUCE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: *Salmonella enterica* outbreaks are frequently linked to the consumption of fresh produce. Strategies to reduce microbial load before consumption at home are crucial for mitigating health risks to consumers.

Purpose: Determine the effectiveness of washing procedures using hot water and free available chlorine in the reduction of *Salmonella enterica* on tomatoes and cucumbers.

Methods: Seventy cucumber (*Cucumis sativus*) and seventy tomato (*Solanum lycopersicum*) samples were used for the experiment. Pieces of fresh produce (3.5x5 cm^2) were spot inoculated with 10^8 CFU/mL of *Salmonella* Newport, dried for 2 h, and stored overnight under refrigeration (4°C). Each sample was washed by immersion in 300 mL of hot water (HW: 60°C), free available chlorine (FAC: 25 ppm), and water treatment control (WTC) at four contact times (5, 15, 30, and 60 s). A no-treatment control (NTC) was used to determine microbial reduction. After washing and rinsing with water at room temperature, samples were homogenized, and microbial density was enumerated by plating on XLD agar. Data was analyzed using Welch's test due to unequal variances, and post hoc comparisons were performed using Games-Howell.

Results: Hot water (HW) and free available chlorine (FAC) reduced *Salmonella* on tomatoes by up to 1.48 and 1.56 log CFU/g, respectively, with HW at 15 s and FAC at 5 s being the most effective treatments compared with NTC ($p<0.0001$). In cucumbers, HW applied for 60 s achieved a 2.37 log CFU/g reduction and was more effective than FAC at the same contact time ($p<0.0035$), whereas no difference was observed between treatments at 5 s. Overall, HW and FAC did not differ from the WTC in cucumbers, whereas both treatments significantly reduced bacterial loads in tomatoes at 30 and 60 s.

Significance: These findings shed light on household practices that may help reduce pathogenic bacteria on fresh produce immediately before consumption, thereby decreasing the risk of foodborne illness.

P2-120: TRANSFERENCE OF SALMONELLA TYPHIMURIUM TO TOMATOES (SOLANUM LYCOPERSICUM 'MICRO-TOM') USING DIFFERENT CONTAMINATION ROUTES WITH POULTRY MANURE-AMENDED SOIL

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Foodborne illness outbreaks are reported annually, with many associated with the consumption of fresh produce, including tomatoes. Tomatoes are particularly susceptible to contamination from multiple sources, such as irrigation water, soil, wild-animal feces, and the use of manure as a fertilizer. Preharvest interventions are crucial for controlling pathogenic bacteria, prompting research into the effects of agricultural practices that lead to the contamination of fresh produce with foodborne pathogens.

Methods: Two hundred twenty pots with amended soil (poultry manure: 80, commercial fertilizer: 80, control: 60) were used to grow seeds and seedlings of tomato var. Micro-Tom under greenhouse conditions. Inoculated amended soil was used to assess the transference of *Salmonella* Typhimurium to tomatoes over a 70-day post-inoculation period. Analyses were conducted at eight time points (0, 7, 14, 21, 28, 42, 56, and 70 days post-inoculation) to determine *Salmonella* persistence in soil for up to 70 days. Data were analyzed using one-way ANOVA (p<0.05).

Results: Poultry manure led to significantly lower bacteria survival in soil compared to commercial fertilizer at multiple time points during analysis.
Although *Salmonella* Typhimurium was found in soil throughout the 70-days period, no bacterial presence was detected in tomatoes. Contamination at different stages of tomato plant growth did not increase the likelihood of *Salmonella* internalization in the fruit.

Significance: These findings provide insights into controllable agricultural practices that may lead to the reduction of human pathogenic bacteria on fresh produce.

P2-121: WHOLE-GENOME SEQUENCING ANALYSIS OF ANTIBIOTIC RESISTANCE AND VIRULENCE IN SALMONELLA ENTERICA ISOLATED FROM MID-ATLANTIC CREEK SEDIMENTS

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: Water-overlying sediments in agricultural watersheds serve as critical reservoirs for *Salmonella enterica*, posing a contamination risk to fresh produce via irrigation.

Purpose: This study utilized whole-genome sequencing (WGS) to characterize serotype diversity, virulence profiles, and antimicrobial resistance (AMR) of *Salmonella* isolates from Mid-Atlantic creek sediments.

Methods: Sixteen *Salmonella* isolates obtained from Conococheague Creek, PA (2019–2022) underwent WGS on an Illumina NovaSeq platform followed by de novo assembly. Bioinformatic analysis (SeqSero2, SPIFinder, ResFinder, PlasmidFinder, and CRISPRCasFinder) was utilized to characterize serotypes, pathogenicity islands (SPIs), AMR genes/mutations, plasmids, and CRISPR/Cas loci.

Results: Eight distinct serotypes were identified: Newport was most prevalent (43.8%), followed by Give and Braenderup. All isolates harbored 9 to 11 SPIs; Newport isolates uniquely carried a consistent 10-SPI profile and higher SPI-2 gene counts than other serotypes. Regarding AMR, the aminoglycoside resistance gene *aac(6')-Iaa* was detected in

100% of isolates, and 87.5% (14/16) carried a *parC* (T57S) chromosomal mutation associated with quinolone resistance. Six plasmid types were identified in 10 of 16 isolates (62.5%), including IncFII and IncI2, associated with virulence and beta-lactamase dissemination. Diverse Type I–IV restriction-modification systems and complex CRISPR/Cas loci were identified, reflecting high genomic plasticity.

Significance: These high-resolution genomic insights reveal that environmental *Salmonella* in agricultural watersheds harbor significant virulence and emerging resistance potential, necessitating targeted water quality monitoring to prevent foodborne illness.

P2-122: DEVELOPING, OPTIMIZING, AND COMMERCIALIZING A SILVER ION-BASED WASH PROCESS FOR WHOLE CANTALOUPE USING A CLOTH-BASED AGGREGATED SAMPLING TOOL

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Building on success with silver ions for leafy greens, this study assessed their effectiveness for washing whole cantaloupe—a fruit frequently linked to outbreaks due to its netted surface harboring pathogens. Previous USDA methods like steam and nisin have not seen industry adoption.

Purpose: Characterize the key operating parameters and evaluate the effectiveness at commercial scale of a silver ion-based wash process (SmartWash® Fruit Boost™) for washing the exterior of cantaloupes**.**

Methods: Using cloth-based aggregated sampling (MicroTally® Mitt samplers) to reduce sample count and facilitate microbial assessments, pilot plant experiments were done to characterize the key operating parameters for a silver ion wash process for cantaloup. These parameters were used to design and install a wash line in a commercial fresh-cut facility for a verification trial.

Results: Experiments indicate that a 30 second residence time at 25-30 ppm silver ion

concentration in a system that provides light protection and ensures complete coverage without wasting chemistry can deliver significant lethality (3+ log for inoculated generic *E. coli* and ~1.5 log for lactic acid bacteria (LAB), a natural more resistant surrogate for in plant verification). During commercial operation over several months on 21 sampling days, 61 pairs of pre and post process samples of 5 melons each were non-destructively sampled with mitt samplers to measure the LAB recovery. These results confirmed the expected lethality of LAB under commercial conditions. The results also indicate that simple pair wise comparison of pre- and post-process LAB is not a suitable metric for process verification due to the natural variation.

Significance:

Silver ion washing can help control cantaloupe pathogens.
Cloth-based aggregated sampling improves supply chain microbial assessments.
LAB is a valid process indicator if sufficient result aggregation addresses variability.

P2-123: ASSESSING THE ENHANCED SAMPLING POWER OF A PENETRATIVE SAMPLING DEVICE, MICROTALLY® MICROBROOM™ SAMPLER, OVER CONVENTIONAL TISSUE SAMPLING FOR PREHARVEST MICROBIAL ASSESSMENT OF ROMAINE LETTUCE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Romaine lettuce has been identified as causative in many fresh-cut produce related outbreaks. Pre-harvest testing data indicates that conventional tissue sampling may underreport pathogen presence. It is speculated that solarization is an important mitigation which does not impact the interior of the head. Penetrative sampling can potentially overcome this limitation.

Purpose: To compare a penetrative cloth-based aggregative sampling to conventional composite tissue grabs for microbial assessment of Romaine lettuce given the expected absence of pathogens.

Methods: During the regular pre-harvest assessment, 88 penetrative aggregative samples where 60 heads were spiked and sampled with the cloth sampler in parallel to 88 150g tissue

composites where 60 2.5g specimens were collected. Samples were enriched and screened for pathogens (*stx* and *invA*). Purified DNA was assayed for 6 rare markers (*tetA*, *tetB*, *ybbW*, *phoE*, *EB-set1*, and *fliC*) similar to those used for validating cloth sampling of protein foods. The markers are loosely associated with pathogens making suitable surrogates for the extremely rare pathogens to examine sampling efficacy. Prevalences, tissue versus cloth, for each marker were compared with Fisher's exact test for difference and PSI correlation coefficients were examined pairwise to assess correlation.

Results: No pathogens were detected as expected in this number of samples. Prevalence for the markers reached up to 100%. Cloth sampling did as good or better for all markers with no significant differences (*P's* > 0.2). The results for *fliC* and *tetA* showed significant positive correlations (*P's* < 0.05)

Significance: These results suggest that sampling the interior of Romaine lettuce would better detect pathogens than conventional tissue grabs. The MicroTally® MicroBroom™ sampler is on approach to sampling the interior of Romaine lettuce.

P2-124: CLOTH-BASED FINISHED PRODUCT SAMPLING OF FRESH-CUT PRODUCE WITH MICROTALLY® MICROCAP™ SAMPLERS COMPARED TO CONVENTIONAL TISSUE GRABS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Fresh-cut produce is often linked to outbreaks. Many customers require finished product microbial testing before packaging, typically by hand-collecting specimens every minute for an hour to create a 150g structured sample. This method approximates randomness but does not target the product surface, where contamination is most likely, so any contaminants may be diluted by internal tissue.

Objective: Compare cloth-based aggregated sampling to conventional tissue composites for 5 fresh-cut products at commercial scale given the extremely low prevalence of pathogens.

Methods: During commercial operation, extra tissue samples were collected for five fresh-cut items (diced carrots, diced tomatoes, cut onions, diced peppers, and cut Romaine lettuce) alongside routine pathogen sampling. At the same time, a cloth sampler was installed on the weigh scale's distribution cone. A total of 473 sample pairs were enriched and tested for six markers that are loosely linked to pathogens (*tetA*, *tetB*, *phoE*, *ybbw*, Entero-set 1, and *fliC*), as

no actual pathogen detections were expected. Prevalence for each item-marker pair were compared using the Fisher's exact test for difference, and pair-wise correlations were analyzed with the PSI Correlation Coefficient.

Results: Among five commodities, only diced carrots had significantly different prevalence with higher detection of *tetA* and *tetB* with cloth-based sampling (P=0.05 and P=0.03, respectively). Overall, cloth-based sampling was as effective as, or better than, tissue grabs. Positive correlations appeared in some cases, while other correlations may reflect random detection patterns.

Significance: These findings show that processors can use cloth-based aggregated sampling with the MicroTally® MicroCap™ sampler to save time and costs without affecting results. With its continuous approach and complete coverage, this method provides a random sample.

P2-125: 254 NM UV-C LED DISINFECTION OF DRIED PRODUCE: MICROBIAL INACTIVATION PERFORMANCE AND QUALITY IMPACTS FOR SMALL-SCALE PROCESS ADOPTION

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Small-scale dried produce manufacturers often lack cost-efficient, validated disinfection options that reduce microbial risks without compromising product quality. 254 nm UV-C LED is a practical nonthermal surface intervention, but its performance on irregular, low-moisture produce and its effects on shelf-life quality require product-specific validation.

Purpose: To evaluate the microbial inactivation and quality impacts of 254 nm UV-C LED on dried produce relevant to small-scale processing operations.

Methods: Representative Indiana-produced dried produce are obtained from small processors and inoculated with pathogenic *Salmonella* Typhimurium, *Listeria monocytogenes*, *E. coli* O157:H7, and *Staphylococcus aureus* targeting ~7 log CFU/sample. Samples are treated in a 254 nm UV-C LED chamber at multiple lamp intensities and exposure times(30s–5 min). Survivors are recovered and enumerated by plate counting on selective media. Product quality (a_w, moisture, pH, and color/ΔE) is measured pre/post treatment. Effect of treatments on shelf life is also measured for all samples. Biological triplicates and technical duplicates of treatments are performed, and differences analyzed using ANOVA (α=0.05). Inactivation kinetics are quantified with the Weibull model.

Results: Efficacy was matrix-dependent: smoother surfaces achieved higher log reductions

faster than textured surfaces. Between 4.1–6.2-log reductions are achievable among all samples within 5min of continuous UV-C LED treatments, with good fit with Weibull models across treatments (R^2>0.95). Quality screening suggests minimal changes in a_w and pH, with small, dose-dependent color shifts at highest exposures. Shelf-life data shows significant quality deterioration difference (p<0.05) between treated and untreated controls.

Significance: This work delivers practical implementation guidelines of UV-C LED for small-scale dried produce manufacturers to improve safety and shelf life with minimal quality impact.

P2-126: A CCP-BASED ASSESSMENT OF MYCOTOXIN RISK IN MEDICINAL FOOD MATERIALS

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Category: Produce

Abstract:

Introduction: Inadequate post-harvest handling of medicinal herbs can promote fungal growth and mycotoxin formation, posing food safety risks.

Purpose: To evaluate post-harvest steps associated with Ochratoxin A (OTA) contamination during production of *Angelicae Gigantis Radix* (AGR) to identify practical control measures for mycotoxin risk reduction.

Methods: Sixty AGR samples were collected from small-scale farms using either conventional handling or an official guideline-based protocol across field-drying, washing, and thermal drying stages. OTA was quantified by validated HPLC with fluorescence detection (limit of detection, 0.122 μg/kg; limit of quantification, 0.485 μg/kg; recovery, 89.1–94.4%). Drying load size, duration, and inner versus outer dryer positions were compared. Statistical significance was assessed using Student's *t*-test and ANOVA with Newman–Keuls post hoc analysis.

Results: Most pre-harvest AGR samples contained detectable OTA, but levels remained below the European Union guidance range for medicinal herbs and spices (15–80 μg/kg). Field-dried AGR showed significantly higher fungal contamination and OTA levels than thermally dried AGR. Moisture content after thermal drying was 10.09–13.72% versus 11.11–17.47% after field-drying. Washing did not significantly reduce OTA in final dried products.

Approximately 50% of official protocol-based products exceeded 80 μg/kg OTA. Under overloaded thermal drying, fungal counts and OTA increased, and inner dryer portions contained approximately twofold higher OTA concentrations than outer portions. Ochratoxigenic fungi were detected under inadequate drying conditions.

Significance: Avoiding field-drying and maintaining appropriate thermal drying loads are critical, low-cost interventions to reduce mycotoxin risk in herbal materials and improve food safety in small-scale production systems. Supported by NRF grants funded by the Ministry of Education (RS-2018-NR035639, RS-2023-00240942, RS-2024-00462450).

P2-127: ASSESSING THE REACH AND USE OF OFRR SURVEY DATA AMONG PRODUCE SAFETY EDUCATORS AND REGULATORS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Since 2018, data from the On-Farm Readiness Review (OFRR) post-visit surveys has been shared with produce safety educators and regulators to inform educational and regulatory responses. In late 2025, a follow-up survey was conducted to evaluate the reach and use of this data.

Purpose: This study aimed to assess how OFRR survey data influences educational and regulatory activities nationwide and to identify opportunities to increase the impact of future data-sharing efforts.

Methods: An anonymous online survey was administered via Qualtrics™ (December 2025–January 2026) to educators and regulators who completed the NASDA OFRR training or who participate in the FSMA PSR regional centers. Responses were analyzed using descriptive statistics to evaluate data utilization and its impact on state-level produce safety programs.

Results: Respondents (n=33) represented all four FSMA PSR regional centers, primarily comprising State Department of Agriculture staff (63%, n=17) and Extension faculty or University researchers (36%, n=12). The most valuable data points identified were "top three Walk Around Questions (WAQ) areas requiring improvement" (70%, n=23), "specific issues within those WAQ areas" (67%, n=22), and "farm-specific needs" (55%, n=18). 52% (n=17) of respondents indicated that they used the OFRR data to inform the content or focus of their educational programming. OFRR reported data most significantly impacted the development of hands-on training (42%, n=14), PowerPoint presentations (39%, n=13), and 24% (n=8) webinars offered. Notably, multiple respondents requested that inspectional data be shared in a similar format to better align resources with farm compliance needs.

Significance: Post-OFRR data collection provides important insights for both regulatory and educational sectors, directly shaping the resources developed to assist farmers with FSMA PSR compliance.

P2-128: ASSESSMENT OF CONSUMER-DRIVEN SALMONELLA ENTERICA CROSS-CONTAMINATION FROM AVOCADO PEEL TO PULP AND KNIFE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Avocados can be contaminated with *Salmonella enterica* during cultivation, post-harvest handling, or domestic preparation.

Purpose: This study aimed to characterize consumer avocado handling habits and evaluate exposure to *S. enterica* through a domestic cross-contamination scenario (CCS).

Methods: Consumer-handling behavior was characterized by an online survey. A domestic cross-contamination scenario involving *S. enterica* transfer from avocado peel to pulp via a knife was experimentally evaluated by simulating cutting. Four *S. enterica* strains (two isolated from chicken, one from avocado, and ATCC 14028) were inoculated (100 µL) onto a 1 cm^2 area of avocado peel and allowed to dry for 2 h to simulate bacterial attachment. Cutting was performed using a sterile scalpel to simulate a knife, and bacterial transfer to the blade and pulp was quantified. A Kruskal–Wallis's test was used to evaluate the effect of strain on transfer rates.

Results: Between 2.3% and 15.2% of respondents reported never washing or disinfecting cutting boards and knives after handling raw meat or avocado, while higher proportions

(11.3–21.2%) reported the same behavior when cutting other vegetables. Attachment to avocado surfaces ranged from 0.22% to 6.62%, showing significant differences among strains, with avocado isolate exhibiting the lowest adhesion capacity. Transfer rates to knives (0.8–13.9%) and pulp (3.9–9.5%) showed no significant differences, likely due to high variability.

Significance: Despite the absence of strain-dependent differences, the observed transfer rates underscore the importance of cross-contamination during domestic handling.

P2-129: ASSESSMENT OF THE ATTACHMENT STRENGTH OF SALMONELLA ENTERICA ON THE SURFACE OF VARIOUS TOMATOES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: *Salmonella enterica* is among the top 5 foodborne pathogens that contribute to the number of recorded cases, hospitalizations, and deaths. Salad vegetables, such as tomatoes, are prone to *Salmonella* outbreaks due to the lack of a “kill-step”. Published studies on the attachment strength of *Salmonella* on the surface of various types of tomatoes are limited.

Purpose: To assess the attachment strength of *Salmonella* on the surface of Roma tomatoes and tomatoes-on-the-vine.

Methods: The exocarp of tomatoes was cut into 3x3 cm squares, washed, and exposed to UV light. Then, these samples were placed in petridishes with overnight *Salmonella* culture, skin side facing down. The inoculated samples were transferred into centrifuge tubes containing phosphate buffered saline and vortexed for 10 sec to recover loosely-attached *Salmonella*. The remaining strongly-attached *Salmonella* on tomato surface were recovered by stomaching for 2 min. Both loosely and strongly-attached *Salmonella* were plated on Xylose Lysine Deoxycholate Agar for enumeration. The attachment strength was calculated using: S_R

= [strongly-attached *Salmonella* recovered after stomaching/(strongly-attached *Salmonella* recovered after stomaching+ loosely-attached *Salmonella* recovered after vortexing)]. The background microbiota on unwashed tomatoes was enumerated via aerobic plate count.

Results: From Roma tomatoes, 6.11±0.11 log CFU/g of loosely-attached and 6.67±0.14 log CFU/g of strongly-attached *Salmonella* were recovered. From tomatoes-on-the-vine, 6.51±0.32 log CFU/g of loosely-attached and 6.57±0.37 log CFU/g of strongly-attached *Salmonella* was recovered. Overall, the attachment strength of *Salmonella* was higher on Roma tomatoes (S_R = 0.79) than on tomatoes-on-the-vine (S_R = 0.54). Roma tomatoes had APCs of 6.1±0.07 log CFU/g, and tomatoes-on the vine had 3.1±0.1 log CFU/g.

Significance: The results can be used to determine if one variety could be more risky for *Salmonella* contamination than others.

Award Application: Developing Scientist Award

P2-130: BIOCONTROL POTENTIAL OF NATIVE SPROUT-ASSOCIATED PANTOEA AGGLOMERANS FOR REDUCING SALMONELLA DURING ALFALFA SPROUTING

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Sprouts are widely consumed due to their high nutritional value; however, they are often associated with foodborne outbreaks that are frequently caused by *Salmonella*.

Purpose: This study aims to isolate and characterize native microbiota from alfalfa sprouts and evaluate their biocontrol potential in reducing *Salmonella* growth without affecting seed germination during sprouting.

Methods: Four different sprouts (alfalfa, beans, broccoli, and mixed (alfalfa and kale)) were purchased from the market, processed for metagenomic DNA extraction, shotgun sequencing and this native microbiome of sprouts was defined using Kaiju tool. Meanwhile, the market sprouts were homogenized, and the native microbiota were plated on tryptic soy agar. Whole genomes of colonies with unique morphologies were sequenced using AVITTI sequencing, and bacterial taxa belonged to *Pantoea* species were used for biocontrol experiments. For biocontrol evaluation, 10 g of alfalfa seeds were surface-sanitized, inoculated with 10^3 CFU/g of *Salmonella enterica* serovar Enteriditis, followed by inoculation with 10^7 CFU/g of four *Pantoea agglomerans* strains and *Salmonella* population was determined by plating on XLT4 agar and compared with *Salmonella* populations in untreated

sprouts (*Pantoea*-free). One gram of sprouts was sampled on Day 2 from each group and the ratio of germinated to non-germinated sprouts was calculated.

Results: On-day 5 of sprouting, *Salmonella*-only inoculated sprouts had an average of 5.1 ± 0.8 log CFU/g of *S.* Enteritidis, whereas the *P. agglomerans*-treated sprouts showed a significantly lower *Salmonella* population of 2.7 ± 0.2 log CFU/g indicating a 2.4 log reduction ($p < 0.01$, n=3). The germination percentage in the *Salmonella*-only group was 92.3 ±1.91%, compared with 94.2 ± 1.3% in the *Pantoea*-treated group (n=3).

Significance: *Pantoea agglomerans* as biocontrol agents can significantly reduce *Salmonella* growth during alfalfa sprouting without affecting germination.

Award Application: Developing Scientist Award

P2-131: CROSS-CONTAMINATION OF COMMERCIAL HARVESTER-SURFACE COUPONS FOLLOWING CONTACT WITH BLUEBERRIES INOCULATED WITH LISTERIA MONOCYTOGENES AND ESCHERICHIA COLI

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Category: Produce

Abstract:

Introduction: Mechanical harvesters efficiently harvest berries, but complex designs can impact food safety. Quantitative data on pathogen transfer from contaminated berries to harvester surfaces are lacking, limiting risk assessment and sanitation validation.

Purpose: Quantify cross-contamination of lower belt (LB) and catcher plate (CP) harvester-surface coupons by *L. monocytogenes* (*Lm*) and *E. coli* (*Ec*) from inoculated blueberries.

Methods: Blueberries (n=40/microbe) were dip-inoculated with 3-strain rifampicin-resistant *Ec* or 6-strain *Lm* cocktail (~5 log CFU/mL; 0.85% saline) and dried (30 min). Sterile coupons (2x2 cm; 4/surface) in 6-well plates were covered with inoculated berries (n=4), shaken (150 rpm) for 3 min, berries removed, and coupons dried (15 min). Coupons were transferred to 50 mL tubes with 25 mL saline and sonicated (5 min). *Ec* and *Lm* were enumerated from berries (10 mL saline) and coupons (25 mL saline) on eosin methylene blue agar with rifampicin and Harlequin agar, respectively (37°C, 24 h). Blueberry dip inoculum was enumerated as an exposure check. Experiments were repeated twice.

Results: Blueberry dip concentrations were 3.9-4.5 log (*Ec*) and 4.3-4.6 log CFU/mL (*Lm*). Post-contact with coupons, mean recoveries on berries were ~4.2 log CFU/ml (*Ec*) and ~4.5 log

(*Lm*). Coupon recoveries ranged from 2-2.9 log CFU/mL for *Ec* and 2-4.1 log CFU/mL for *Lm.* On CP coupons, *Ec* was largely at/below the detection limit (LOD 2.0 log CFU/mL; 3/4 <LOD; 1 at LOD). *Lm* contamination on coupons (CFU/coupon; log-transformed) did not differ between CP and LB (Welch's *t*-test; $p>0.05$), though geometric mean loads were higher on CP (~$2.5x10^4$) than LB (~$8.2x10^3$).

Significance: This study provides quantitative data on *Lm* and *Ec* contamination of representative harvester surfaces following contact with inoculated berries, supporting the need for sanitation optimization and informing future work pairing berry and different harvester surfaces to estimate transfer rates.

P2-132: DEVELOPMENT AND VALIDATION OF A UV-C DECONTAMINATION TUNNEL FOR CONTROLLING LISTERIA BIOFILMS AND OTHER MICROBIAL POPULATIONS ON FOOD-CONTACT SURFACES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Food-contact surfaces used in the fresh produce industry may serve as reservoirs for *Listeria* biofilms and other microorganisms, posing significant challenges for control under a single sanitation intervention.

Purpose: This study aimed to develop and validate a decontamination tunnel that provides 360-degree UV-C light exposure along with a 4% lactic acid spray to food-contact surfaces to control *Listeria* biofilms and reduce the overall microbial population.

Methods: *Listeria innocua* (ATCC #33660) was used to form biofilms on stainless steel (SS) and high-density polyethylene (HDPE) coupons (3” x 3”). Coupons were inoculated with an initial inoculum of ~8.48 log CFU/mL. Four treatment combinations, including individual and combined applications of UV-C and 4% lactic acid, were evaluated by varying conveyor belt

speed and UV module configuration, and control treatments received no UV-C exposure or lactic acid spray. A single UV-C module delivered approximately 50–60 mJ/cm^2, while four modules delivered a cumulative dose of 216–320 mJ/cm^2. Field validation studies were conducted on harvest bins (100 cm^2 sampling area) to assess the effectiveness of the combined treatments in controlling microbial populations, including aerobic bacteria, coliforms, yeast, and mold.

Results: All experiments were replicated three times with three samples per treatment. Significant microbial reduction of 4–5 log CFU/cm^2 was observed on both SS and HDPE coupons across all treatment combinations compared with their control treatments (6.64 log CFU/cm^2) and (6.78 log CFU/cm^2), respectively (p<0.05). Field validation studies on harvest bins also revealed significant reductions (2–3 log CFU/100 cm^2) for coliforms, yeast, and mold (p<0.05).

Significance: Using UV-C light in combination with lactic acid as a hurdle technology offers an effective way to control *Listeria* biofilms and overall microbial populations on difficult-to-access food-contact surfaces.

Award Application: Developing Scientist Award

P2-133: VALIDATION OF A 360° HYBRID SPRAY UV-C TUNNEL SYSTEM FOR WHOLE-APPLE DECONTAMINATION

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Microbial contamination of apples remains a major food safety concern for the fresh produce industry, while conventional chemical sanitizers can leave residual traces, promote cross-contamination, and adversely affect product quality, alternatives such as UV-C light may help reduce chemical use.

Purpose: This study evaluated a hybrid tunnel system equipped with a 360-degree exposure to UV-C light and an aqueous sanitizer spray for whole-apple decontamination.

Methods: Fresh whole apples (local farm) were washed, weighed, and surface-inoculated with approximately 6.48 log CFU/ml of *E. coli (*ATCC 25922). Whole apples were treated using a 360° integrated tunnel system that delivered a peracetic acid (PAA, 80 ppm) spray and UV-C light exposure, with treatments including PAA spray alone, UV-C alone, a combined treatment of UV-C and PAA, and an untreated control. Microbial reductions were expressed as $\log_{10}$ CFU g^{-1} relative to untreated controls.

Results: Three independent trials with four samples per treatment were conducted, and treatment effects were evaluated using ANOVA with Tukey's post hoc test ($p < 0.05$) on log-

transformed data. The combined treatment produced the greatest reduction (3.48 ± 1.07 $\log_{10}$ CFU g^{-1}), compared with PAA alone (2.94 ± 0.55 $\log_{10}$ CFU g^{-1}) and UV alone (2.90 ± 0.78 $\log_{10}$ CFU g^{-1}). These findings demonstrate that integrating chemical and non-thermal disinfection within a full-coverage tunnel configuration can enhance apple decontamination efficacy and support the potential of hybrid tunnel systems for postharvest produce sanitation.

Significance: The 360-degree exposure UV-C tunnel system effectively reduced *E. coli* population on whole apples, demonstrating that the combination of UV-C light with peracetic acid spray offers promising technology for sanitizing fresh produce surfaces.

P2-134: EFFECT OF EPIPHYTIC BACTERIA ON LISTERIA MONOCYTOGENES GROWTH ON PRODUCE FROM FARMERS' MARKETS IN ALLEGANY COUNTY, MARYLAND

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: *Listeria monocytogenes* is a serious foodborne pathogen that has caused major outbreaks linked to a variety of foods in the U.S. There have been several recalls and contamination alerts of *L. monocytogenes* tied to products typically sold at or associated with farmers' markets. There is a growing interest among consumers who prefer locally grown food products, and they are willing to pay a premium for fresh food products that are grown without the use of chemicals and antibiotics. That said, there is an increased concern about food safety due to the transfer of pathogens between animals and crops commonly sold at farmers markets.

Purpose: Our primary objective is to identify *L. monocytogenes* isolated from farmers' market products in Allegany County, Maryland, and to determine the effect of epiphytic bacteria on *L. monocytogenes* growth.

Methods: Various farmers' market products were collected and transported to the microbiology lab at Frostburg State University and stored at 4°C until used. Cubed samples were incubated for 24 to 48 hours in tryptic soy broth and Buffered Listeria Enrichment Basal to isolate bacteria. The streak plate was conducted on Oxford *Listeria* Agar media, and a spectrophotometer was used to observe the relationships of bacterial growth.

Results: Positive *L. monocytogenes* was observed after 24-hour incubation from the salad mix, swiss chard, broccoli sprouts, mushrooms, and lettuce, with a positive black color colonies on Oxford Agar, and cantaloupe had negative results with no black colonies. Absorption readings of salad mix, cantaloupe, swiss chard, lettuce, broccoli sprouts, mushrooms, and tomato were 1.16, 0.45, 0.86, 0.97, 0.13, 0.12, and 0.11, respectively. There was no direct effect found between the total epiphytic bacteria on the growth of *Listeria monocytogenes* ($P<0.05$). Observations of the research will be shared with USDA farmer training programs.

P2-135: ESCHERICHIA COLI TRANSFER ONTO STRAWBERRIES DROOPED ONTO NEW AND USED MULCH OVER TIME

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: The USFDA Produce Safety Rule (PSR) prohibits distribution of dropped produce due to ground contamination, but does not address drooped produce (produce in contact with the ground before harvest) which may also be prone to contamination.

Purpose: This study evaluated *Escherichia coli* transfer onto strawberries drooped over new (N) and used (U) plastic mulch (PM) over time.

Methods: Unwashed strawberries (n = 192) were weighed and drooped onto NPM or UPM inoculated with GFP-tagged *E. coli* (ca. 8 log CFU) resistant to ampicillin (Amp;250 ppm) for 0 (touch), 0.16, 1, and 24 h following a 30-min drying period. Bacterial survival (BS) on PM and bacterial transfer (BT) to strawberries were quantified by plate counts on tryptic soy agar with Amp (TSAAmp). Three independent experiments with four replicates were conducted. Effects of PM type and time on BS and BT were analyzed using a generalized linear mixed model. Linear regression evaluated the relationship between fruit weight and BT.

Results: BS was not significantly affected by PM ($p = 0.37$), whereas time had a significant ($p < 0.0001$), with the greatest reductions at 24 h. On NPM, BS decreased from 7.5±0.7 log CFU/mulch at 0 h to 0.9±1.1 log CFU/mulch at 24 h, while on UPM it declined from 6.9 ± 0.4 to 0.6±1.3 log CFU/mulch. BT on both PM types varied significantly over time ($p < 0.0001$),

increasing slightly at 0.16 h, decreasing at 1 h, and increasing markedly by 24 h (NPM: 3.8±2.4 to 5.2±1.8 and UPM: 2.1± 2.0 to 3.9± 2.0 log CFU/ strawberry). BT was consistently higher on NPM than UPM. No correlation was observed between fruit weight and BT.

Significance: Bacterial transfer to drooped strawberries depends on surface type and contact time, indicating that contamination risk is not solely dependent on surface populations.

P2-136: EVALUATING THE IMPACT OF PRODUCT STRUCTURE ON SALMONELLA THERMAL RESISTANCE IN APPLES AND MANGOES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Prior research shows that product structure impacts *Salmonella* thermal resistance in low-moisture foods; however, little is known about product structure impacts on *Salmonella* thermal resistance in high sugar fruits, which often are processed from high- to low-moisture with varying levels of structural integrity.

Purpose: The purpose of this study was to evaluate *Salmonella* lethality during isothermal treatment of sliced and pureed apples and mangoes.

Methods: Fresh apples and mangoes were sourced locally and stored at ~4°C for up to 1 week. Fruits were washed, peeled, sliced (~4 mm thickness), and arranged on a rack in a biosafety cabinet. Using a lawn-grown 6-serovar *Salmonella* cocktail, fruit slices were spot inoculated (~500 µL) on each side, resting 15 min before flipping. Fruit slices were stamped into disks for thermal treatment. Other fruit slices were blended until a smooth consistency was achieved (~40 s). Fruit disks and slurry samples (~1 g, ~4 mm thick) were loaded into aluminum test cells and treated at 60°C using a circulating water bath for 160 s. Samples were quenched in an ice-water bath, diluted, stomached, serially diluted, plated on

differential/non-selective media, incubated (48 h, 37°C), and then survivors enumerated. Log-transformed data were analyzed using ANOVA and *t*-tests.

Results: Initial inoculation levels for both apple and mango slices and slurries were not statistically different (~8.32 log CFU/g; $p > 0.05$). *Salmonella* reductions on apple slices were negligible ($p > 0.05$) after 160 s. However, greater *Salmonella* reductions ($p < 0.05$) were observed in mango and in blended fruit matrices, resulting in 2.78, 3.54, and >5.50 log reductions for mango slices, apple slurry, and mango slurry, respectively.

Significance: The results suggest that application of pathogen inactivation data should be product structure specific. Further research should further elucidate the structural phenomena impacting pathogen inactivation resistance.

Award Application: Undergraduate Student Award

P2-137: EVALUATION OF RAPID DETECTION KITS FOR LISTERIA SPP. ON FOOD CONTACT SURFACES

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Claire Murphy, Washington State University

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Packinghouses routinely conduct environmental monitoring for *Listeria* spp. while rapid detection kits offer faster turnaround times and lower costs, limited data exists on their performance on complex surfaces.

Purpose: This study evaluates the sensitivity and specificity of two commercially available rapid detection kits compared to culture-based methods for detecting *Listeria* spp. on brush surfaces.

Methods: Two brush types were evaluated: 100% nylon and mixed (50% horsehair/50% polyethylene). Treatments included a negative control, a low inoculum (~2 log CFU/brush), and a high inoculum (~4 log CFU/brush) of a *Listeria* spp. cocktail or *Bacillus cereus*. Two biological and three technical replicates were conducted per treatment. Brushes were spot inoculated to ensure even distribution across bristles and the brush core. Surface sampling was performed using two rapid detection kits (visual and antigen-based). Samples were processed according to manufacturer instructions, with sponge swabs analysed in parallel using the FDA BAM method. DNA extraction and PCR were used for confirmation.

Results: For *Listeria* spp., the FDA BAM method demonstrated high sensitivity (83.3–100%)

with low false negative rates. The visual rapid test showed comparable sensitivity to FDA BAM at high inoculum levels but reduced sensitivity at low inoculum, particularly on nylon brushes (16.7%). The antigen-based rapid test showed lower sensitivity (~50%) across all brush types and inoculum levels, resulting in higher false negative rates. No false positives were observed for any method when tested against *Bacillus cereus* (100% specificity).

Significance: Culture-based methods were the most reliable for detecting *Listeria* spp. across conditions. Reduced sensitivity of rapid tests may limit their usefulness for routine environmental monitoring. These findings support informed selection of monitoring tools.

Award Application: Developing Scientist Award

P2-138: FROM LAB TO PACKING HOUSE: GAS PHASE HYDROXYL-RADICAL PROCESS FOR ENHANCING FOOD SAFETY OF STONE FRUIT

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Jerin Kliewer, Kliewer Farms

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Stone fruits have been implicated in several foodborne outbreaks due to contamination with *Listeria monocytogenes*. A risk management option is the Clean Works gas phase Hydroxyl-Radical Process (HRP) generated from the UV-C mediated degradation of H_2O_2 and O_3.

Purpose: To provide an overview on how laboratory-based validation for inactivating *Salmonella*, *Listeria* & toxigenic *E. coli* associated with stone fruit translated to in-facility performance of a unit processing 80,000 lbs of fruit/hr.

Methods: Peaches & nectarines inoculated with *S.* Enteritidis, *Listeria monocytogenes*, or *E. coli* O157:H7 were passed through the HRP unit at different parameters for optimization. These parameters were applied to the commercial unit installed over a brush bed. *Lactobacillus fructivorans*, identified as a suitable surrogate, was introduced onto batches of fruit passed through the unit. Surface rinse samples of the fruit were recovered and

enumerated on MRS agar.

Results: Laboratory studies identified the treatment parameters of H_2O_2 (3%) delivered 120ml/min combined with 20 ppm O_3 and 119 mJ/cm² UV-C dose, supporting 2.27 ± 0.17 and 2.31 ± 0.16 log reductions for *S.* Enteritidis on peaches & nectarines, respectively; 2.32 ± 0.17 and 2.36 ± 0.17 log reductions for *L. monocytogenes*; and 1.59 ± 0.03 and 1.23 ± 0.14 log reductions for *E. coli* O157:H7. In comparison, reduction of *L. frutivorans* was 0.72 ± 0.18 log in peaches and 1.11 ± 0.27 log in nectarines. Commercial validation confirmed the scalability of the technology, yielding *L. fructivorans* log reductions of 2.07 ± 0.42 in peaches, 3.48 ± 0.11 in plums, and 2.90 ± 0.08 on nectarines. That suggested that the commercial unit exhibited greater decontamination efficacy compared to the laboratory-based unit.

Significance: The study illustrated that laboratory-based validation studies could be translated to large commercial units & provides supporting evidence of the HRP as a preventative control in stone fruit processing.

P2-139: EVALUATING VIRAL CROSS-CONTAMINATION AND INTEGRITY IN WASH WATER SYSTEMS: DETECTION OF INTACT AND FRAGMENTED PARTICLES

Primary Author:
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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Cross-contamination in post-harvest wash water systems is an undervalued risk. Little is known of potential for cross-contamination of foodborne viruses among and between batches of produce in wash water.

Purpose: This study quantifies cross-contamination potential in wash water determined by presence of intact and fragmented genetic material and virus infectivity.

Methods: Cherry tomatoes (n=2) were inoculated with hepatitis A virus (HAV) at 10^6 log/TCID50 or Tulane virus (TV) at 10^4 log/TCID50, and added to wash water systems (200mL) containing 80ppm peracetic acid (PAA) with varying turbidity across 12 experimental trials. Uninoculated tomatoes (n=2) were added to the system and washed for 1, 5, or 10 min. After initial sampling, a second batch of uninoculated tomatoes (n=2) was added to the same system and washed for 1 min to evaluate cross-contamination between batches. Controls included untreated and treated tomatoes. Water samples (100mL) were collected and neutralized. qPCR with and without RNase treatment was performed to quantify viral presence and cell culture TCID50 for infectivity. Statistical analysis was completed using the JMP Pro 17 software.

Results: PAA treatment at 80ppm reduced virus presence on inoculated tomatoes by 2.5 log for HAV and 2.7 log for TV ($p < 0.0001$). Cross-contamination between batches was detected, at 2–3 log/TCID50/mL for HAV and at <1.6 log/TCID50/mL for TV. No significant differences were observed for time or turbidity. Detection of viral genetic material in tomato and water samples across all treatments (n = 52), regardless of virus type, when coupled with RNase treatment was significantly lower (96% at $p < 0.05$ and 4% at $p<0.06$), indicating much of the virus detected were not intact.

Significance: These findings reveal viral cross-contamination risks, as well as a means for risk reduction, as PAA was efficient at degrading virus particles, as evidenced by RNase assay.

Award Application: Developing Scientist Award

P2-140: FROM SOIL TO STORAGE: TRACKING PATHOGENS ACROSS THE FARM

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Hazard identification and risk assessment in a farm production environment are vital in limiting the potential of foodborne pathogen transmission.

Purpose: This study presents data from weekly farm visits in the Mid-Atlantic U.S. in 2025 as a part of a multi-year study, including microbial assessment of soils growing raw agricultural commodities and swabs from harvesting, packing, and storage environments.

Methods: Soil samples (n=97) were collected at a 7cm depth from active fields (each a composite of one 0.56 m^2 area) and swab samples (n=585) from harvesting and packing equipment were collected from July–Oct. 2025 at 6 farms. Samples were analyzed for *E. coli* and coliforms using Charm Peel Plates and for *Listeria* using Buffered Listeria Enrichment Broth (30°C for 24h) and RAPID agar (37°C for 24h). For *Salmonella* detection, soil and swabs were enriched in Universal broth (37°C for 24h), TET and RV (42°C for 24h), and then enumerated onto XLT-4 (37°C for 24h and 48h). Statistical analysis to determine correlations between environmental factors and bacterial levels was performed using JMP software.

Results: Coliforms were detected on most soils and swabs (88%) from all farms. *E. coli* was

detected in soil and swab samples from four farms, at a maximum of 8.5 x 10^2 CFU/g in soil. *L. monocytogenes* was detected in swabs from two farms and soils from three farms. *Salmonella* was detected on all farms, totaling 25% of swabs and 26% of soil samples. Longitudinal assessment including metadata analysis informs food safety case studies, i.e. crop cover use in between rows reduces *Salmonella* presence (p = 0.0005), changes in soil with change in irrigation water source, and cold storage cleaning and sanitizing practices.

Significance: On-farm surveillance offers unique opportunities for real-time and longitudinal education and feedback to reduce risks of foodborne pathogen transmission.

P2-141: GROWTH AND SURVIVAL OF FOODBORNE PATHOGENS ON FRESH-CUT PRODUCE: A SYSTEMATIC REVIEW

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Laura Strawn, Virginia Tech

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Fresh-cut fruits and vegetables may support the survival and growth of foodborne pathogens due to tissue damage, moisture, and nutrient availability.

Purpose: This systematic literature review sought to identify and characterize published data on the growth and/or survival of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* on fresh-cut produce products.

Methods: Literature searches were conducted in AGRICOLA, CABI, FST Abstracts, ProQuest, PubMed, Web of Science, and Google Scholar using Boolean terms related to fresh-cut produce, foodborne pathogens, and microbial growth or survival. Over 6,000 records were screened at the title/abstract and full-text levels. Eligible studies were laboratory-based inoculation experiments reporting quantitative pathogen growth and/or survival during storage; studies of intact or otherwise treated produce were excluded. Extracted data included commodity type, pathogen type, inoculum level, storage conditions, and microbial growth outcomes.

Results: Eighty studies met inclusion criteria, representing 34 fresh-cut produce

commodities, primarily leafy greens, melons, and tropical fruits. Storage temperatures ranged from 4 to 35°C, with durations ranging from 10 hours to 28 days. Pathogen growth and survival varied substantially by commodity and storage conditions. However, frequent omission of key parameters, such as water activity, relative humidity, and packaging conditions, limited cross-study comparability.

Significance: This review identifies gaps in experimental design and commodity coverage that limit predictive modeling for fresh-cut produce risk assessment. Improved standardization of storage and environmental parameters will support food safety decision-making, including time temperature control for safety (TTCS).

Award Application: Developing Scientist Award

P2-142: GROWTH INHIBITION OF MONILINIA FRUCTICOLA, MONILINIA LAXA, AND BOTRYTIS CINEREA EXPOSED TO ESSENTIAL OIL EXTRACTS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Postharvest fungal spoilage contributes substantially to fresh produce losses and food safety concerns, particularly in organic production systems where postharvest intervention options are limited.

Purpose: The objective of this study was to evaluate the antifungal activity of essential oil–based formulations (EO) under produce-relevant pH conditions and assess their potential incorporation into edible coating systems.

Methods: Fungal isolates were collected from infected produce samples, donated cost-free by local grocers. Four isolates of *Monilinia* spp. and two isolates of *Botrytis cinerea* were confirmed using molecular methods. Extracts of thyme, cinnamon, clove, peppermint, and rosemary were added to Potato Dextrose Agar (PDA) at concentrations of 0.1%, 1%, or 3% v/v and adjusted using lactic acid or sodium hydroxide to pHs of 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, and 7. After agar plug transfer and incubation, fungal colonies on petri dishes were

measured for growth rate and diameter to determine growth suppression. Mycelial growth inhibition was determined by:

MGI (%) = ((Growth on Non-Amended - Growth on Amended)/Growth on Non-Amended) * 100.

Results: MGI values at all tested pH conditions were 100% for cinnamon, clove, and thyme oils. Peppermint and rosemary EOs exhibited MGI values under 50% at pH conditions of 4.0 or above, with differences observed among fungal species. Growth suppression for peppermint and rosemary EOs was enhanced at pH 2.0 conditions, where MGI values were 100%.

Significance: These findings support the potential use of essential oil-based edible coatings as natural antifungal interventions for postharvest produce, particularly in organic systems. This work provides foundational data to inform development of practical coating strategies to reduce fungal spoilage and improve postharvest quality.

Award Application: Developing Scientist Award

P2-143: IMPACT OF REAL-TIME FEEDBACK ON CLEANING AND SANITATION EFFICACY ON MICROBIOLOGICAL LOADING ON A LEAFY GREEN HARVEST MACHINE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Harvest equipment has been identified as potential sources of microbiological contamination; support to identifying opportunities to improve cleaning and sanitation (C/S) programs is needed.

Purpose: Assess efficacy of onsite feedback on leafy green harvester (LGH) C/S programs.

Methods: An assessment of C/S practices, as well as swabs of food contact and non-contact surfaces evaluating microbiological quality (total count, coliforms, *E.coli,* and *Listeria*) was performed using AOAC methods for a 3-point LGH before and after C/S practice feedback. Swabs (n=60) were collected at three timepoints: Post-Harvest (PH), Post-Detergent (PD), and Post-Sanitation (PS) with five sampling zones (A-E). Metadata were recorded for each event, including C/S chemistries and practices used. Feedback was provided in real time; *t*-tests and

ANOVAs were performed (n=2 visits).

Results: LGH was initially cleaned in the field with a non-chlorinated alkaline detergent and sanitized with chlorine. Following location and chemistry feedback, the LGH was moved from the harvest area and sanitized with a quat. Considerable improvement was observed between samplings. Despite a similar volume of water (567 L) being used, notable differences were observed including: cleaning location change; additional staff; a top-down approach; addition of a hygienic brush; and an increased volume of detergent. Presumptive *Listeria* populations decreased from 51.7% of all swabs (n=60: 48%PH, 60%PD, 80%PS) at the first event, to 8.3% of all swabs (n=60: 8%PH, 8%PD and 10%PS). *E. coli* detection (□10 CFU/swab) was infrequent and decreased from: 8%PH, 8%PD, 0%PS at the first event, to 4%PH, 0%PD, 0%PS. Total and coliform counts significantly decreased ($p < 0.05$) between PH and PD, and PH and PS.

Significance: Simple changes after real-time feedback can impact the effectiveness of LGH cleaning practices, resulting in significant improvements in microbiological quality of food contact and non-contact surfaces.

Award Application: Developing Scientist Award

P2-144: IMPROVING THE MICROBIOLOGICAL SAFETY OF RECYCLED DRENCHING SYSTEMS: EFFICACY OF SANITIZER-FUNGICIDE COMBINATIONS ON THE REDUCTION OF BACTERIAL PATHOGENS

Primary Author:
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Claire Murphy, Washington State University

Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Recycled drenching systems (RDS) are an effective method of fungicide application used by the apple industry; however, the recirculation of solution may increase the risk of microbiological accumulation and subsequent bacterial cross-contamination.

Purpose: The objective of the current study was to evaluate the efficacy of sanitizer-fungicide combinations on the reduction of bacterial pathogens for future application in commercial RDS.

Methods: Individual fungicides (fludioxonil [FDL], pyrimethanil [PYR], thiabendazole [TBZ]) were selected and supplemented with peroxyacetic acid (PAA; 0–55 ppm). Resulting solutions were inoculated with a 5-strain Shiga-toxigenic *Escherichia coli* (STEC*)* or *Listeria monocytogenes* cocktail to achieve a target cell density of 7 log CFU/mL. Samples were left undisturbed for 0–180s, neutralized using sodium metabisulfite (12.5%), and enumerated on TSAYE (37°C, 24–48 h). A linear model was fitted to evaluate the impact of treatments (PAA, fungicide, PAA+fungicide), PAA concentrations, and contact time on bacterial reduction.

Results: As PAA concentrations increased (25–55 ppm), STEC (-2.95±0.27 to -5.35±0.27 log

CFU/mL) and *L. monocytogenes* (-1.58±0.28 to -4.66±0.28 log CFU/mL) populations decreased. Similarly, as contact time duration (60–180s) increased, STEC (0.10±0.51 to -1.74±0.51 log CFU/mL) and *L. monocytogenes* (-0.08±0.59 to -1.59±0.59 log CFU/mL) decreased. Low PAA concentrations (10 ppm) and contact time (30s) did not result in measurable reductions compared to controls (0 ppm; 0 s). Bacterial populations between treatment groups did not significantly differ between PAA and PAA+fungicide treatments (P>0.05), suggesting fungicide addition had minimal impact on PAA efficacy.

Significance: These findings suggest that FDL, PYR, and TBZ have minimal impact on PAA efficacy against STEC and *L. monocytogenes,* and PAA may help reduce bacterial cross-contamination in RDS.

Award Application: Developing Scientist Award

P2-145: ASSESSMENT OF PACKINGHOUSE FLUME SYSTEMS AND INFLUENCE OF WATER PHYSICOCHEMICAL PROPERTIES ON CHERRY MICROBIOLOGICAL POPULATIONS

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Claire Murphy, Washington State University

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Cherry packinghouses (PH) rely on flumes to transport fruit; however, inadequate water management may facilitate cross-contamination events.

Purpose: This study assessed how water physicochemical properties influenced microbial populations in water and on cherries.

Methods: Four cherry PHs in Washington state were each visited once during the 2025 packing season. Cherry samples were collected before entering the packing line and after exiting each flume (PH 1/2: 4 flumes; PH 3/4: 3 flumes). Water samples were collected from each flume, and sanitizer levels, pH, turbidity, and water temperature were measured. All samples were collected in triplicate across the duration of water hold time (4–10h). Peptone (20mL) was added to a cherry sample, followed by 30 sec rub, 30 sec shake, 30 sec rub. Cherry rinsate and water were plated on aerobic plate count (APC; 37°C, 48h), Enterobacteriaceae (EB; 37°C, 24h), and Total Coliform/generic *Escherichia coli* (TC/EC; 37°C, 48h) Petrifilms. Linear regression models were used to assess microbial population dynamics and the influence of physiochemical properties, flumes, and PHs.

Results: TC/EC were rarely detectable, with 138/437 and 5/437 water and 40/522 and 3/522 cherry samples, respectively, above detection limits (0 log CFU/mL water; <1 log CFU/cherry). Minimal deviation in APC/EB were observed by flume (<0.05 log CFU/cherry; <1 log CFU/mL water). In cherry/water models, an increase in sanitizer levels corresponded to reduced APC (P<0.05). In water, lower temperatures and higher pH corresponded to a decrease in APC (P<0.05). PHs were significant predictors for both systems (P<0.05). Microbial populations were stable across flume location, and APC varied by PH. For water/cherries, sanitizer levels were the strongest predictor of APC.

Significance: These findings highlight the importance of proper water quality management and monitoring to maintain water quality in cherry PHs.

Award Application: Developing Scientist Award

P2-146: INACTIVATION OF LISTERIA MONOCYTOGENES ON RASPBERRIES AND POSTHARVEST WATER BY UV-C LIGHT

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Claire Murphy, Washington State University

Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Frozen berries are typically washed prior to freezing; however, water represents a potential source of microbial contamination. Ultraviolet light (UV-C; 254 nm) is FDA-approved for produce surfaces and has been shown to effectively inactivate microorganisms in process water.

Purpose: This study evaluated the reduction of *Listeria monocytogenes* on raspberries and raspberries in postharvest water at varying turbidity levels treated with UV-C light.

Methods: Raspberries (10g; 5.5 log CFU/g) or postharvest water (100mL; 6.47 log CFU/mL) were inoculated with a five-strain *L. monocytogenes* cocktail and treated using a conveyor system with integrated UV-C bulbs at dosages of 232, 478 and 843 J/m^2. Treatments included: (1) dry inoculated raspberries, (2) inoculated raspberries in uninoculated water at varying turbidities (0, 150, 300, and 450 NTU) and (3) inoculated water at varying turbidity levels containing uninoculated raspberries. Two trials were conducted with three replicates per treatment, and surviving populations were enumerated on tryptic soy agar with rifampicin.

Results: Exposure to UV-C dosages resulted in significant reductions of 1.45±0.63, 3.36±0.69, and 4.18±0.88 log CFU/g on dry raspberries, respectively. Raspberries in postharvest water

ranged from 1.52±0.30 to 3.96±0.97 log CFU/g. In inoculated postharvest water, reductions ranged from 2.13±0.20 to 3.44±1.21 log CFU/mL. UV-C effectively reduced cross-contamination from water to berries and berries to water compared to untreated controls. The three-way interaction between turbidity, dosage, and contamination source was significant ($p<0.05$), indicating that the combined effect of water clarity and UV-C intensity varies depending on whether *L. monocytogenes* is present on fruit or in wash water.

Significance: Optimized UV-C treatments can provide an additional layer of risk reduction, improving the microbial safety of ready-to-eat berries while helping to maintain postharvest water quality.

Award Application: Developing Scientist Award

P2-147: INACTIVATION OF SALMONELLA TYPHIMURIUM AND SURROGATE BACTERIA ENTEROCOCCUS FAECIUM ON CUCUMBERS BY TRIPLE-WASH WITH PEROXYACETIC ACID

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Fresh produce washing can reduce microbial contamination but may also facilitate cross-contamination if not properly controlled.

Purpose: This study evaluated a triple-wash system using peroxyacetic acid for reducing *Salmonella* Typhimurium and *Enterococcus faecium* on cucumbers.

Methods: Fresh cucumbers were subjected to a triple-wash process conducted in three experimental phases, each performed in triplicate. A 1:2 ratio of one *Salmonella* or *E. faecium* inoculated cucumber to two uninoculated cucumbers was used to simulate cross-contamination. Phase I consisted of three potable water washes. In Phase II, the second wash contained 24 or 96 ppm of peroxyacetic acid, with potable water used for the first and third washes. Microbial recovery was quantified using a most probable number (MPN) method. After enrichment, samples were plated using a multi-channel pipette onto XLT-4 or bile esculin agars as the selective medium for confirmation of *Salmonella* and *E. faecium*, respectively. Mixed model procedure was used for data analysis with P=0.05.

Results: Water-only washing resulted in limited *Salmonella* reduction (0.51–0.62 $\log_{10}$MPN/

ml) and substantial cross-contamination to uninoculated cucumbers (3.56 $\log_{10}$MPN/ml). Inclusion of 24 or 96 ppm of peroxyacetic acid in the second wash significantly reduced *Salmonella* populations (3.00 to 3.56 $\log_{10}$MPN/ml) than the water-only washing ($P < 0.05$), also reduced the cross-contamination cells counts from 3.04 to 0.78–1.48 $\log_{10}$MPN/ml ($P < 0.05$). *E. faecium* indicated lower reductions (1.08 to 1.62 $\log_{10}$MPN/ml, $P < 0.05$) and greater cross-contaminated cells counts (2.75 to 3.47 $\log_{10}$MPN/ml, $P < 0.05$) than *Salmonella*.

Significance: These findings demonstrate that applying peroxyacetic acid within a triple-wash system plays a critical role in controlling *Salmonella* and limiting cross-contamination on cucumbers. *E. faecium* is a promising surrogate for *Salmonella*.

P2-148: PERCEPTIONS OF PRODUCE RECALL RESPONSIBILITY AND ON-FARM FOOD SAFETY COMPLIANCE AMONG SMALL AND MEDIUM-SIZED GROWERS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Produce outbreaks and recalls involve multiple actors across the food supply chain, and responsibility is often perceived differently by stakeholders. Understanding produce growers' perceptions of responsible stakeholders can inform more effective food safety engagement strategies.

Purpose: This study examined produce growers’ perceptions of responsibility for produce-linked foodborne outbreaks and on-farm food safety compliance.

Methods: An online cross-sectional survey was conducted among small and medium-sized produce growers across the United States (N=500). The survey captured information on who was attributed responsibility for produce outbreaks related to a spring mix blend and jalapeños. These findings were descriptively compared with those from a previous consumer study to identify any alignments in perceptions. The influences of on-farm food safety compliance were also explored.

Results: Growers predominantly attributed a jalapeño-related outbreak to retailers (58%) and a spring mix blend-related outbreak to government agencies (90%). Further analysis

revealed that approximately 50% of small-sized produce growers perceived retailers to be more responsible for an outbreak related to the spring mix blend, while 57% of medium-sized farms attributed the same outbreak to produce transportation. For a jalapeño outbreak, a significant difference between small and medium-sized farms was observed only for attribution of responsibility to consumers. Both growers and consumer study groups perceived the responsibility of stakeholders for an outbreak associated with spring mix blends differently ($p<0.0001$). Regarding on-farm food safety compliance, medium-sized produce growers were more likely than small-sized growers to be influenced by their values (OR=1.605; $p=0.0104$).

Significance: This study provides complimentary evidence on how growers perceive responsibility of produce recalls, supporting the development of more targeted communication strategies.

Award Application: Developing Scientist Award

P2-149: PERSISTENCE OF SALMONELLA TYPHIMURIUM IN NUTRIENT FILM TECHNIQUE HYDROPONIC SYSTEMS FOR FRESH HERB PRODUCTION

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Hydroponic systems, particularly Nutrient Film Technique (NFT), are increasingly used for fresh herb production but pose unique food safety risks due to recirculating water.

Purpose: This study evaluated the survival and distribution of *Salmonella enterica* serovar Typhimurium in NFT systems producing basil, cilantro, and arugula following a simulated sporadic contamination event.

Methods: Three independent NFT systems were inoculated with *Salmonella* (10^3 CFU/mL) in the nutrient solution to simulate a single sporadic contamination. Samples of recirculated water (from channels and reservoirs), edible tissues, and roots with plugs were collected at 3 h, 12 h, 24 h, 48 h, and at weekly intervals up to 35 days. Microbial populations (*Salmonella*, aerobic plate counts, yeast, and molds) were enumerated using culture-based methods. Data

were analyzed using linear mixed-effects models.

Results: *Salmonella* was recovered from all matrices within 48 h post-inoculation, peaking at 4.78 log CFU/mL in channels and 3.7 log CFU/mL in reservoirs at 3 h. Edible tissues showed transient contamination (□2.7 log CFU/g) within 24 h but none after 48 h. Roots with plugs supported persistence throughout the 35-day cycle, with levels fluctuating between 3 and 4 log CFU/g. Background microflora remained stable, with aerobic plate counts exceeding 5 log CFU/g and yeast/molds >6 log CFU/g in roots. Herb type did not significantly affect *Salmonella* survival ($p > 0.05$).

Significance: NFT systems can promote *Salmonella* survival in roots and recirculated water for extended periods after a single contamination event, underscoring the need for preventive water management and sanitation strategies in controlled environment agriculture.

P2-150: PREHARVEST APPLICATION OF NATURAL ANTIMICROBIALS TO REDUCE ESCHERICHIA COLI O157:H7 ON SPINACH GROWN IN A CONTROLLED ENVIRONMENT

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Leafy greens such as spinach are frequently consumed raw and have been implicated in foodborne illness outbreaks associated with *Escherichia coli* O157:H7, highlighting the need for effective preharvest pathogen control strategies such as natural antimicrobial treatments.

Purpose: This study evaluated three natural antimicrobials, 1% benzyl isothiocyanate (BIT) nanoemulsion, 2% Sporan emulsion, and 1% eugenol nanoemulsion, as preharvest interventions to reduce *E. coli* O157:H7 on spinach under controlled environmental conditions.

Methods: Two spinach varieties (Space and Tundra) were grown for four weeks in a controlled environment growth chamber. Plants were spray-inoculated with a four-strain *E. coli* O157:H7 cocktail (~6 log CFU/mL) prepared in sterile bovine fecal slurry, followed by preharvest treatment with BIT, Sporan, or eugenol spray. Samples were collected at 1 h (Day 0), 24 h (Day 1), and Day 7 post-treatment, and pathogen populations were enumerated on sorbitol MacConkey agar. Experiments were conducted in triplicate with four plants per treatment per sampling day. Data were analyzed using ANOVA with post hoc comparisons in

R.

Results: Preharvest antimicrobial treatments significantly reduced *E. coli* O157:H7 populations compared with untreated controls ($p < 0.05$), whereas sampling day, spinach variety, and interaction effects were not significant ($p > 0.05$), indicating consistent treatment performance across time and varieties. BIT nanoemulsion significantly reduced *E. coli* O157:H7 by 1.57 log CFU/g on spinach after treatment compared to 1.24 and 1.06 log reduction with Sporan and eugenol treatment, respectively. At 7 days post-treatment, *E. coli* O157:H7 recovered from treated spinach plants were ~1 log lower than in control plants. Overall, BIT showed greater efficacy than Sporan followed by eugenol.

Significance: Preharvest application of BIT nanoemulsion provides a promising and sustainable strategy for reducing *E. coli* O157:H7 on spinach.

Award Application: Developing Scientist Award

P2-151: PREHARVEST CONTROL OF SALMONELLA ENTERICA IN MICROGREENS USING NATURAL ANTIMICROBIALS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Microgreens are commonly consumed raw as fresh, nutrient-dense produce. They lack validated postharvest pathogen reduction steps, making preharvest control critical for food safety.

Purpose: This study evaluated the efficacy of two natural antimicrobial formulations 1% (v/v) benzyl isothiocyanate (BIT) nanoemulsion and 2% (v/v) Sporan emulsion as preharvest interventions to control *Salmonella enterica* in microgreen production systems.

Methods: Seeds of five Brassicaceae microgreen species (daikon radish, broccoli, mustard, red cabbage, and red Russian kale) were inoculated with a *S. enterica* cocktail (4–5 log CFU/mL) prior to antimicrobial treatment. Inoculated seeds were treated with antimicrobials for 20 min and air-dried prior to planting. Seed decontamination efficacy was determined by bacterial enumeration following treatment. Treated seeds were cultivated for 14 days in environmentally controlled growth chamber; bacterial populations on plant tissues and in the growing media were quantified on days 7 and 14. Germination rates and yield were also evaluated. Data from three independent replicates were statistically analyzed using SAS

software.

Results: BIT treatment resulted in a 1.6 log CFU/g reduction of *S. enterica* on seeds, while Sporan achieved a 1.2 log CFU/g reduction relative to untreated controls ($p < 0.05$). During cultivation, BIT-treated microgreens exhibited significantly lower bacterial populations on plant tissues, whereas no significant reductions were observed in growing media. Both treatments-maintained germination rates between 60–80% for most species with minimal yield impacts, although BIT caused a slight yield reduction in daikon radish and delayed germination relative to controls.

Significance: BIT nanoemulsion demonstrated effective preharvest control of *S. enterica* in microgreens while maintaining acceptable germination and yield, supporting its potential as a sustainable food safety intervention.

Award Application: Developing Scientist Award

P2-152: PROLIFERATION OF SALMONELLA DURING POSTHARVEST STORAGE OF ALFALFA SPROUTS AS AFFECTED BY STORAGE TEMPERATURE AND PATHOGEN LOAD

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: The U.S. Food Code defines sprouts as a “Time/Temperature Control for Safety” food. However, little is known about the growth profile of foodborne pathogens in sprouts stored at different temperatures.

Purpose: Examine the proliferation of *Salmonella* during storage of alfalfa sprouts, as affected by storage temperature and pathogen load.

Methods: Alfalfa sprouts grown from uninoculated seeds and harvested after 5 days of sprouting were divided into 25-g portions. Each portion was inoculated with a cocktail of 5 *Salmonella* serovars at various levels (approximately -1, 1, 3, or 5 log CFU/g) prior to storage at 4, 7, or 10°C for 21 days or at 25°C for 7 days. At defined time points (Days 0, 1, 2, 3, 4, 7, 10, 14, and 21), levels of *Salmonella* in stored sprouts were determined by plate count, 3-tube MPN or culture enrichment.

Results: No *Salmonella* proliferation was observed when inoculated sprouts were stored at 4,

7, or 10°C, regardless of initial pathogen load. *Salmonella* proliferated in sprouts during storage at 25°C, and the extent of growth varied depending on the initial pathogen population. In sprouts contaminated at levels of 0.9 log MPN/g, 3.1 log MPN/g, and 4.8 log CFU/g, the *Salmonella* population increased and reached levels of 3.7 log MPN/g, 5.6 log CFU/g, and 5.7 log CFU/g, respectively, after 7 days of storage. However, for sprouts inoculated at a level of -0.9 log MPN/g, no *Salmonella* growth was observed.

Significance: No pathogen growth was observed in sprouts stored at 10°C or below. There appeared to be a minimal contamination level below which pathogen growth may not occur even when sprouts are stored at a high temperature (25°C). Knowledge gained will inform development of controls that minimize pathogen proliferation during storage of sprouts and Food Code recommendations.

P2-153: STRUCTURAL AND MOLECULAR PROFILES OF SALMONELLA SPP. BIOFILMS IN HYDROPONIC LEAFY GREEN PRODUCTION

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: *Salmonella* spp. persists in leafy green hydroponic production systems in controlled environments. Understanding the role of *Salmonella* biofilms is essential to reduce food safety risks.

Purpose: This study assessed the ability of *Salmonella* spp. to form biofilms in hydroponic leafy green nutrient solution and characterized the biofilms at different temperatures and carbohydrate concentrations relevant to hydroponic leafy green production.

Methods: Biofilms of 13 *Salmonella* serovars were grown in hydroponic leafy green nutrient solution (LG), LG with lettuce exudates (LGE), and LG amended with glucose, fructose, sucrose, or inositol (0.1–0.4%). Biofilms were assessed in vitro (25°C/28°C/37°C) and under controlled environment conditions. Total biofilm production was quantified by crystal violet (OD570) and categorized into weak/moderate/strong. Viable cells were enumerated, and RDAR (red-dry-and-rough) morphotype analyzed. Genetic analysis was performed.

Results: *Salmonella* Anatum and Newport formed weak biofilms at 25°C. In the presence of lettuce exudates, all strains formed moderate biofilms at 25°C. At 28°C, 6/13 strains formed

moderate biofilms, while 5/13 formed weak biofilms. *S.* Enteritidis and *S.* Heidelberg formed strong biofilms at 37°C. In the presence of sucrose, glucose, and inositol, >70% of the strains formed moderate-strong biofilms under all test conditions. Five strains exhibited RDAR morphology in LG, while 9 had RDAR morphology in LGE at 28°C. Molecular profile of each strain was generated. Biofilm-associated genes gcpA, csgD, and adrA were detected in all tested *Salmonella* strain.

Significance: *Salmonella* spp. can form biofilms in leafy green nutrient solutions, especially in the presence of plant exudates at temperatures specific to leafy green production. While the characteristics of biofilms varied, growth at 28°C and the presence of sucrose were the most favorable conditions for biofilm production among the tested strains.

P2-154: SURVEY OF POSTHARVEST WASHING PRACTICES IN COMMERCIAL FRESH PRODUCE PACKINGHOUSES

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Presentation type/Format: Technical

Category: Produce

Abstract:

Introduction: Postharvest water can cross-contaminate fresh produce, particularly when water is reused. Although FSMA regulations mandate the absence of *Escherichia coli* in postharvest water, its quality is influenced by physicochemical properties, antimicrobials, and reuse frequency, with limited data available on postharvest washing practices in produce packinghouses.

Purpose: To assess postharvest water management practices in commercial fresh produce packinghouses via a survey tool.

Methods: A skip-logic, multiple response survey was developed in Qualtrics to collect data on postharvest washing practices from fresh produce packinghouses across the United States. Responses were summarized using frequency distributions, and differences by operation size based on annual sales were evaluated using the Rao-Scott corrected Chi-square test in JMP 19.0.

Results: A total of 114 valid responses were obtained, representing 28 states and the packing of 12 crop types. Water quality was assessed by most packers using *E. coli* microbiological testing (41%; n=103), with no differences observed across operation sizes (P>0.05). Recirculated or batch systems were frequently used in dump tanks, flumes, and

hydrocoolers, primarily employing chlorine, whereas single-pass systems were typically used in spray bars with peracetic acid as predominant antimicrobial. Sanitizer concentrations were most often monitored using test strips (42%; n=73); however, larger operations (annual sales > $1,000,000) used oxidation-reduction potential (ORP) probes and titration kits more frequently ($P<0.05$). Water reuse practices varied widely, with change intervals ranging from <4h to biweekly; however, daily changes were most common (59%; n=39). Water change decisions were primarily based on measured or visual turbidity (48%; n=26).

Significance: Data on washing practices across diverse operations help prioritize targeted interventions, resources, and research needs to improve produce safety.

P2-155: SURVIVAL OF ESCHERICHIA COLI O157:H7, LISTERIA MONOCYTOGENES, AND SALMONELLA ENTERICA AND GROWTH OF NATIVE MICROBIOTA ON WORK-IN-PROCESS FRESH-CUT CARROT AND ONION DURING STORAGE AFTER CHLORINE AND PERACETIC ACID WASHES

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Work-in-process (WIP) fresh-cut produce undergoes partial processing and temporary holding that can create conditions conducive to pathogen survival and growth.

Purpose: This study evaluated the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and native microbiota on WIP fresh-cut carrot and yellow onion under different storage temperatures after sanitization.

Methods: Carrot and onion were processed into WIP ingredients and inoculated with *E. coli* O157:H7, *L. monocytogenes*, or *S. enterica*. Inoculated WIP samples were either unwashed or washed with water, sodium hypochlorite (>10 ppm free chlorine), peracetic acid (80 ppm), and stored at 4, 8, or 12°C for seven days. Pathogen populations and native microbiota (mesophiles, psychrophiles, and yeast and mold) were enumerated using standard

microbiological methods. The effects of commodity type, sanitizer treatment, and storage temperature on microbial survival and growth were evaluated over time.

Results: Pathogen survival was significantly (P<0.05) influenced by storage temperature, washing, and WIP type. Washing with either water, chlorine or PAA significantly (p<0.05) reduced initial populations of all three pathogens. Washing with chlorine or PAA achieved >1.5 log reductions of *E. coli* O157:H7 on carrots and ca. 1.0 log of *L. monocytogenes* on onions. At 4°C, *S. enterica* on carrots declined by up to 2.27 log. Conversely, at 12°C, *S. enterica* grew by 3.45 log on onions. Chlorine suppressed *E. coli* O157:H7 growth on carrots even at 12°C (-0.63 log), whereas PAA-treated samples grew by 1.19 log. *L. monocytogenes* never proliferated on carrots. Native microbiota reached ca. 9.0 log on onions at 12°C, regardless of sanitization.

Significance: These findings identify the WIP holding temperature and time as a critical control point in minimizing pathogen growth on WIP fresh-cut produce items. Maintaining the cold chain and using appropriate sanitizers are key to produce safety.

P2-156: THE EFFECT OF ONION VARIETY ON SALMONELLA AND E. COLI O157:H7 IN DICED ONIONS

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Onions grown in different regions of the U.S. are exposed to different photoperiods depending on latitude. Notably, these onions vary in physicochemical properties and shelf life. We hypothesized that these differences would impact the survival and growth of *Salmonella* and *E. coli* O157:H7 in diced onions of different varieties.

Purpose: To determine the effect of onion variety on *Salmonella* and *E. coli* O157:H7 behavior on diced onions.

Methods: Diced onions (½ x ½ “, 50 g) were inoculated with a three-strain cocktail of *Salmonella* or *E. coli* O157:H7 (3.99 ± 0.25 log CFU/g), then incubated at 6, 7, 8, 9 and 10°C for 14 days and sampled on days 0, 3, 7, 10, and 14. The pH and total soluble solids were measured on days 0, 7 and 14. Microbiological data were analyzed by linear mixed-effects models with emmeans contrasts, and physicochemical data by three way ANOVA with emmeans ($p < 0.05$).

Results: The minimum temperature required for *Salmonella* growth was 8°C on both onion varieties, while *E. coli* O157:H7 grew at 8 or 9°C on short- and long-day onions, respectively. At 6°C, both *Salmonella* and *E. coli* counts were higher on short-day onions by day 14 (1.22 and 0.48 log CFU/g; $p<0.05$). For *E. coli*, levels were higher on short-day at 7°C on

days 10 and 14 (0.96 and 2.07 log CFU/g; $p<0.05$), and also higher at 8°C from day 7 onward (1.39 to 1.80 log CFU/g; $p<0.05$). Overall, pH increased, and the total soluble solids decreased during the incubation period.

Significance: Quantification of how *Salmonella* and *E. coli* O157:H7 behavior changes based on onion variety may help the industry better target risk mitigation activities to improve food safety.

Award Application: Developing Scientist Award

P2-157: THE POST-HARVEST ROOT SANITIZER TREATMENT ON SALMONELLA TYPHIMURIUM IN HYDROPONICALLY GROWN LIVING BASIL

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Hydroponically grown living basil is increasingly marketed for its extended freshness due to attached roots, which retain water and nutrients but may also accumulate pathogens capable of transferring to edible tissues.

Purpose: This study evaluated the efficacy of peracetic acid (PAA), lactic acid (LA), and sodium hypochlorite (NaClO) sanitizers against *Salmonella enterica* serovar Typhimurium inoculated at 6 $\log_{10}$ CFU/mL onto hydroponic living basil roots.

Methods: Inoculated living basil plant roots were dipped in PAA (40 or 80 ppm), LA (1% or 2%), or NaClO (100 or 200 ppm) for 30 or 60 seconds, rinsed in Dey-Engley neutralizing broth

for 1 minute, stored at 8°C for 14 days. Untreated inoculated roots served as controls. *S.* Typhimurium was enumerated on XLT4 agar at 0 h, 6 h, 12 h, 24 h, 48 h, 5 d, and 14 d.

Results: *Salmonella* population increased significantly during storage. At lower sanitizer concentrations (1% LA, 40 ppm PAA, 100 ppm NaClO), reductions ($p < 0.05$) were observed only through day 1, after which counts did not differ from controls. Higher concentrations (2% LA, 80 ppm PAA, 200 ppm NaClO) extended reductions through day 5 ($p < 0.05$), with no differences thereafter. By day 14, *Salmonella* increased by 2.44–2.71 $\log_{10}$ CFU/g for PAA, 2.61–2.62 $\log_{10}$ CFU/g for NaClO, and 1.94–3.60 $\log_{10}$ CFU/g for LA, compared with 0.60–0.92 $\log_{10}$ CFU/g in controls.

Significance: Contrary to expectations, *Salmonella* did not decline during storage and instead proliferated on living basil roots. These findings highlight the need for enhanced postharvest strategies to mitigate the food safety risks associated with marketing hydroponic herbs with roots attached.

P2-158: UNDERSTANDING AQUAPONICS: HOW FOOD SAFETY CONCERNS AND LABEL RECOGNITION SHAPE U.S. CONSUMER ACCEPTANCE

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Presentation type/Format: Poster

Category: Produce

Abstract:

Introduction: Aquaponics, which integrates hydroponics and aquaculture, has gained attention as a sustainable food production system, yet consumer awareness remains limited, and concerns about food safety persist.

Purpose: This study explores U.S. consumers' perceptions of aquaponics, focusing on their understanding and food safety concerns. It assesses consumers' recognition of existing food certification logos and evaluates their attitudes toward a hypothetical aquaponically grown logo.

Methods: A nationwide U.S. online survey (Purdue IRB-2023-1305) was administered via Qualtrics in October 2025. Participants were adults, primary grocery shoppers who had purchased fish and/or leafy greens in the past year. Descriptive statistics were computed using RStudio to determine frequencies and distributions of participants' responses.

Results: Among 2,011 survey respondents, 70% had never heard of aquaponics. After receiving a definition, 66% expressed food safety concerns. For fish (n=1205), primary concerns were bacterial contamination (78%), antibiotics (63%), and chemical use (62%). For produce (n=995), bacterial contamination was also the main concern (78%), followed by chemicals (67%), antibiotics (56%), and pesticides (51%). Limited familiarity with aquaponics

appeared to contribute to these concerns. Regarding labeling, 58% reported paying attention to certification logos, and 69% rated them as moderately to extremely important during grocery shopping. When presented with a hypothetical "aquaponics grown" logo, it was associated with sustainability (61% agree or strongly agree), or safety (57%), and 71% indicated that additional information on the logo's meaning should be provided at the point-of-sale.

Significance: This study illustrates major gaps in U.S. consumers' awareness and food safety perceptions related to aquaponics food systems and suggests that communication and clear labeling may help build consumers' trust and support informed purchasing decisions.

Award Application: Developing Scientist Award

P2-159: ANTIBIOTIC RESIDUES IN RAW COW MILK COLLECTED FROM SMALLHOLDER DAIRY PRODUCERS IN KASAMA AND MBALA, ZAMBIA

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The deposition of antibiotic residues in animal source foods such as milk has become a global public health threat and food safety concern.

Purpose: Assess antibiotic residues in milk samples among smallholder dairy farmers in Mbala and Kasama, Zambia with a view to ascertain its food safety and compliance with antibiotic withdraw period.

Methods: A cross-sectional study was conducted, in which 93 milk samples (54 from Mbala and 39 from Kasama) were randomly collected from lactating cows on 56 farms between May and June, 2025. The samples were analyzed using the Charm II assay for beta-lactams, tetracyclines, macrolides, sulfonamides, and aminoglycosides. A total of 100 mL of milk was collected in sterile plain tubes, placed in a cooler box with ice packs, and transported to the district laboratory's freezer and then delivered to the Central Veterinary Research Institute at (□18 to □20□C), where they were stored at □2□C. Statistical significance between districts was determined using Pearson's chi-square, and associations between a district and the occurrence of antibiotic residues in milk were evaluated using logistic regression. Data were analyzed using Stata 14.2 at a 95% confidence level (p = 0.05).

Results: A total of 91.4% (n = 85) of samples had antibiotic residues above EU/MRLs, with mean positive samples being 0.91 ± 0.28 and a significant association between a district and residue occurrence (OR = 0.086; p = 0.025). Approximately 44.1% of the samples had multiple antibiotic residues. Approximately 82.1% of samples from Kasama and 98.1% from Mbala had antibiotic residues (p = 0.006). Approximately 68.8% of samples had sulphonamides, and 58.1% macrolides, indicating their widespread use. Tetracyclines were 12.9%, beta lactams 9.7%, and aminoglycosides 2.2%.

Significance: These findings pose public health risks and food safety concerns among consumers like antimicrobial resistance, allergies, cancer among others.

Award Application: Developing Scientist Award

P2-160: THE ONE HEALTH LANDSCAPE OF BACTERIAL ANTIMICROBIAL RESISTANCE IN VIRGINIA: 2007–2021

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) is an escalating global concern under the One Health framework, driven by extensive antimicrobial use in human medicine and agriculture. Effective regional surveillance is critical for guiding strategies to curb this threat.

Purpose: This study examines AMR patterns in 1,824 bacterial isolates—including *Campylobacter* (99), *Escherichia coli* (1,485), *Listeria* (121), and *Salmonella* (119)—collected from farm animals, wildlife, environmental samples, and food sources in Virginia between 2007 and 2021.

Methods: Antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method with 12 FDA-approved antimicrobials across nine categories. Isolates were classified as susceptible, intermediate, or resistant; non-susceptibility included intermediate and resistant. Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more categories.

Results: 80% of isolates were non-susceptible to at least one antimicrobial. Non-susceptibility was highest in *Listeria* (99.2%), followed by *Campylobacter* (99%), *Salmonella* (93.2%), and *E. coli* (76.6%). Significant resistance ($p < 0.05$) was observed for ampicillin in *Campylobacter* (66.7%), streptomycin in *E. coli* (39.1%), nalidixic acid in *Listeria* (87.6%), and tetracycline in *Salmonella* (25.2%). No single antimicrobial was universally effective.

Significance: This research provides essential regional data that complements the FDA's NARMS program, strengthening AMR surveillance across food, livestock, wildlife, and environmental sources. While region-specific, the approach offers a scalable model for national monitoring under One Health. The widespread detection of broad-spectrum resistance underscores the urgent need for judicious antimicrobial use in healthcare and agriculture.

P2-161: UPSCALING THE PRODUCTION OF A NATURAL ANTIMICROBIAL PEPTIDE, PAENIBACILLIN, UTILIZING A FOAM SEPARATION-BASED BIOREACTOR

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The rising demand for clean-label foods favors natural antimicrobial preservatives over synthetic ones. Paenibacillin, a potent antimicrobial peptide produced by *Paenibacillus polymyxa* OSY-DF, could be a natural alternative. However, low productivity of the producer strain limits paenibacillin's industrial application. Utilizing foam fractionation technology in a bioreactor set-up could help upscale paenibacillin production, as paenibacillin can be concentrated by foam fractionation due to its amphiphilic nature.

Purpose: Optimizing bioreactions in a custom-built foam separation-based bioreactor for increasing paenibacillin production.

Methods: A 2-L bioreactor designed with a foam collector was used to optimize paenibacillin production. Tryptic soy broth+0.6% yeast-extract (TSBYE, 400 or 1000mL) was inoculated with a 24hrs culture of *P. polymyxa* OSY-DF (0.1 or 1%). Bioreactor process parameters were set at 0.8L/min airflow, 450rpm stirring, and 30°C. Throughout the 24hrs bioreaction run, bulk medium was sampled using a fraction collector, and foam was collected separately. Paenibacillin concentration in bulk medium and foam was quantified using the agar-spot antimicrobial bioassay with *Listeria innocua* as indicator.

Results: The foam collected from the bioreactor using 400ml TSBYE and 1%

inoculum produced high paenibacillin titer (64ug/ml) compared to the bulk medium (4ug/ml). The foam-based paenibacillin yield was 1280ug/20ml. Paenibacillin was detected in the bulk medium starting at 12.5hrs of incubation. No detectable paenibacillin activity was observed when 1000mL culture and 0.1% inoculum was used. The foam separation-based bioreactor substantially increased paenibacillin yield. Developing a continuous foam-separation bioreactor to prolong and further increase paenibacillin yield is underway.

Significance: Optimizing and maximizing the production of paenibacillin will speed up its application as a natural preservative for maintaining food safety.

Award Application: Developing Scientist Award

P2-162: PRETREATMENT OF ESSENTIAL OILS TO REDUCE ODOR WITHOUT IMPACT ON ANTIMICROBIAL EFFECTIVENESS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Essential oils (EOs) have been intensively studied as antimicrobial agents for food preservation. They can be directly applied to food or encapsulated into coatings and films to inactivate foodborne pathogens or spoilage microorganisms. However, their strong unpleasure odor negatively impacts consumer acceptance.

Purpose: The purpose of this study was to investigate the effectiveness of two pretreatments on the odor reduction and antimicrobial activity of three essential oils.

Methods: Allyl isothiocyanate (AIT), Carvacrol (CAR) and Cinnamaldehyde (CIN) were utilized in their original form (O), autoclaved (121^{o}C for 15 min) (A), or vacuumed evaporation (60^{o}C for 12h) (V). Their antimicrobial effectiveness was evaluated against *E. coli* (G-), *Listeria innocua* (G+), and fungi by two methods. Liquid Method: 100 µL of EO was added to 10 ml inoculated peptone water and reductions in microbial population were determined; Headspace method: 100 µL of EO was applied to a paper film which was placed on the inside of a petri dish covering an inoculated agar plate, and the colony forming density on the agar plate was determined. Odor was estimated by sensory smell evaluation.

Results: Both pretreatments effectively reduced the odor of the three EOs. Both pretreatments also significantly (p<0.05) reduced microbial populations in greater numbers

in liquid media than their original EOs. Taking *Listeria* treated by autoclaved EOs as an instance, the reductions achieved were 3.28 and 5.70 log CFU/ml by AIT(O) and AIT (A), 6.11 and 6.70 log CFU/ml by CAR (O) and CAR (A), and 1.79 and 3.20 log CFU/ml by CIN (O) and CIN (A), respectively. Similar results were obtained using the headspace method.

Significance: This study demonstrates a creative approach to apply EOs without a strong odor in foods to enhance food safety and reduce food spoilage. Future studies will investigate the mechanisms behind the enhanced antimicrobial activities after pretreatments.

P2-163: NOVEL PEPTIDES GENERATED BY DAIRY FERMENTATION EXHIBIT ANTIBACTERIAL ACTIVITY AND GUT MICROBIOTA MODULATION

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The overuse of antibiotics and chemical disinfectants represents a major global challenge, and there is an increasing demand for natural, sustainable alternatives capable of controlling pathogenic microorganisms while preserving or promoting a healthy human microbiota. Bioactive peptides generated through microbial fermentation have emerged as promising candidates to address these challenges, in line with One Health principles.

Purpose: This study aimed to characterize antibacterial activities and gut microbiota modulation potential of peptides produced through a novel fermentative hydrolysis process.

Methods: Fermentation was conducted in milk for 48 hours, followed by removal of coagulated proteins and isolation of peptide-rich fractions by ultrafiltration. Antibacterial activity was evaluated against clinically and food-relevant pathogens, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157, and *Bacillus cereus*,

using broth microdilution assays to determine minimum inhibitory concentrations (MICs). The ability of the peptides to modulate the gut microbiota was assessed *in vivo* in a murine model through 16S rRNA gene sequencing.

Results: The fermentation-derived peptides exhibited dose-dependent antibacterial activity against all tested pathogens, with MIC values ranging from 3–6 µg/mL. In vivo administration resulted in a marked increase in *Bacteroidota*, suggesting enhanced polysaccharide fermentation capacity and a potentially improved metabolic profile. Concurrently, a clear reduction in *Deferribacterota* and *Firmicutes* was observed, leading to a favorable modulation of the *Firmicutes/Bacteroidota* ratio, commonly associated to improved metabolic status. Overall microbial diversity was also enhanced.

Significance: These findings demonstrate that fermentation-derived bioactive peptides display a dual functional profile, combining effective pathogen inhibition with beneficial modulation of the gut microbiota.

P2-164: ALGINATE-INULIN HYDROGEL BEADS FOR DELIVERY OF BACTERIOPHAGE AGAINST CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Klebsiella pneumoniae* is a carbapenem-resistant WHO priority pathogen. Its increasing detection in food raises food safety concerns by promoting intestinal colonization and infection in immunocompromised hosts.

Purpose: This study developed a bacteriophage (phage) delivery system using calcium alginate-inulin hydrogel beads as an antibiotic alternative to mitigate intestinal colonization by carbapenem-resistant *K. pneumoniae*.

Methods: A lytic phage, HJK2, isolated from influent water, was characterized by host range analysis (25 strains), antibacterial activity at MOIs of 0.1 and 1, and stability at pH 2–11 and -18–80℃. Genome sequencing used Illumina MiSeq. Phage (8.9 log PFU/g) was encapsulated into ~2 mm hydrogel beads of 1.5% (w/v) sodium alginate and 3–6% (w/v) inulin via extrusion in 0.1 M $CaCl_2$. Encapsulation efficiency was determined after dissolution in sodium citrate buffer. Phage release was evaluated at 37°C, pH 7 (120 rpm, small intestine; 50 rpm, colon). Viable phages were quantified by double-layer agar assay (n=3). Data were analyzed by ANOVA and Duncan's test ($p < 0.05$).

Results: Phage HJK2 formed plaques (2 mm) against *K. pneumoniae* NMS 1120 and inhibited bacterial growth in 2 h at an MOI of 0.1 with activity maintained for 9 h. The phage remained stable at pH 4–11 and -18–50°C. Genome analysis revealed a 40,685 bp dsDNA genome without toxin or lysogeny genes. Calcium alginate-inulin beads containing 4% (w/v) inulin (AGI4) showed the highest encapsulation efficiency (8.7 log PFU/g). Under simulated intestinal conditions, AGI4 released 0.78 log PFU/g less phage in the small intestine but 0.98 log PFU/g more phage in the colon than alginate-only beads, indicating colon-targeted release.

Significance: This study demonstrates a microbiome-conscious phage delivery system using alginate-inulin hydrogel beads as an antibiotic alternative, reducing carbapenem-resistant *K. pneumoniae* and enhancing food safety and public health.

Award Application: Developing Scientist Award

P2-165: IMPACT OF NOVEL LACTOFERRIN, LACTOPEROXIDASE, AND THEIR COMBINATIONS ON INACTIVATION OF FOODBORNE PATHOGENS AND SPOILAGE MICROORGANISMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Derived from milk, lactoferrin, and lactoperoxidase could enhance the safety and quality of an array of products against microbial pathogens and spoilage organisms.

Purpose: The current study investigated the impact of lactoferrin and lactoperoxidase at the level approved by the GRAS-list for reducing nontyphoidal *Salmonella* serovars, *Listeria monocytogenes*, Shiga toxin-producing *E. coli*, and background microbiota of dairy-based products, and in a buffered environment.

Methods: The study is a complete randomized block design with two biologically independent replications. Each block was further consisted of three replications. The antimicrobial compounds were tested alone and in combination at levels of up to 300 ppm in buffered medium, 3% milk, and liquid whey. Samples were neutralized on D/E broth prior to microbial analyses. The results were statistically analyzed using Tukey- and Dunnett ’s-

adjusted ANOVA. Exposure times were up to 48 hours, and samples were plated on non-selective and selective media.

Results: Both lactoferrin and lactoperoxidase, and their combination, were effective ($P<0.05$) to reduce up to 90% of inoculated Gram-negative pathogen cocktails (nontyphoidal *Salmonella* serovars and *E. coli* O157: H7), Gram-positive pathogen cocktail (*Listeria monocytogenes*), and background microbiota of milk and liquid whey. Separately and in combination at 300 ppm concentration, they additionally illustrated effectiveness for the reduction ($P<0.05$) of the pathogens in phosphate-buffered saline.

Significance: Our results illustrate that application of the novel lactoferrin and lactoperoxidase compounds derived from milk could lead to up to >90% reduction in both spoilage microorganisms and common pathogens of public health concern, and thus could be used in the context of multiple hurdle technology for ensuring the safety and enhanced quality of dairy-based products.

Award Application: Developing Scientist Award

P2-166: SPOILAGE CHALLENGE STUDIES OF HIGH BRIX SAUCES TREATED WITH NATURAL PRESERVATIVE PROVONTAGE® 399

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: High brix sauces typically have a low risk of microbial spoilage due to their low pH and low a_w, however some lactic acid bacteria (LAB) and yeast can grow.

Purpose: A teriyaki sauce (35° brix, pH 3.7, a_w 0.87) with spoilage identified as *Lactobacillus parabuchneri* and a duck sauce (65° brix, pH 3.5, a_w 0.85) suspected to be spoiled with yeast, were made new with natural preservative, proVONTAGE® 399 @ 0.5% (T), and without (UT) and challenged with their respective spoilage organisms.

Methods: Sauces were portioned for challenge testing over 18 days in duplicate for teriyaki sauce and over 24 days in triplicate for duck sauce. Teriyaki sauce was challenged with *L. parabuchneri* at 4.4E+07 CFU/mL and repeated at 2.5E+08 CFU/mL. The duck sauce was challenged with a 2.4E+04 CFU/mL cocktail of three yeasts (*Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and Z. *bailii*), which are known to spoil high brix foods. All microbial counts were enumerated in duplicate/sample/day on MRS agar for LAB or PDA for yeasts.

Results: In teriyaki sauce, D0 counts averaged 1.6E+05 CFU/mL and rose to 1.2E+06 CFU/mL by D18 in UT samples, whereas T samples did not rise above 1.0E+02 CFU/mL through D18

(p<0.05). When repeated with a higher inoculum, UT samples averaged 1.4E+05 CFU/mL at D0 and stayed above 1E+04 CFU/mL through D18 while T sample counts fell below detection (<10 CFUmL) at D5 through D18 (p<0.05). In duck sauce, yeast counts on D0 averaged 6.2E+02 CFU/mL in UT samples and 4.6E+02 CFU/mL in T samples (NS). The UT samples averaged 1.6E+06 CFU/mL on D24, but T samples averaged <10 CFU/mL at D9 through D24 (p<0.05).

Significance: The natural preservative significantly reduced both yeast and LAB growth in high brix sauces.

P2-167: CLEAN-LABEL REPLACEMENT OF POTASSIUM SORBATE AND SODIUM BENZOATE IN READY-TO-EAT DELI SALADS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Producers of ready-to-eat (RTE) deli salads are moving away from conventional preservatives like potassium sorbate and sodium benzoate due to clean-label market demands.

Purpose: Evaluate clean-label alternatives for shelf-life extension and *Listeria* control in RTE deli salads.

Methods: Three types of salad: coleslaw, potato salad, and chicken salad were prepared with conventional controls (0.3% potassium sorbate + sodium benzoate for coleslaw and potato salad; 0.75% market control for chicken salad) and a clean-label treatment (0.8% D100 + 0.2% 5400L). Samples stored at 4°C for 70 days, and analyzed for aerobic plate count (APC), lactic acid bacteria (LAB), and yeasts/molds. Challenge studies were conducted with *Listeria monocytogenes* inoculated at 2–3 log CFU/g ; data analyzed by ANOVA ($p < 0.05$).

Results: In chicken salad, APC and LAB remained at or below ~3 log CFU/g, with clean-label treatments 0.5–1 log lower than market control. Yeast counts in market control samples increased to ~6 log by Day 40, while clean-label treatments remained undetectable (P>0.05). Both treatments reduced *Listeria* to below detection by Day 5, with levels remaining below the inoculum thereafter (□3 log). For coleslaw and potato salad, clean-label treatments matched conventional control, maintaining APC and LAB below 2–3 log. Yeast counts in coleslaw dropped below detection by Day 35 for both treatments, while potato salad remained below 4 log. *Listeria* in coleslaw exhibited a cidal effect by Day 4 and stayed undetectable, while potato salad-maintained levels below 2 log through Day 70.

Significance: Clean-label system matched benzoate/sorbate and outperformed market control in limiting spoilage and *Listeria* in deli salads, confirming its suitability for reformulation.

P2-168: CLEAN-LABEL ALTERNATIVES TO SODIUM BENZOATE AND POTASSIUM SORBATE FOR SHELF-LIFE EXTENSION AND LISTERIA CONTROL IN READY-TO-EAT CHICKEN SALAD

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: There is an increasing demand the removal of conventional preservatives such as sodium benzoate and potassium sorbate from ready-to-eat (RTE) products, creating a need for effective clean-label solutions.

Purpose: Evaluate clean-label antimicrobials as alternatives to sodium benzoate and potassium sorbate for shelf life and *Listeria monocytogenes* control in RTE chicken salad.

Methods: Chicken salad was formulated with a control (0.08% sodium benzoate + potassium sorbate) and two clean-label treatments: Treatment 1 (0.4% Flavoset 8300L + 0.5% DuraFresh 5970) and Treatment 2 (0.2% NouriShield D4040 + 0.4% Flavoset 8300L + 0.5% DuraFresh 5970). Samples were stored at 4°C for 35 days. Microbial analyses included aerobic plate count (APC), lactic acid bacteria (LAB), yeasts/molds, psychrotrophs, and presumptive

Pseudomonas. Challenge studies were conducted using a five-strain *L. monocytogenes* cocktail (2 log CFU/g inoculation) and LAB cocktail (3 log CFU/g inoculation). Data were analyzed by one-way ANOVA (p □ 0.05).

Results: Both clean-label treatments-maintained APC, LAB, and *Pseudomonas* below detection throughout storage, whereas control samples reached 2–3 log CFU/g. Psychrotrophs in control samples started at ~5 log CFU/g and remained high (4.5-5 log), while treatments stayed below detection till Day 7, and Treatment 2 increasing slightly (2–3 log) till Day 35. In challenge studies, both treatments produced an immediate cidal effect against *L. monocytogenes* (~1 log reduction at Day 0 and ~2 log by 48 h). For LAB inoculation study, both treatments achieved a 3 log cidal effect by 48 h (from 3 to log to below detection), while the control showed minimal change, remaining near 3 log till Day 12 and declining to ~2 log by end of shelf life (Day 35).

Significance: Clean-label antimicrobials outperformed conventional preservatives in controlling spoilage and *Listeria* in RTE chicken, supporting their adoption to meet clean-label market requirements.

P2-169: CLEAN-LABEL, BROAD-SPECTRUM ANTIMICROBIALS PRODUCED BY FERMENTATION OF FOOD INGREDIENTS USING THE GRAS FUNGUS ASPERGILLUS ORYZAE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Growing consumer demand for clean-label foods has intensified efforts to replace synthetic preservatives with natural alternatives. Previous work showed that the GRAS fungus *Aspergillus oryzae* produces a broad-spectrum antimicrobial fermentate in malt extract broth, termed Natural Protectants (NPs), but the substrates inducing antimicrobial production remained unclear, limiting clean-label translation.

Purpose: This study aimed to identify food-grade substrates that induce antimicrobial production by *A. oryzae* and to assess the activity of the resulting clean-label antimicrobials against major foodborne pathogens.

Methods: NPs were fermented from defined media or food-grade ingredient solutions (20 g/L) with *A. oryzae* NRRL 3483 for six days. Individual components of malt extract broth, including yeast extract, maltose, dextrose, and malt extract, were fermented separately to

identify inducers of antimicrobial activity. Additional food-grade substrates were evaluated, including whey protein concentrate, plant-based protein blends, bone broth, and essential amino acid mixtures. Fermented products were extracted with ethyl acetate and screened using disk diffusion assays against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*.

Results: Yeast extract was identified as an inducer of antimicrobial secondary metabolites production by *A. oryzae*. Fermented yeast extract exhibited broad-spectrum inhibitory activity against all tested pathogens and reduced *E. coli* O157:H7 strain by 3-log within 48 hours, suggesting bactericidal effect. Antimicrobial activity was consistently observed following fermentation of protein- and amino acid-rich food ingredients, with inhibition zone ranging from 13 to 23 mm against *S. aureus.*

Significance: GRAS fungus *A. oryzae* can reliably produce antimicrobials from diverse food substrates. Future work will characterize the antimicrobial molecules (aspergillic acid analogs) from *A. oryzae*.

Award Application: Developing Scientist Award

P2-170: EFFICACY OF A NATURAL VEGETABLE FERMENTATE FOR CONTROLLING MICROBIAL SPOILAGE IN PET FOOD

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Microbial contamination—particularly from spoilage microorganisms—remains a major challenge in the pet food industry, leading to shorter shelf life, recalls, and reduced consumer trust. As natural preservation approaches gain traction, it is essential to rigorously assess their effectiveness and their impact on product quality.

Purpose: This study presents an innovative strategy for controlling microbial spoilage in pet food, evaluating the antimicrobial efficacy of a novel natural preservative derived from fermented vegetable juice. Challenge tests were carried out on two types of application models that are prone to yeast and mold contamination: viscera-based and non-meat liquid palatants.

Methods: In the experimental design, both treated and untreated liquid palatants (incorporating vegetable-derived fermentate at 0.5% to 1%) were inoculated with a blend of 6 yeast and 6 mold species frequently involved in the spoilage of pet food products. A positive control using potassium sorbate at 0.3% was also included. Inoculation was standardized at 10^4 CFU/g, with all samples incubated at 25°C. Yeast and mold populations were monitored via microbial plate counts over a 90-day period.

Results: Findings from the meat-based palatant model indicated that vegetable fermentate

at a dosage of 0.75% demonstrated comparable efficacy to potassium sorbate in inhibiting yeast and mold growth. In the no-meat palatant model, a lower dosage (0.5%) yielded similar inhibition results. Notably, both models exhibited a sustained reduction in yeast and mold populations (a 5-log reduction within the first 30 days).

Significance: These results suggest that this innovative, vegetable-based fermentate provides effective microbial control for pet food products, supporting the industry's transition toward natural preservation methods while upholding high standards of product quality and consumer satisfaction.

P2-171: EVALUATION OF THE EFFICACY OF CALCIUM PROPIONATE AND VINEGAR-BASED SOLUTIONS IN EXTENDING THE SHELF LIFE OF WHITE AND WHEAT BREAD

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Growing consumer demand for clean-label bakery products is reshaping preservation strategies. Combining a clean-label solution of calcium propionate and vinegar offers a natural approach to mold inhibition while maintaining product quality.

Purpose: Evaluate the efficacy of clean-label calcium propionate (CalPro) and vinegar solutions as a preservation system for mold inhibition on white and wheat bread.

Methods: Loaves of wheat and white bread were used to evaluate the impact of varying levels of vinegar and CalPro solutions on mold growth. Four treatments of loaves were prepared for both bread types: (1) positive control (conventional CalPro solution), (2) negative control (no preservative solution), (3) 12% vinegar and CalPro solution, and (4) 15% vinegar and CalPro solution. The treatments were spot inoculated with common bakery molds *(Aspergillus niger, Cladosporium cladosporioides, Penicillium commune*, and *Penicillium chrysogenum*), sealed, and stored at 20°C for >30 days. Mold growth was monitored daily; differences among the treatments were determined using one-way ANOVA $P<0.05$.

Results: Negative controls showed mold growth within 5 days (wheat) and 6 days (white). The positive control and fermented wheat flour solutions (T3 and T4) significantly ($P< 0.05$) extended mold-free shelf life for *A. niger*, *C. cladosporioides*, and *P. chrysogenum* (>30 days) in both bread types. *Penicillium commune* exhibited extended inhibition, with T4 achieving >30 days in wheat bread and both T3 and T4 >30 days in white bread, compared to 12 days for positive control in wheat.

Significance: Combining clean-label calcium propionate with vinegar effectively extended mold-free shelf life in both wheat and white bread, offering a natural alternative to conventional preservatives that supports consumer demand for clean-label bakery products.

P2-172: EVALUATION OF AN ORGANIC AND CLEAN-LABEL FERMENTED WHEAT FLOUR SOLUTION FOR MOLD INHIBITION IN WHEAT BREAD

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Mold spoilage in bread contributes to significant food waste and economic loss. Consumers increasingly demand natural preservation systems, driving interest in clean-label and organic alternatives to conventional preservatives.

Objective: Evaluate the impact of Kerry's fermented wheat flour solutions – organic and clean-label - extending mold-free shelf-life in wheat bread compared to conventional calcium propionate.

Materials: Two sets of wheat bread loaves were prepared: (1) organic formulation with 1.4% organic fermented wheat flour, and (2) clean-label formulation with 1.4% fermented wheat flour solution. Each set included a positive control (conventional calcium propionate) and a negative control (no preservative). Loaves were spot inoculated with common bakery molds (*Aspergillus niger, Cladosporium cladosporioides, Penicillium commune*, *Penicillium Chrysogenum*), sealed, and stored at 20°C for >30 days. Mold growth was monitored daily; differences among the treatments were determined using one-way ANOVA $P<0.05$.

Results: Negative controls showed mold growth within 3-8 days. Both organic and clean-label fermented wheat flour treatments significantly ($P<0.05$) extended mold-free shelf-life (>30 days) for *A. niger, C. cladosporioides, P. commune*, and *P. chrysogenum*, comparable to conventional calcium propionate.

Significance: Organic and clean-label fermented wheat flour solutions demonstrated equivalent efficacy to conventional preservatives, supporting natural preservation strategies that reduce food waste and meet consumer demand for clean-label and organic bakery products.

P2-173: LACTIC ACID BACTERIA FERMENTATION OF SORGHUM ENHANCES ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SORGHUM

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Sorghum grain is increasingly used in poultry production; however, its unprocessed form exhibits limited antimicrobial and antioxidant activity. Fermentation enhances the availability of bioactive compounds yet its impact on sorghum antimicrobial activity across microbial groups remains unexplored.

Purpose: This study aimed at evaluating lactic acid bacteria (LAB)-mediated fermentation as a bioprocessing strategy to enhance sorghum's bioactive properties.

Methods: Sorghum varieties (n = 3) were anaerobically fermented at 37°C for 72 h using LAB isolated from fermented food and identified by phenotypic and 16S rRNA sequencing methods. Fermentation was monitored via pH, titratable acidity (TTA), and LAB counts. Sorghum extracts and cell-free fermentation supernatants were evaluated for antimicrobial activity against feline calicivirus (FCV) and different bacteria groups. Antioxidant activity of fermented sorghum was assessed by DPPH assay in triplicate and analyzed by independent *t*-test.

Results: LAB-fermentation reduced pH from ~6.3 to ~3.2, increased TTA to ~2.82% lactic acid (w/v), and raised LAB counts to ~9 $\log_{10}$ CFU/g compared to unfermented control. Sorghum extracts reduced FCV by 1.75 $\log_{10}$ $TCID_{50}$, decreased *Salmonella enterica*, *Staphylococcus*

aureus and *Listeria monocytogenes* counts by 6.69±0.78, 6.89±0.29, and 5.23±0.78 $\log_{10}$ CFU/ml, respectively, with no activity on *Escherichia coli*. In contrast, fermented supernatants reduced counts of all the bacteria tested including *E. coli* by ~8 $\log_{10}$ CFU/ml. Antioxidant activity of fermented samples reached ~41% DPPH inhibition compared to unfermented controls ($p < 0.05$).

Significance: These findings show that LAB fermentation enhanced antimicrobial and antioxidant activities with potential application as a non-antibiotic intervention in poultry.

Award Application: Developing Scientist Award

P2-174: MICROGREEN PRODUCTION: IMPACT OF SANITIZATION TREATMENTS ON GERMINATION PERCENTAGE AND MOLD SUPPRESSION ON ALLIUM PORRUM L. SEEDS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Seed sanitization is critical for microgreen production, it helps prevent growth of microorganisms that can negatively impact the quality of microgreens and consumer health. Improperly sanitized seeds can harbor foodborne pathogens which can cause seedling diseases, reduce seedling vigor, and ultimately impact the final product. Proper sanitization often includes a combination of techniques to mitigate risk.

Purpose: The experiment determined the impact of sanitization methods on the germination percentage and mold suppression on leek (*Allium porrum* L.) seeds used for microgreen production.

Methods: Leek seeds (100 seeds) were exposed to various treatment concentrations: Hydrogen Peroxide (HP) at 9% for 10 min, Hot Water (HW) at 85 ± 2°C for 10 sec, Tsunami (TS) at 0.006% for 5 min, Vinegar (VN) at 2% for 15 min, and Deionized Water (DI) soaked for 10 minutes. After treatment exposure, seeds were strained and rinsed with DI water and placed in sterile Petri dishes containing a felt mat soaked with 2 ml of DI water. For germination, treated seeds were incubated at 24 ± 2°C with 70–80% relative humidity and no light

exposure. Germination percentage and mold detection were recorded daily for 5 days.

Results: An initial evaluation was performed to determine the effectiveness of treatments to suppress fungal growth by measuring the proportion of seeds from which fungal growth was observed and the proportion of leek seeds that germinated. Germination percentages on day 5 were: 75.5% (HP), 74.5% (HW), 68% (TS), 63.3% (DI), and 60.33% (VN). The HW treatment was the only treatment to completely suppress mold growth ($P<0.05$). On the other hand, HP, VN, TS, and DI treatments mold was detected Day 1of germination ($P>0.05$).

Significance: Seed sanitization is a critical aspect of microgreens production, as it helps to ensure that microgreens are safe for consumption, however impact on germination rates and mold suppression should be considered.

P2-175: IMPACT OF RELATIVE HUMIDITY ON CHLORINE DIOXIDE GAS EFFICACY AGAINST CRONOBACTER SAKAZAKII ON STAINLESS STEEL SURFACES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Cronobacter sakazakii* has shown sanitation challenges in low-moisture environments due to its desiccation tolerance and biofilms that can protect the bacteria from the action of disinfectants. Chlorine dioxide (ClO_2) gas offers a promising dry sanitation solution for microbial control.

Purpose: This study evaluated the efficacy of ClO_2 gas in inactivating *C. sakazakii* on stainless steel surfaces at three relative humidity (RH) levels – low (22.7–26.7%), medium (37.0–39.9%), and high (56.7–72.5%). The objective was to determine the impact of RH on microbial reduction, aiming to identify parameters for effective sanitation in dry manufacturing environments.

Methods: Stainless steel coupons were inoculated with a cocktail of five *C. sakazakii* strains and air-dried for 2 h to simulate dry processing conditions. Coupons were exposed to ClO_2

gas (1,550 PPM-H; 2 mg/L) using the Minidox M system. Each RH condition was tested in duplicate**.** After ClO_2 treatment, coupons were added to 20 ml Butterfield's Phosphate Buffer (BPB) with Tween80, hand massaged, and plated onto tryptic soy agar. Plates were incubated at 37°C for 24 h.

Results: After 2 h of air-drying post-inoculation, microbial loads ranged from 7.2–8.0 log CFU/coupon. Average reductions (±SD) were 3.2 ± 0.2 log CFU/coupon at low RH, 4.5 ± 0.5 log CFU/coupon at medium RH, and 7.4 ± 0.2 log CFU/coupon at high RH (complete inactivation). Results indicated ClO_2 can achieve measurable reductions across different RH ranges similar to those in dry processing facilities.

Significance: Although high inoculation levels were used as worst-case scenarios to determine maximum reductions, other parameters may influence overall efficacy. These findings help establish a basis for optimizing treatment parameters to improve microbial control and provide options to incorporate ClO_2 treatment as clean breaks within a dry manufacturing environment.

P2-176: MILK-DERIVED LACTOPEROXIDASE CAN AUGMENTING DECONTAMINATION EFFICACY OF HIGH-PRESSURE PROCESSING AGAINST C. SAKAZAKII IN INFANT FORMULA

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Category: Antimicrobials

Abstract:

Introduction: Application of high-pressure processing is gaining increased adoption in various sectors of the food industry. The addition of novel bioactive compounds could augment the efficacy of this treatment and further protect commodities during shelf life and against post-processing cross-contamination.

Purpose: Current study investigated the impact of a novel milk-derived lactoperoxidase compound for enhancing the effectiveness of high-pressure processing against *C. sakazakii* in infant formula.

Methods: A four-strain cocktail of the pathogen was used to inoculate powdered infant formula and after reconstitution, the product was exposed to mild hydrostatic pressure of 300 MPa with and without 1.5% (w/v) lactoperoxidase. The study was a randomized complete block design, with two biologically independent repetitions as blocking factors, each

consisting of three replications. The study is statistically analyzed using Tukey-adjusted ANOVA. All treatments were conducted at 25°C in a high-pressure processing chamber surrounded by a stainless steel jacket connected to a circulating water bath.

Results: Treatments with 300 MPa of hydrostatic pressure alone, resulted in no reduction (P > 0.05) of the pathogen after 3 minutes and resulted in nearly 2 log reduction (P<0.05) of the pathogen after 7 minutes. Addition of 1.5% lactoperoxidase to great extent augmented the decontamination efficacy of the treatment at 300 MPa, resulting in significant reductions (P<0.05) after 1, 3, and 7 minutes of treatment, reducing >99.9% of the inoculated pathogen.

Significance: Results of the current study, thus, indicate lactoperoxidase is an effective antimicrobial compound to enhance the effectiveness of high-pressure processing against *C. sakazakii,* an important and opportunistic pathogen of public health concern.

Award Application: Undergraduate Student Award

P2-177: WITHDRAWN

WITHDRAWN

P2-178: IDENTIFICATION OF SYNERGISTIC BINARY ESSENTIAL OIL COMBINATIONS WITH IN VITRO ACTIVITY AGAINST SALMONELLA AT PH 5–7 ACIDIFIED WITH LACTIC ACID

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Essential oils (EOs) exhibit antimicrobial activity against *Salmonella* but can negatively impact organoleptic properties of the product. Combining EOs at acidic pH may increase their efficacy and thus reduce their application concentrations and negative organoleptic impacts in food systems.

Purpose: To evaluate synergy in binary combinations of EOs: cinnamon bark (CNB), clove bud (CB), peppermint (P), rosemary (R) and thyme (T), in tryptic soy broth (TSB) at pH 7.0, 6.0 and 5.0 against *Salmonella*.

Methods: A *Salmonella* cocktail was prepared by combining equal portions of five strains

implicated in produce-related outbreaks. TSB (pH = 7.4) was adjusted with lactic acid to pH 7.0, 6.0 or 5.0 when mixed with EO or water. A checkerboard assay was used to study EO interactions, where one EO was diluted along rows while another EO was diluted along columns at 1X, 1/2X, 1/4X, and 1/8X of their minimum inhibitory concentrations (MICs). Each well (200 µL total) consisted of: 100 µL double strength TSB, 20 µL of 6.0 log CFU/mL inoculum, and the balance made of distilled deionized water and EO stock solutions to achieve the desired concentrations. After incubation at 37°C for 24 h, the lowest antimicrobial concentration with DOD_{630nm} below 0.05 was recorded as the MIC of each EO combination. The Fractional Inhibitory Concentration Index (FIC_I) was calculated from the summation of the ratios of MICs of EOs in combination to individual EO MIC. The synergistic, additive, and antagonistic interactions corresponded to FIC_I of <0.5, 0.5–1.5, and >1.5, respectively. Each experiment consisted of two biological and technical replicates (n=4).

Results: Synergy (FIC_I = 0.28–0.45) was found in 2/10 combinations at pH 7.0 (CNB-CB and P-R), and 3/10 (CB-T, P-R, T-R) at pH 5.0, with the remaining EO combinations showing additive effects.

Significance: Combining EOs and acidic pH demonstrates the potential for synergistic effects in EO combinations in food systems.

Award Application: Developing Scientist Award

P2-179: NOVEL COMPOUNDS PLAY A SIGNIFICANT ROLE IN REDUCING THE SURVIVAL OF PENTADRUG-RESISTANT SALMONELLA TYPHIMURIUM

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: The evolution of antibiotic resistance in foodborne *Salmonella* is a significant concern for food safety due to the lack of effective control strategies.

Purpose: To evaluate the efficacy of 4-hydroxycinnamic acid (4-HCA) and novel synthetic compounds in mitigating drug-resistant *Salmonella* Typhimurium.

Methods: We used minimal inhibitory concentrations (MIC), checkerboard, and time-kill kinetic assays to assess the antimicrobial activity of 4-HCA and 270 synthetic compounds. The penta-resistance *S.* Typhimurium DT104 was used as our model organism. Testing was also

performed on an antibiotic-sensitive *Salmonella* Typhimurium strain ST4/74 to compare efficacy. Appropriate uninoculated and antibiotic controls were used in all assays. The experiments were always performed in biological triplicates, and Student's *t*-test was used to assess statistical significance in the kinetic growth assays. Additionally, the efficacy of 4-HCA was evaluated against *Escherichia coli* strains to assess their antimicrobial potential toward other microorganisms.

Results: 4-HCA exhibited strong synergy with chloramphenicol by inhibiting the survival of *Salmonella* Typhimurium DT104. Screening of the 270 synthetic compounds revealed varied efficacy, with several compounds significantly reducing bacterial survival at low concentrations. Testing the antibiotic-sensitive strain ST4/74 showed similar compound activity, indicating their effect was independent of the resistance profile. Selected compounds also enhanced the effectiveness of conventional treatments by further lowering bacterial survival, not only for *Salmonella* strains but also for *E. coli*.

Significance: 4-HCA and selected compounds can reduce *Salmonella* survival, offering promising tools to enhance food safety and guide new contamination control strategies.

Award Application: Developing Scientist Award

P2-180: MONITORING ANTIBIOTIC-RESISTANT SALMONELLA IN POULTRY CARCASS RINSES THROUGHOUT THE UNITED STATES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) in foodborne pathogens is a public health concern due to the risk of exposure to resistant pathogens. Poultry is the most consumed animal protein in the U.S. and has been implicated in many cases of salmonellosis.

Purpose: To survey the incidence of antibiotic-resistant *Salmonella* from poultry across the United States.

Methods: The profiles of antibiotic-resistant *Salmonella* from chicken and turkey carcass rinses were evaluated using the FSIS data (2015–2023) and compared with retail isolates using the Sensititre system. Pearson's Chi-square test was used to analyze the data in SAS (p□0.05).

Results: A total of 3,650 (chicken) and 101 (turkey) *Salmonella* were evaluated, with a higher prevalence (p<0.05) in the South (56%) for chicken and the Midwest (57%) for turkey. Overall, 63% chicken and 57% turkey isolates were AMR, with the highest in the Northeast (chicken) and the South (turkey). For both, 30% were multi-drug resistant (MDR), and this varied (p<0.05) across all regions for chicken but was similar for turkey. There were 24 distinct

resistant serotypes identified for each poultry, with *S*. Kentucky (chicken) and *S*. Reading (turkey) being the highest, while *S*. Infantis and *S*. Reading displayed the most MDR. Resistance patterns showed high levels of streptomycin and tetracycline with a distinct pattern for penicillins, cephems, and quinolones in the South (chicken) and Midwest (turkey). The retail isolates showed 82% (79/96) AMR and 67% (53/79) MDR, with resistance to folate pathways and quinolones common to these isolates, but not to therapeutic antibiotics like azithromycin. The overall resistance to quinolones in both datasets remains concerning.

Significance: This data provides a critical insight into the circulation of antibiotic-resistant *Salmonella* in chicken from processing plants and at the retail level, which further emphasizes the importance of proper handling of chicken products.

P2-181: SEQUENTIAL APPLICATION OF SODIUM BISULFATE AND PERACETIC ACID AGAINST MULTI-DRUG-RESISTANT SALMONELLA IN CHICKEN THIGHS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Salmonellosis is a major foodborne illness associated mainly with handling and consumption of undercooked poultry. Higher prevalence of multi-drug resistant (MDR) *Salmonella* in poultry is a serious public health concern. Sodium bisulfate (SBS) and peracetic acid (PAA) are commonly used antimicrobials for post-harvest *Salmonella* control in food.

Purpose: To evaluate the efficacy of SBS alone and as a sequential application with PAA, both in vitro and on chicken thighs, to mitigate MDR *Salmonella*.

Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SBS were determined for nalidixic acid-resistant (NAr) *S.* Typhimurium; pre-harvest isolate (B3C4), and ATCC (14028) strains via the microdilution method. In vitro study used SBS (0.5, 1, 2, 4, and 10)× MIC over exposure times of 0, 2, 6, 12, 24, and 48h. Chicken thighs inoculated with NAr *Salmonella* were dipped for 20 s in SBS alone (1, 2, or 3)%, PAA (100 or 200 ppm), or sequential SBS followed by PAA. Fourteen treatments, including positive and negative controls, were evaluated. Samples were processed for *Salmonella* reduction on d0, d1, d2, and d3. Experiments were conducted in triplicate and

analyzed using one-way ANOVA (p<0.05).

Results: MIC and MBC values ranged from 0.4–0.433% and 0.66–0.7%, respectively. In vitro, 1.6 and 4% SBS significantly reduced *Salmonella* by ~2.5–5.5 log CFU/ml depending on exposure time. On chicken thighs, 2% SBS achieved a 0.68 log CFU/g reduction on day 1 compared to controls (p < 0.05). PAA alone was not significantly effective. Sequential treatment with 3% SBS followed by 100 or 200ppm PAA resulted in a 0.56–1.37 log CFU/gm reduction (p<0.05). No synergistic effect observed, though an additive effect occurred at higher concentrations.

Significance: The findings indicate that SBS, particularly when applied sequentially with PAA, may serve as an effective intervention to mitigate *Salmonella* contamination in poultry products.

Award Application: Developing Scientist Award

P2-182: PREDICTIVE MODELING AS A DECISION-SUPPORT TOOL FOR CONTROLLING LISTERIA MONOCYTOGENES IN PLANT-BASED MEAT PRODUCTS

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: Control of *Listeria monocytogenes* in ready-to-eat foods is strictly regulated worldwide. Its growth is influenced by product attributes such as organic acids and storage conditions. With neutral pH, vacuum or MAP packaging, and refrigeration, plant-based meats are high-risk products where *L. monocytogenes* can grow, driving the need for control strategies.

Purpose: To evaluate the Corbion® *Listeria* Control Model (CLCM) for the prediction of *L. monocytogenes* growth in plant-based meat products.

Methods: Several types of plant-based products were formulated with either no antimicrobial (negative control), 0.6%–1.0% Verdad® Powder N6, or 0.25%–0.40% Verdad® Powder N30. Samples were inoculated with a cold-adapted 5-strain *L. monocytogenes* cocktail (2–3 log CFU/g), vacuum-packaged, and stored at 4°C. On sampling day, three replicates of

samples were diluted with buffered peptone water, and the homogenate was spread-plated on PALCAM (37°C for 24 h). The CLCM model was used to predict the time to a one-log increase (TTG1) for each formulation, and the predictions were compared with the results from the challenge study.

Results: The negative control samples exhibited a 1-log increase in *L. monocytogenes* within 5 days. Formulation with ≥0.8% Verdad® Powder N6 effectively inhibited *L. monocytogenes* outgrowth for >100 days, while 0.25% Verdad® Powder N30 delayed a 1-log increase to 76 days. Comparison of observed TTG1 values with CLCM predictions showed that experimental results fell within the ranges defined by both conservative (population majority) and typical (most probable growth) scenarios, demonstrating strong agreement between predicted and observed outcomes.

Significance: Validated predictive microbiology models like CLCM support compliance with *Listeria monocytogenes* requirements by enabling science-based risk assessment, reducing the need for extensive challenge studies, and speeding formulation optimization and time to market for plant-based products.

P2-183: CLEAN LABEL ANTIMICROBIAL ALTERNATIVES FOR SHELF-LIFE EXTENSION OF COLESLAW

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Post-processing contamination and naturally occurring microflora can limit the refrigerated shelf life of coleslaw. Traditional preservatives, such as benzoates and sorbates, are effective but are less preferred by consumers.

Purpose: To evaluate the efficacy of clean-label antimicrobials as an alternative to the traditional antimicrobials for extending the shelf life of coleslaw.

Methods: Coleslaw was formulated with either no antimicrobial (negative control), 0.05% sodium benzoate + 0.05% potassium sorbate, 0.75% Verdad® F340 [Vinegar], or 0.5% Verdad® Opti Powder N370 [Cultured Dextrose], packaged aerobically and refrigerated at 4.4°C. At each sampling point, triplicate units were diluted in buffered peptone, stomached, and spread-plated on de Man, Rogosa, and Sharpe agar for lactic acid bacteria (LAB; 30°C, 48 h) and on tryptic soy agar for aerobic bacteria (35°C, 48 h). Microbial growth curves were fit using the Baranyi and Roberts model to derive growth rate and lag estimates. A one-way

ANOVA (α=0.05) was used to compare treatment effects on growth rate and lag parameters.

Results: The initial LAB population in coleslaw was 1-1.5 log CFU/g. The negative control reached 6-log LAB and aerobic bacteria counts on days 17 and 24, respectively. In contrast, all antimicrobial-treated samples maintained LAB and aerobic bacterial populations at levels below 6 log throughout the 60-day period. The tested treatments had a significantly longer lag time (p<0.05) and a lower growth rate compared to the negative control sample. There was no significant difference (p>0.05) in lag time or growth rate between the conventional and clean-label antimicrobials.

Significance: Clean-label antimicrobials can match the performance of conventional preservatives by prolonging shelf life, extending lag time, and slowing microbial growth. These findings support industry transitions toward cleaner formulations while maintaining microbial control in fresh refrigerated foods.

P2-184: SALMONELLA CONTROL ON PORK BONES USING CLEAN-LABEL ANTIMICROBIALS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Salmonella* contamination remains a key food safety concern during carcass fabrication. The porous structure of animal bones makes the application of antimicrobials challenging. Although post-harvest sprays, such as peroxyacetic and lactic acid, are widely used, interest is growing in clean-label interventions to achieve *Salmonella* reduction.

Purpose: To assess the efficacy of clean-label antimicrobials as an intervention for *Salmonella* reduction in pork bones.

Methods: Pork shoulder and loin chops were purchased from retail stores, and bones were trimmed free of lean tissue. Bones weighing 80–100 g were selected and surface-inoculated with a five-strain *Salmonella* cocktail (*S*. Enteritidis ATCC 13076 and 31194; *S*. Typhimurium ATCC 13311 and 14028; *S*. Heidelberg ATCC 8326) to achieve 5.0 log CFU/g, then held in a biosafety cabinet for 20 min to allow bacterial attachment. Inoculated bones were treated with: untreated control, 200 ppm peracetic acid [N1], 10% Verdad® N100 [N2], or Purac® BF S/210 [N3]. Treatments were applied using a pressurized spray (20 psi; ~3 mL). Samples were drained at a 45° angle for 5 minutes, then diluted with buffered peptone water,

homogenized, and spread-plated on Xylose Lysine Tergitol-4 agar (35°C, 24 h).

Results: The untreated control had a *Salmonella* population of 5.1 log CFU/g. Treatment N3 showed the highest *Salmonella* reduction (0.79 log), yielding significantly lower counts ($p<0.05$) at 4.39 log CFU/g compared to the untreated control. N1 resulted in the next lowest counts at 4.61 log CFU/g, which were also significantly reduced (0.49 log) compared to the untreated control. Treatment N2 lowered *Salmonella* to 4.75 log CFU/g, representing a 0.35-log reduction.

Significance: Treatment N3 significantly reduced *Salmonella* on porous pork bones, offering a practical intervention that enhances food safety during fabrication and downstream processing while aligning with consumer demand for cleaner ingredients.

P2-185: BEYOND POTASSIUM SORBATE: CLEAN-LABEL SOLUTIONS FOR LISTERIA MONOCYTOGENES CONTROL IN REFRIGERATED DELI SALADS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Refrigerated deli salads are susceptible to *Listeria monocytogenes* contamination due to their nutrient-rich matrices, minimal heat treatment, and the risk of post-processing contamination during handling and packaging, particularly during extended refrigerated storage. Although potassium sorbate is widely used, demand for clean-label foods is increasing.

Purpose: To assess the effectiveness of clean-label antimicrobials, compared with a conventional antimicrobial, in controlling *Listeria monocytogenes* outgrowth in refrigerated deli salads (chicken and potato salad).

Methods: Chicken (CS) and potato salad (PS) were prepared with five treatments: no antimicrobial (negative control), 0.1% potassium sorbate, Verdad® Opti Powder N370 (0.75% PS; 0.5% CS), Verdad® Opti Powder N105 (0.75% PS; 1.5% CS), and Verdad® F340 (0.75% PS;

0.5% CS). Portions (50 ± 2 g) were inoculated with five acid-adapted *L. monocytogenes* strains (targeting 2.5–3 log CFU/g), aerobically packaged, and stored at 4.4°C (40°F). On each sampling day, three replicates per treatment were diluted in buffered peptone water and spread plated on Modified Oxford agar (35°C, 48 h).

Results: Initial *L. monocytogenes* levels were 2.2–2.5 log CFU/g in CS and 2.5–3 log CFU/g in PS. The negative control samples reached a 1-log *L. monocytogenes* outgrowth by day 11 (PS) and day 19 (CS). In contrast, all clean-label antimicrobials limited the outgrowth of *L. monocytogenes* to <1 log CFU/g for up to 75 days. Clean-label antimicrobials performed comparably to potassium sorbate, with no significant differences ($p > 0.05$) in *L. monocytogenes* control.

Significance: Clean-label antimicrobials can control *L. monocytogenes* as effectively as potassium sorbate in refrigerated deli salads. These findings support safer product formulation, reducing reliance on conventional preservatives, and help manufacturers meet consumer demand for clean-label foods without compromising microbial safety or shelf-life performance.

P2-186: ASSESSING THE EFFICACY OF CONSISTENT USE OF PLANT-BASED AQUEOUS EXTRACT IN PREVENTING BIOFILMS FORMED BY SALMONELLA ENTERICA AND LEAFY GREEN NATIVE MICROBIOTA ON DIFFERENT FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Biofilms are formed over time when microorganisms attach to a surface and start proliferating, indicating poor cleaning and sanitizing practices. Food processing plants provide an ideal environment for biofilm formation by foodborne pathogens along with native microbiota on food contact surfaces. This can potentially be prevented by consistent use of effective sanitizers on these surfaces.

Purpose: The effectiveness of consistent use of a plant extract on food contact surfaces (stainless steel 304, high density polyethylene, polyvinylchloride, polycarbonate and buna-N-rubber) against *Salmonella enterica* and leafy green native microbiota from spinach and cut romaine was evaluated.

Methods: Coupons of different surface materials were dipped for 2 min in different concentrations of plant extract A (25, 50, 75 and 100%) and then dried for 30 min. The treated coupons were dipped in tryptic soy broth (TSB), then inoculated with either *S. enterica* or leafy

green (cut romaine or spinach) native microbiota to simulate surface contamination during processing. These coupons were then washed, diluted in 0.1% peptone water and enumerated by spread plating on tryptic soy agar at 0 h, followed by treatment and immersion in TSB for 14-18 h. These steps were repeated at 14-18 h, 28-36 h (not enumerated) and 42-54 h.

Results: Plant extract A at all concentrations was very effective in reducing *S. enterica* and leafy green native microbiota (from spinach and cut romaine) to below detection levels (<1 log CFU/coupon) within 14-18 h for all surface types. Consistent application of plant extract A at all concentrations maintained the microbial reduction to below detection levels at 42-54 h as compared to untreated surfaces that had average microbial population of 7 logs at all time points including 54 h.

Significance: Results imply that plant extract A at various concentrations can potentially be used to prevent biofilm formation on different types of food contact surfaces.

Award Application: Developing Scientist Award

P2-187: NATURAL ANTIMICROBIAL STRATEGIES FOR SPOILAGE AND PATHOGEN CONTROL IN MEAT PRODUCTS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Meat products are highly susceptible to microbial growth due to high moisture, near-neutral pH, and nutrient availability. Spoilage organisms limit refrigerated shelf life, while *Salmonella* spp. in raw meat and *Listeria monocytogenes* in ready-to-eat (RTE) products pose significant food safety risks. Effective antimicrobial interventions are essential to maintain safety, quality, and shelf life.

Purpose: The study evaluated the efficacy of a clean-label antimicrobial, DuraShield™ NPV (cultured dextrose, rosemary extract, and buffered vinegar), for controlling spoilage microorganisms and foodborne pathogens in meat applications.

Methods: Spoilage control was evaluated in ground turkey stored at 4°C with either no antimicrobial (control) or 1% NPV. A *Salmonella* challenge study was conducted in ground turkey inoculated with a *Salmonella* cocktail and stored at 10°C, with and without 1% NPV. In *Listeria* study, efficacy of three treatments: control, control + 1% NPV, and control + 2% sodium lactate/diacetate were compared in cured hot dogs after inoculated with *L. monocytogenes* and stored at 4°C. Studies were repeated twice with duplicate samples (n = 4).

Results: In ground turkey, control samples reached spoilage limit (7.0 log CFU/g) by day 10, while NPV extended shelf life by 8 days ($P < 0.05$). In the *Salmonella* study, *Salmonella* counts in control increased from 3 to 6.0 log CFU/g by day 5, whereas NPV suppressed growth and maintained levels below 3.0 log CFU/g throughout 20 days ($P < 0.05$). In cured hot dogs, *Listeria* counts in controls increased to 6.2 log CFU/g by day 80, while the efficacy of NPV and sodium lactate/diacetate was comparable and maintained *L. monocytogenes* levels below 3.0 log CFU/g ($P < 0.05$).

Significance: NPV effectively controlled spoilage microorganisms, *Salmonella*, and *L. monocytogenes* in meat applications, demonstrating its use as a clean-label alternative to synthetic antimicrobials.

P2-188: CLEAN-LABEL ANTIMICROBIAL SOLUTION FOR SPOILAGE CONTROL IN SAUCES AND DRESSINGS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Hot-filled and cold-filled sauces and dressings present distinct microbial stability challenges. While hot filling reduces initial microbial loads, preservatives are often required for extended shelf life. Cold-filled products lack post-formulation heat treatment and are more susceptible to yeast and mold spoilage during storage. Clean-label antimicrobial strategies are needed to address spoilage risk in both systems.

Purpose: The study evaluated the efficacy of a clean-label antimicrobial, DuraShield™ NP2 (cultured dextrose and rosemary extract), for controlling yeast and mold in tomato sauce and vegan mayonnaise.

Methods: NP2 efficacy against yeast and mold was assessed in tomato sauce inoculated separately with a cocktail of acid-tolerant yeasts (*Zygosaccharomyces bailii, Z. rouxii,* and *Saccharomyces cerevisiae*) and mold (*Rhizopus oryzae*). Treatments included a control (no antimicrobial), 0.3% and 0.5% NP2, and then stored at 5–8°C. For vegan mayonnaise, treatments included a control (no antimicrobial), 0.2% and 0.5% NP2, and 0.1% potassium sorbate. All samples were inoculated with mold (*Penicillium roqueforti*) and stored at 23°C. Experiments were repeated twice with duplicate samples (n = 4).

Results: In tomato sauce, NP2 inhibited acid-tolerant yeast growth ($P < 0.05$), resulting in a 2.0–2.5 log CFU/g reduction compared with the control over 21 days of refrigerated storage. NP2 also suppressed visible growth of *R. oryzae*, extending shelf life by 50 days relative to the control ($P < 0.05$). In vegan mayonnaise, control spoiled by day 196 and reached 6.9 log CFU/g mold growth by day 270 at 23°C. NP2 performed comparably to potassium sorbate, achieving up to a 6.0 log CFU/g inhibition in mold growth by day 270 ($P < 0.05$).

Significance: NP2 inhibited yeast and mold in sauces and dressings, demonstrating its use as a clean label antimicrobial in cold-filled and hot-filled applications.

P2-189: COPPER RESISTANCE DRIVES RESISTANCE TO NATURALLY OCCURRING AMINOGLYCOSIDE IN SALMONELLA ENTERICA

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Category: Antimicrobials

Abstract:

Introduction: Copper, a transition metal, has been widely used as an antimicrobial in health care and agricultural settings for decades. It has been extensively used in animal diet to support growth and enhance immune defense mechanisms. Despite its essentiality in cellular processes, excess copper is detrimental to prokaryotic and eukaryotic cells. Therefore, bacteria and host cells have evolved mechanisms to maintain copper homeostasis. For example, *S. enterica* has acquired the copper-resistance (*pco* and *sil*) clusters under selection pressure. The spread of heavy metals in the environment has led to co-selection of multidrug and heavy metal-resistant *S. enterica*, leading to co-resistance and cross-resistance.

Purpose: The aim of this study was to examine the link between copper resistance and aminoglycoside resistance in *Salmonella enterica*.

Methods: The *pco* cassette in *Salmonella* Senftenberg was disrupted through deletion of *pcoABCD* genes using allelic exchange mutagenesis. The MICs of aminoglycosides were evaluated by Alamar Blue assay and Etest. Bacterial membrane permeability was assayed

using propidium iodide (PI) staining.

Results: Deletion of the copper-resistance genes (*pcoABCD*) resulted in more than 10-fold increase in susceptibility to tobramycin, kanamycin, streptomycin, gentamicin, and spectinomycin. However, there was no increase in susceptibility to amikacin, netilmicin, or plazomicin. Remarkably, *S.* Anatum N31410 that carried the *pco* and *sil* copper-resistance clusters, but lacks known aminoglycoside-resistance genes, is resistant to tobramycin (MIC = 125 µg/mL), gentamicin (MIC = 620 µg/mL), kanamycin (MIC = 156 µg/mL), streptomycin (MIC 2000 µg/mL), and spectinomycin (MIC = 620 µg/mL).

Significance: Understanding the connection between copper resistance and aminoglycoside susceptibility in bacteria could open new avenues for revitalizing existing antibiotics and highlights the urgent need to address the spread of copper-resistance genes.

P2-190: COMPARATIVE ANALYSIS OF TWO ORGANIC ACID PRE-PROCESSING RINSES ON SALMONELLA ENTERICA SURVIVAL ON FREEZE DRIED APPLES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Freeze drying to preserve food products (e.g., produce) has increased. These products do not undergo a kill step prior to freeze drying, which can conserve microbial contamination for extended periods before consumption. Generally Recognized as Safe (GRAS) organic acids can effectively reduce microbial load on fresh produce, but their efficacy with freeze dried produce is unknown.

Purpose: Compare efficacy of three pre-processing washes (acetic acid [A], citric acid [C], water [W] only) on concentration of *Salmonella enterica* on ‘Granny Smith’ apples over 180d in ambient storage.

Methods: A five-strain *S. enterica* cocktail (Javiana, Tennessee, Typhimurium, St. Paul, and Montevideo) was cultured in Tryptic Soy Broth (TSB) for 24h at 35°C. Each serovar was grown

on Tryptic Soy Agar (TSA) for 24h at 35°C, flooded with 5 mL 0.1% peptone water, and scraped with a sterile spreader. Cultures were combined, centrifuged at 10,000 x g for 10 min, and resuspended before inoculation. Apples (10g) were spot inoculated (100µL; 9.54 log CFU/sample) and dried for 40 min. Samples (*n* =198) were submerged in 2 L of W, 0.21% C, or 4% A for 60s. Samples were freeze dried and stored at 23°C for 180d. *Salmonella* populations were enumerated at 0, 3, 7, 14, 21, 30, 60, 120, 150, and 180d. A two-way ANOVA and Tukey's HSD were run in RStudio to determine treatment efficacy by wash.

Results: *Salmonella* concentrations were significantly reduced by 3.07 and 2.35 log CFU/g for A (p= 0.0204) and C (p<0.0001). *Salmonella* concentrations were not significantly reduced for W (p=0.1910). *Salmonella* concentrations persisted throughout 180d in storage with reductions of 2.03 and 0.89 log CFU/g for A (p=0.0490) and C (p=0.0387). At 7d, significant reductions were observed for A compared to W (p<0.0001), and A maintained higher efficacy through 21d (p<0.0001) and 30d (p<0.0001).

Significance: Acid washes can reduce *Salmonella* on fresh produce prior to freeze drying and during storage.

Award Application: Developing Scientist Award

P2-191: EFFICACY OF SEQUENTIAL ALKALINE-ACIDIC ELECTROLYZED WATER TREATMENTS FOR SALMONELLA SPP. REDUCTION ON ALFALFA SPROUT SEEDS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Sprout seeds have been associated with foodborne illness outbreaks, and seed decontamination remains challenging due to strong bacterial attachment and protected surface niches that limit sanitizer access.

Purpose: This study quantified reductions of a multiple serovar cocktail of *Salmonella* spp. on alfalfa sprout seeds following sequential alkaline-acidic EW applications and tracked EW physicochemical changes during use.

Methods: Alfalfa sprout seeds were inoculated with *Salmonella* spp. according to an FDA-validated protocol. Seeds were exposed at a 1:10 (w/v) seed-to-solution ratio to alkaline EW, acidic EW, or DI water (control) for 7.5 min, drained, then exposed to fresh solution for another 7.5 min. For sequential alkaline-acidic EW, different EW solutions were used in the first and second exposure periods. Bacterial counts were enumerated by culture-based plating and expressed as $\log_{10}$ reductions relative to the initial *Salmonella* level on seeds. All

treatments and controls were replicated three times; results are reported as mean ± SD. Differences were assessed by one-way ANOVA (α = 0.05) using JMP Pro 17.0.

Results: The initial *Salmonella* level on seeds was 6.71 ± 0.05 $\log_{10}$ CFU/g. Log reductions (CFU/g) for acidic EW (1.00–1.48 ± 0.03) and sequential alkaline-acidic EW (up to 1.51 ± 0.02) were similar and significantly greater ($p < 0.05$) than alkaline EW (0.65–0.88 ± 0.01) and DI water (0.11 ± 0.01). EW properties changed most during acidic EW exposures, with ACC depletion and decreases in ORP; alkaline EW showed minor shifts in pH and ORP and maintained low ACC.

Significance: This research establishes a baseline for comparing sequential antimicrobial EW treatments with industry-standard high-chlorine seed treatments (20,000 ppm $Ca(OCl)_2$), and research is ongoing to optimize pathogen reduction. Monitoring pH, ORP, and ACC during treatment informs solution factors affecting antimicrobial activity and guides optimization of operating conditions.

Award Application: Developing Scientist Award

P2-192: EVALUATION OF ANTIMICROBIAL FOGGING TECHNOLOGY FOR CONTROLLING SALMONELLA ENTERICA NEWPORT ON ROMA TOMATOES AND EXTENDING THE SHELF LIFE OF BERRIES USING ANTIMICROBIAL EDIBLE FILMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Fresh fruits and vegetables are increasingly consumed worldwide due to their nutritional value. Food safety and shelf-life represent two critical challenges during their handling and distribution, with a growing demand for clean-label products that meet consumer expectations. *Salmonella* is a foodborne pathogen of concern in fresh produce, causing 1.2 million illnesses annually in the U.S. Berries have the shortest shelf-life among fresh fruits, lasting 3–7 days at 4°C. Improving safety and shelf life of fresh produce will benefit industry and consumers.

Purpose: To improve the safety of Roma tomatoes and extend the shelf life of berries using plant-based antimicrobials applied via fogging and edible films, respectively.

Methods: Roma tomatoes were inoculated with *S.* Newport and treated with five plant-based antimicrobial microemulsion formulations, A, B, C, D, and E, at 0.5% concentration each, applied via a fogging equipment producing 10–30 micron droplets, refrigerated and sampled

at Days 0, 3 and 7. Whole and cut strawberries and whole raspberries were placed in Ziploc bags and exposed to apple-based edible films containing combinations of antimicrobials at various concentrations. Fruits were refrigerated and visually assessed at various timepoints until 60 days.

Results: Results showed 1–2 log reductions in *Salmonella* population with various antimicrobial treatments. On Day 7, Treatment D (4.88 ± 0.04) showed the strongest antimicrobial activity against *S.* Newport compared to the untreated (7.23 ± 0.21) and water (7.21 ± 0.10) controls. Whole and cut strawberries and whole raspberries demonstrated a shelf life of up to 45 and 60 days, respectively, maintaining their color and appearance compared to controls that turned moldy and mushy.

Significance: Plant-based antimicrobials applied via fogging and edible films have the potential to improve both food safety and shelf-life, while aligning with the growing consumer demand for clean-label products.

Award Application: Developing Scientist Award

P2-193: METABOLOMICS PROFILING REVEALS THE SANITIZER MECHANISMS AGAINST SALMONELLA ENTERICA BIOFILM

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The biofilm-forming ability of *Salmonella enterica* on food contact surfaces amplifies the cross-contamination risk of food. Despite sanitization, reported *Salmonella* outbreaks continue to increase, suggesting that current sanitization regimes are not satisfactory and poorly understood mechanistically.

Purpose: To identify key metabolic targets disrupted by sublethal levels of sodium hypochlorite and peracetic acid against *Salmonella* Newport biofilms.

Methods: Stainless steel coupons (n=54) were inoculated with *S.* Newport (10^6 CFU/coupon) in Lauria Bertani (LB) for 24 hours at 21°C for biofilm development. After incubation, planktonic cells were aspirated. For sanitizer treatment, 18 coupons were untreated (control), while the remaining were divided equally and treated with sublethal levels of either sodium hypochlorite (10 ppm) or peracetic acid (10 ppm) for one minute. Biofilm cells were scraped off all coupons, and intracellular metabolites were extracted with a 60% methanol solution. Six replicates of both treatments and controls were dried and reconstituted in tributylamine. Untargeted ion pairing LC-MS analysis was conducted using Waters Xevo G2-XS UPLC/MS/

MS, whereas a targeted analysis was conducted on Waters Xevo TQ-S UPLC/MS/MS instrument for quantifying cyclic nucleotides (e.g., c-di-GMP).

Results: Untargeted metabolomic analysis revealed 855 identifiable compounds (e.g., Asp-Ile-Lys) that are enriched ($p < 0.05$; fold change > 2) in the untreated, as well as 797 identifiable compounds that were enriched in the treated biofilm cells with both sanitizers. Both sanitizers significantly ($p < 0.01$) reduced the production of c-di-GMP, c-di-AMP, and cAMP in comparison to the untreated biofilm cells.

Significance: We can then infer that the utilization of sanitizers that significantly inhibit the production of key biofilm metabolites would be beneficial to use for reducing safety risks associated with *Salmonella* biofilms.

Award Application: Developing Scientist Award

P2-194: LONG-READ SEQUENCING IDENTIFIES PLASMID-MEDIATED ANTIMICROBIAL RESISTANCE AND VIRULENCE DETERMINANTS IN SALMONELLA ENTERICA FROM RETAIL PORK

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Retail pork is an important reservoir for antimicrobial-resistant (AMR) *Salmonella enterica*, posing a potential risk to food safety and public health. Within the National Antimicrobial Resistance Monitoring System (NARMS), genomic surveillance of retail meat isolates provides critical insights into the genetic mechanisms driving resistance dissemination.

Methods: A *Salmonella enterica* isolate recovered from retail pork was characterized following NARMS protocols. Long-read whole-genome sequencing was performed to generate high-quality assemblies. Genomic analyses were conducted using tools from the Center for Genomic Epidemiology, including MobileElementFinder, ResFinder, VirulenceFinder, and plasmid replicon detection, to identify antimicrobial resistance genes, mobile genetic elements, plasmid incompatibility groups, and virulence-associated determinants.

Results: The isolate harbored multiple antimicrobial resistance genes, including $bla_{\text{TEM-1B}}$, *dfrA1*, *sul2*, and *aph(6)-Id*, conferring resistance to β-lactams, trimethoprim, sulfonamides,

and aminoglycosides. Notably, *bla*$_{\text{TEM-1B}}$, *dfrA1*, and *sul2* were co-localized on a contig containing an IncI1 plasmid replicon and embedded within a Tn2 transposon, providing strong evidence of plasmid-mediated resistance. Additional resistance genes detected on other contigs lacked associated plasmid replicons and were considered likely chromosomal. A large IncF-type plasmid (IncFIB/IncFIA/IncFIC) encoded numerous virulence-associated genes, including *tsh*, *iroN*, *iucC*, *hlyF*, *iss*, and *ompT*, consistent with virulence plasmids commonly described in foodborne *Salmonella*.

Significance: These findings demonstrate plasmid-mediated AMR in *Salmonella enterica* from retail pork and highlight the role of mobile genetic elements in resistance dissemination. Continued long-read genomic surveillance within NARMS is essential for monitoring plasmid-borne resistance in the food supply.

P2-195: PLANT-BASED PREBIOTICS ENHANCE LACTOBACILLUS RHAMNOSUS METABOLITE PRODUCTION TO INCREASE ANTIMICROBIAL ACTIVITY AGAINST STAPHYLOCOCCUS EPIDERMIDIS

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: *Lactobacillus rhamnosus* (LR), a widely used probiotic with known antimicrobial/ beneficial properties, produces metabolites to inhibit bacterial pathogens such as *Staphylococcus epidermidis* (SE).

Purpose: To investigate if plant-based prebiotics enhance LR's antimicrobial activity against (SE) and identify key bioactive metabolites.

Method: LR was co-cultured with multidrug-resistant SE to determine its activity with and without raffinose, stachyose, or inulin. Antimicrobial activity was assessed using cell-free cultural supernatants (CFCSs) from LR supplemented with prebiotics. Bioactive components

in CFCSs were extracted and analyzed using mass spectrometry.

Results: Low concentrations of inulin and stachyose in co-culture resulted in complete SE exclusion by 48hr (p<0.0001), while raffinose required needed 72hr (p<0.0001). CFCSs from LR supplemented with prebiotics significantly decreased SE growth within 6hr (p=0.0003) and caused complete exclusion by 72hr. CFCSs also displayed antibiofilm activity, with a significant decrease in biofilm formation after 24hr (p=0.0211). Gene expression analysis showed significant inhibition of cell wall-associated genes (p<0.05), confirmed by fluorescent microscopy. Crude metabolic extract showed complete exclusion of SE at 0.625-2.625mg/mL and promoted keratinocyte proliferation by 165.45-215.87%. Mass spectrometry revealed small peptides, benzenes and their derivatives, and heterocyclic compounds as major bioactive components of CFCS. Of the identified compounds, the benzene derivative nimesulide was the most secreted, with an average fold increase of 3161.5, followed by the small peptide sequence Arg-Ile-Val-Leu-Val (2892.05-folds), and leucinic acid (2,587.5-fold).

Significance: Prebiotic supplementation enhanced LR antimicrobial activity against SE with minimal cytotoxicity. The identification of major LR-derived bioactive compounds can provide more insight into LR’s mechanisms and further practical applications.

Award Application: Developing Scientist Award

P2-196: CORIANDER ESSENTIAL OIL VS. ITS MAJOR COMPONENT LINALOOL: EFFECTS OF ARID CONDITIONS ON COMPOSITION AND THE PERFORMANCE OF FREE AND MICROENCAPSULATED LINALOOL AGAINST STAPHYLOCOCCUS AUREUS IN SHEEP MEAT

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Natural preservation strategies using essential oils are increasingly explored in meat systems; however, the impact of arid conditions and water stress on coriander essential oil (CEO) composition and the efficacy of multilayer microencapsulated linalool for controlling *Staphylococcus aureus* in sheep meat remain insufficiently studied.

Purpose: This study evaluated the composition and antimicrobial activity of coriander essential oil produced under environmental stress and assessed the effectiveness of free and multilayer microencapsulated linalool in controlling *S. aureus* in refrigerated minced sheep meat.

Methods: CEO was extracted from environmentally stressed Qatari grown plants. Minimum inhibitory concentrations (MICs) of CEO, free linalool (FL), and multilayer microencapsulated linalool (EL) were determined against *S. aureus*. Minced sheep meat portions were inoculated with *S. aureus* and treated with FL or EL at 0.5% or 1% (w/w), with untreated samples serving as controls. Samples were vacuum-packaged and stored at 4°C for 9 days. Data were analyzed using two-way ANOVA followed by Tukey's post hoc test.

Results: CEO was dominated by linalool (76.52%). MIC values indicated greater antimicrobial activity for CEO (0.625 mg/mL) compared with EL (1.25 mg/mL) and FL (10 mg/mL). At 0.5% (w/w), FL and EL produced similar reductions in *S. aureus* by Day 3 (1.4–1.6 log CFU/g; $p > 0.05$); however, only EL sustained antimicrobial activity through Day 9, maintaining an approximately 1-log CFU/g reduction relative to controls ($p < 0.05$), while FL lost effectiveness after Day 3. At 1% (w/w), both treatments achieved comparable reductions by Day 6 (2.5–2.8 log CFU/g; $p > 0.05$). By Day 9, FL resulted in a significantly greater log reduction of 3.3 compared to 1.9 for EL ($p < 0.05$).

Significance: This strategy offers a promising natural approach to improve meat safety and control *S. aureus*, with clear implications for food protection and public health.

P2-197: PREHARVEST-ANTIBIOTIC USE INFLUENCES ANTIBIOTIC RESISTANCE IN SALMONELLA ENTERICA FROM COMMERCIAL POULTRY AND SWINE FARMS IN LAGOS, SOUTHWESTERN NIGERIA

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Presentation type/Format: Poster

Category: Pre-Harvest Food Safety

Abstract:

Introduction: The widespread use of antibiotics in commercial livestock production for growth promotion and prophylaxis is a primary driver of antimicrobial resistance (AMR) globally.

Purpose: This study provides a systematic characterization of the drivers of preharvest AMR in *Salmonella enterica* across farms in Lagos, SW Nigeria.

Methods: We evaluated 3,600 fecal samples from animals on commercial poultry and swine farms for *S. enterica* and for antimicrobial and virulence determinants, using established microbiological, biochemical, and molecular protocols. We further assessed farm management practices and antimicrobial use through structured questionnaires and correlated these data with antimicrobial resistance phenotypes and determinants in *S. enterica* strains recovered from fecal samples across the farms.

Results: Overall, we observed a 1.5% (54/3,600) prevalence of *S. enterica* across the sampled animals, with 0.67%, 1.67%, and 2.2% in layers, broilers, and pigs, respectively. While most *S. enterica* strains showed multidrug resistance, high rates of AMR to erythromycin (80–100%) and tetracycline (75–92.3%) were observed across animal species, with multiple antibiotic resistance indices ranging from 0.2 to 0.9. Molecular analysis revealed widespread distribution of major resistance determinants - *tetA* (70–100%), *ant(3")-Ia* (80.8–100%), and *sul1* (65.4–80%) and high prevalence of *Salmonella*-associated virulence factors, including *iroB* (62.5–75%), *pipD* (37.5–92.3%), and *orfL* (65–88.5%). Furthermore, we found extensive preharvest antibiotic use [as growth promoter (53.8–85.7%) and prophylaxis (71.4–81.3%)] as well as weak positive correlations between antibiotic use and AMR phenotypes across the animal species.

Significance: Our study reveals widespread AMR and virulence determinants in *S. enterica* from commercial farms, suggesting a potential public health risk and a need for enhanced surveillance, antimicrobial stewardship, and a concerted One Health mitigation.

P2-198: PROBIOTIC POTENTIAL AND ANTIMICROBIAL CHARACTERIZATION OF BIOPROTECTIVE LACTIC ACID BACTERIA FROM THE GASTROINTESTINAL TRACT OF FOOD ANIMALS IN SOUTHWESTERN NIGERIA

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The search for effective, naturally derived antimicrobial agents has intensified as antibiotic resistance continues to threaten global food-animal production. This study focused on the identification and probiotic characterization of bacteriocin-producing lactic acid bacteria (LAB) isolated from poultry farms across Southwestern Nigeria.

Methods: A total of 562 LAB were isolated from the gut contents of turkeys, broilers, layers, cattle, and pigs and identified using API 50 CHL and 16S rRNA gene sequencing. The cell-free supernatants from the LAB were characterized for specific antimicrobial substances, including organic acids, bacteriocins, and catalase, and then evaluated for antimicrobial activity against major foodborne pathogens. LAB strains that demonstrated antimicrobial activity against the pathogens were further evaluated for tolerance to simulated gastric juice

(pH 2.0), bile salts, and phenol, and for their ability to competitively exclude pathogens from the chicken ileal epithelium.

Results: Of the 562 LAB isolated from the gut contents of animals, 32 produced heat-stable antimicrobial substances with inhibitory activity against *Escherichia coli* O157:H7, *Pasteuerella multocida*, *Listeria monocytogenes*, *Salmonella enterica*, *Enterococcus faecium*, *E. faecalis*, and *Staphylococcus aureus*. The 32 LAB strains exhibited varying degrees of significant survivability in simulated gastric juice at pH 2.0, bile salts (0.6%), and phenol (0.4%). They also competitively excluded some indicator pathogens while adhering to the chicken ileal epithelium, with viable counts ranging from 3.2 to 6.3 log CFU/mL.

Significance: The results indicated that the selected LAB strains can survive gut stress conditions and antagonize pathogens, and thus may be considered potential probiotic candidates for animals.

P2-199: ANTIMICROBIAL RESISTANCE PROFILES OF ESKAPE(E) GROUP ISOLATES DETECTED ON RETAIL FRESH PRODUCE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The ESKAPE(E) group comprises opportunistic human pathogens responsible for a large proportion of the burden of multidrug-resistant nosocomial infections; however, antimicrobial resistance (AMR) profiling of these ubiquitous organisms in the food system is infrequently carried out.

Purpose: Characterize AMR profiles of ESKAPE(E) group isolates detected on retail fresh produce samples.

Methods: Two *Klebsiella pneumoniae* and 36 *Enterobacter cloacae* complex strains were isolated from 432 retail fresh produce samples and underwent antimicrobial susceptibility testing using Sensititre^{TM} GN7F broth microdilution plates. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control (QC) strains. Cryopreserved samples and QC strains were revived by streaking onto tryptic soy agar, followed by incubation at 37°C for 24±2 h. Strains were inoculated into sterile purified water relative to a 0.5 McFarland standard prior to inoculation of 30 µL into 11 mL of cation-adjusted Mueller Hinton II broth and subsequent inoculation of 50 µL into the GN7F plate wells. Plates were incubated at 35°C for 18–24 h, and antibiotic susceptibility was assessed relative to the CLSI M100 Enterobacterales breakpoints.

Results: Both *K. pneumoniae* isolates tested intermediate to ampicillin and were susceptible to all other first-line antibiotics tested. 97% (35/36) of *E. cloacae* complex isolates were resistant to cefazolin, 92% (33/36) tested intermediate to ampicillin, and 56% (20/36) tested intermediate to ampicillin-sulbactam. One *E. cloacae* isolate tested intermediate to all tetracyclines, and one *E. hormaechei* isolate tested intermediate to nitrofurantoin.

Significance: AMR surveillance of organisms other than major foodborne pathogens (MFPs) is crucial for capturing the scope of AMR along the food chain and characterizing plausible transmissibility of resistance determinants to MFPs through AMR gene harborage on mobile genetic elements.

P2-200: EFFECTIVENESS OF BEEF-BASED ANTIMICROBIAL INTERVENTIONS ON MICROBIAL LOADS OF BISON CARCASSES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Beef-based antimicrobial interventions are used in bison processing, but their effectiveness is poorly defined.

Purpose: To evaluate seasonal changes in microbial loads and pathogen prevalence on bison carcasses before and after antimicrobial treatment.

Methods: Bison carcasses were sampled at two facilities: Facility A (~15 bison/week) and Facility B (~200 bison/week). Sampling occurred in spring and fall with 12 bison per facility per season. After hide removal and splitting, three 4 × 3-inch excision samples were collected from one carcass half before treatment and three from the opposite half after carcass wash, zero-tolerance trimming, 165°F hot water (15 s), and 2.5–3% lactic acid. Microbial indicators were enumerated as log CFU/cm^2, and pathogens were detected using enrichment and BAX. Pre- and post-treatment data were analyzed using paired comparisons (P □ 0.05).

Results: All changes reflect pre- versus post-treatment differences. In spring, aerobic counts were significantly reduced at Facility A (0.52±0.37 log CFU/cm^2) but increased at Facility B

(0.28±0.20). LAB were significantly reduced at Facility A (0.81±0.58) with no change at Facility B (0.11±0.08). Psychrotrophs were below detection at Facility A and slightly reduced at Facility B (0.13±0.09); yeasts and molds increased slightly. In fall, aerobic counts were significantly reduced at Facility A (0.27±0.19) and Facility B (0.29±0.21). LAB were significantly reduced at Facility A (0.97±0.69) but not at Facility B (0.16±0.11). Yeasts and molds were significantly reduced at Facility A (1.05±0.74) and moderately reduced at Facility B (0.38±0.27). Indicator organisms were reduced to below detection limits post-treatment. No pathogens were detected at Facility A, while pathogens were sporadically detected at Facility B.

Significance: Antimicrobial interventions reduced microbial loads on bison carcasses, with facility-dependent effectiveness, supporting bison-specific validation.

Award Application: Developing Scientist Award

P2-201: THERMAL INACTIVATION OF FOODBORNE PATHOGENS AND SURVIVAL OF PROBIOTICS IN PECAN-BASED FROZEN DESSERTS

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Presentation type/Format: Poster

Category: Plant-Based Alternative Products

Abstract:

Introduction: Pecan-based frozen desserts are increasingly popular dairy alternatives, but limited data exist on thermal inactivation of foodborne pathogens and heat tolerance of commercially available probiotics in pecan-based systems.

Purpose: To evaluate the impact of mild heat treatments on survival of foodborne pathogens in pecan milk and pecan ice cream batter, and to assess survival of a commercially available probiotic (*Bacillus subtilis* DE111) following thermal processing.

Methods: Pecan milk and pecan ice cream batter were separately inoculated with a 4-strain mixture of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 at 7–7.3 log CFU/mL. Samples were heated at 63°C for 30 min; 66°C was included based on preliminary studies. Samples were collected at 10, 20, and 30 min and analyzed for pathogen recovery. Separate formulations were inoculated with *Bacillus subtilis* DE111 (~9 log CFU/mL). Experiments were conducted in triplicate with controls and analyzed using SAS (P □ 0.05).

Results: At 63°C, reductions varied by matrix. In pecan ice cream batter, *Salmonella* Typhimurium was reduced by ~3 log after 30 min, while *Listeria monocytogenes* and

Escherichia coli O157:H7 were reduced by 1.8 and 1.48 log CFU/mL. In pecan milk at 63°C, pathogen levels declined by 2.36 and 2.52 log CFU/mL over 30 min. At 66°C, *Escherichia coli* O157:H7 was reduced to 6.2 log CFU/mL within 30 min in batter, while *Salmonella* and *Listeria* showed reductions of 4.2 and 4.80 log CFU/mL. *Bacillus subtilis* DE111 showed ~0.8 log CFU/mL reduction at both temperatures.

Significance: Heat-treatment effectiveness in pecan-based systems is matrix dependent, and *Bacillus subtilis* DE111 demonstrates thermal resilience. Validation of time–temperature combinations is essential to ensure food safety while preserving probiotic viability in nut-based frozen desserts.

P2-202: METAGENOMIC INSIGHTS INTO THE RESISTOME AND MICROBIAL COMMUNITIES OF SWINE AND CATTLE FECAL SAMPLES FROM SLAUGHTERHOUSES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Food-producing animals are recognized as reservoirs of antimicrobial resistance genes (ARGs), and shotgun metagenomics enables the characterization of the resistome within microbial communities.

Purpose: This study aimed to characterize the resistome and microbial composition of swab from swine and cattle across 10 farms/species.

Methods: A total of 100 samples/species were collected and pooled into 10 composite samples, each representing one farm. DNA was extracted and subjected to shotgun metagenomic sequencing. Bioinformatic analyses included quality control, removal of host and human sequences, taxonomic profiling, and resistome characterization (MEGARes Database). Relative abundance data were used for comparisons.

Results: Microbiota analysis revealed distinct structures between cattle and swine (PERMANOVA: $R^2 = 0.281$, $p = 0.001$). Cattle exhibited higher richness (1,091 ± 329) than swine (628 ± 215; $p = 0.006$). Cattle microbiota was dominated by Pseudomonadota (31.4%), Bacteroidota (29.1%), and Actinomycetota (11.0%), whereas swine showed enrichment of Bacillota (41.5% vs. 19.4%). At the genus level, *Lactobacillus* and butyrate-producing bacteria were enriched in swine, while *Vibrio* and *Acinetobacter* predominated in cattle. Resistome analysis identified over 520 resistance genes, with macrolide resistance accounting for 32% of relative hits, followed by aminoglycoside (11%), multidrug (8%), and heavy metal resistance (5–8%). Cattle harbored significantly higher tetracycline resistance (TETQ: 750 vs. 78) and aminoglycoside 16S mutations (641 vs. 30), while swine showed higher aminoglycoside transferases (ANT9: 65 vs. 16). Resistance to multiple metals was also detected.

Significance: These findings show that cattle and swine harbor distinct microbial ecosystems and resistomes, emphasizing the importance of species-specific surveillance to support antimicrobial resistance monitoring in food production systems.

P2-203: INACTIVATION OF CAMPYLOBACTER JEJUNI PERSISTERS USING ZNO NANOPARTICLES

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: *Campylobacter jejuni* is a leading bacterial cause of human gastroenteritis worldwide and can enter a dormant persister state under unfavorable conditions, which contributes to its persistence in the food supply chain. ZnO nanoparticles (ZnO NPs) have emerged as a potential antimicrobial strategy due to their broad-spectrum activity.

Purpose: This study aimed to evaluate the effectiveness of ZnO NPs in activating *C. jejuni* persisters.

Methods: *C. jejuni* strains F38011, NCTC 81116, and ATCC 33560 were cultivated to 8 log CFU/mL and exposed to 100 µg/mL ampicillin and 2 µg/mL ciprofloxacin for 4 hours at 37 °C under microaerobic conditions to induce persister formation. Antibiotics were subsequently removed, and cells were resuspended in Mueller–Hinton broth. ZnO NPs were added at 5 and 10 mg/mL, with untreated controls included for each strain. Bacterial survival was assessed at 2, 4, and 24 h post-treatment by plating on Mueller–Hinton Blood Agar.

Results: After 4 hours of exposure, treatment with 10 mg/mL of ZnO NPs resulted in 3-logs reduction in persisters across all strains. Ciprofloxacin-induced persisters of strain NCTC 81116 reached undetectable levels after 4 hours of ZnO NP exposure. After 24 hours, all strains reached undetectable levels (<2 log CFU/mL) when treated with 5 mg/mL ZnO NPs.

Statistical analysis revealed no significant difference between the 5 and 10 mg/mL treatments in overall persister reduction, except for ciprofloxacin-induced persisters of strains ATCC 33560 and F38011, where the higher ZnO NP concentration led to a more rapid reduction.

Significance: Effective control of *C. jejuni* persister cells is critical to reduce contamination along the food supply chain. ZnO NPs show strong potential as an antimicrobial intervention likely due to their membrane disruptive properties and previously demonstrated bactericidal activity against actively growing *C. jejuni.*

Award Application: Developing Scientist Award

P2-204: REDUCTION OF CAMPYLOBACTER JEJUNI ON POULTRY MEAT BY SIMULTANEOUS APPLICATION OF ANTIMICROBIAL BLUE LIGHT AND PERACETIC ACID

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: *Campylobacter jejuni* is a leading cause of bacterial foodborne infections in the United States. Controlling this pathogen throughout the poultry production chain is challenging.

Purpose: Here, we evaluated the impact of novel interventions, namely antimicrobial blue light (aBL) with and without traditional antimicrobials, on the reduction of *Campylobacter* on chicken meat.

Methods: Chicken breast pieces (11 g) were inoculated with 8 log CFU/mL *C. jejuni* and subjected to aBL with and without peracetic acid (PAA). Two scenarios were investigated; tandem treatments (PAA followed by aBL and vice versa) and simultaneous treatments (PAA and aBL applied together), using different PAA concentrations (200 and 400 ppm) and aBL doses (60 and 120 J/cm^2). Reductions in *C. jejuni* were reported as log reduction CFU/g. Differences were analyzed using one-way ANOVA, followed by the Tukey-Kramer HSD test (α = 5%).

Results: Our results showed that aBL caused a maximum of 0.3 to 1.5 log CFU/g reduction

under different dose and temperature conditions, while PAA alone resulted in 0.7 to 1.3 log CFU/g reduction. In comparison, tandem and simultaneous treatments resulted in reductions of 1.7 to 2.4 and 2.0 to 2.2 log CFU/g, respectively. Statistical analyses ($p < 0.05$) indicated that 400 ppm PAA for 30 min, followed by exposure to 120 J/cm^2 aBL or vice versa, resulted in 2.4 log CFU/g reduction in *C. jejuni*. Although the simultaneous treatments resulted in a 2.2 log CFU/g reduction, this was achieved in 15 min. Therefore, the simultaneous treatments were considered more adequate for achieving at least 2-log reduction. aBL had a limited to negligible impact on the sensory properties of the meat when used for relatively short periods.

Significance: Overall, combining aBL with PAA enhances *C. jejuni* reduction on poultry meat. aBL is a user-friendly and very affordable technology that can lead to improved post-harvest intervention strategies.

Award Application: Developing Scientist Award

P2-205: LIMITATIONS OF CARBOHYDRATE FATTY ACID ESTERS (CFAS) AS FOOD ANTIMICROBIALS AND EMERGING ALTERNATIVE APPLICATIONS: A REVIEW

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Carbohydrate fatty acid esters (CFAs) are non-toxic additives that meet the demand for safer preservatives with reported in vitro antimicrobial activity, yet their application in real food systems remains limited by practical constraints.

Purpose: To assess how food components limit CFA antimicrobial activity and to evaluate alternative application formats, including coatings, antibiofilm, and synergistic combinations.

Methods: Peer-reviewed studies were identified using Scopus, ScienceDirect, and PubMed, with Google Scholar and Semantic Scholar used to supplement retrieval. Only experimental studies were included, with foundational studies considered when proposing concepts relevant to future applications. Studies were selected using a predefined framework addressing: (i) reduced efficacy in real food matrices and (ii) alternative strategies, including edible coatings, antibiofilm applications, and synergistic combinations.

Results: Forty-four studies met the inclusion criteria. Only five evaluated antimicrobial efficacy in real food matrices and reported reduced activity, attributed to lipid-phase partitioning, protein and starch interactions, and reduced solubility at refrigeration temperatures. Twenty-nine studies incorporated CFAs into edible coatings, but only assessed barrier and physicochemical properties rather than antimicrobial function. Eight studies

examined synergistic strategies pairing CFAs with other agents or processes, and all reported improved outcomes versus CFAs alone. Only two studies evaluated CFAs for antibiofilm activity, reporting inhibition of biofilm formation.

Significance: Evidence indicates a translation gap from in vitro to foods. Combination approaches represent a main route to enhance efficacy, while coatings and antibiofilm applications remain promising but under-tested, highlighting the need for studies that quantify antimicrobial and antibiofilm activity under relevant food and processing conditions.

Award Application: Developing Scientist Award

P2-206: SUCROSE LAURATE (SL) ENHANCES CLEANING-IN-PLACE (CIP) CHEMICAL REMOVAL OF E. COLI BIOFILMS WITHOUT ALTERING PLANKTONIC MICS

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Valeria Rodriguez Monge, Utah State University

Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Sucrose laurate (SL) has antimicrobial potential, but efficacy in foods is often reduced by interactions with food ingredients.

Purpose: We evaluated whether SL can be leveraged as a sanitation aid to enhance cleaning-in-place (CIP) chemical control of biofilms, thereby avoiding food-matrix interactions that limit in-food efficacy.

Methods: Planktonic MICs of sodium hypochlorite (NaOCl), NaOH, phosphoric acid (PA), and nitric acid (NA) against *Escherichia coli* K-12 were determined by 96-well broth microdilution with and without a fixed sub-inhibitory (5 mg/ml) sucrose laurate (SL) dose. For antibiofilm assays, 24-h *E. coli* K-12 biofilms were formed in 96-well plates and treated for 24 h at each chemical's MIC with/without SL. Residual biofilm biomass was quantified by crystal violet staining and expressed relative to untreated controls. Experiments used 3 biological replicates with 3 technical replicates per condition. Statistical differences between treatments with and without SL were evaluated using a two-sample *t*-test ($\alpha = 0.05$).

Results: MICs (mg/mL) were 0.01 (NaOCl), 0.125 (NaOH), 0.25 (phosphoric acid), and 0.125 (nitric acid) and did not change with SL. At MIC, NaOCl removed 98.9% of biofilm biomass, which increased to 99.9% with SL ($p<0.05$). Also, SL significantly increased biomass removal

for NaOH (42.6% to 86.1%), PA (47.7% to 91.9%), and NA (27.9% to 56.8%) (all $p<0.05$).

Significance: A fixed SL dose improved antibiofilm performance of multiple CIP chemicals without increasing their planktonic MICs, supporting SL as a practical additive to reduce residual biofilm on food-contact surfaces.

Award Application: Developing Scientist Award

P2-207: PREVALENCE AND GENOMIC CHARACTERIZATION OF ANTIMICROBIAL-RESISTANT ESCHERICHIA COLI IN RETAIL MEATS FROM PUERTO RICO

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) is a major threat to human and animal health, with retail meat recognized as a reservoir and transmission pathway for multidrug-resistant bacteria. *E. coli* serves as a key indicator organism for monitoring AMR trends along the food chain. Despite Puerto Rico's reliance on imported foods, data on AMR in retail meats remain limited.

Purpose: This study evaluated the prevalence and genomic characteristics of antimicrobial-resistant *E. coli* isolated from retail meats in Puerto Rico.

Methods: Between 2022 and 2023, 475 retail meat samples (chicken, turkey, beef, and pork) were collected from retail outlets. *E. coli* was isolated following FDA protocols and characterized using whole-genome sequencing to identify resistance genes, plasmid replicons, and sequence types.

Results: *E. coli* was recovered from 33% of samples. Prevalence varied by season (25–40%), with no significant seasonal differences. Significant differences were observed by meat type,

with the highest prevalence in ground turkey (52%, $p<0.001$) and chicken (44%, $p<0.01$). The country of origin was not associated with prevalence. Packaging influenced recovery, increasing from 13% in plastic film to 36% in modified-atmosphere packaging and 49% in other packaging types. Genomic analysis of 145 *E. coli* isolates revealed frequent resistance genes to aminoglycosides (68%), tetracyclines (50%), sulfonamides (22%), and β-lactams (21%), including determinants (*blaTEM-1B*, *blaCMY-2*, *blaCTX-M-1*). Poultry, particularly turkey, harbored the greatest diversity of resistance genes and plasmid replicon types (IncFIB, IncI1), and high-risk sequence types such as ST117 and ST131 were detected.

Significance: These findings provide the first integrated assessment of *E. coli* prevalence and AMR genomics in retail meats in Puerto Rico and highlight the need for continued One Health genomic surveillance to mitigate AMR transmission through the food supply.

P2-208: TREATMENT OF PSEUDOMONAS AERUGINOSA AND LISTERIA MONOCYTOGENES BIOFILMS WITH 405 NM ANTIMICROBIAL BLUE LIGHT

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The presence of other biofilm bacteria like *Pseudomonas aeruginosa* may influence the persistence of *Listeria monocytogenes*. Antimicrobial blue light (aBL) emerged as a potential intervention technology against biofilms.

Purpose: This study was undertaken to assess the inactivation of biofilms of *L. monocytogenes* and *P. aeruginosa* by aBL on inert surfaces, and to determine the influence of both bacteria on their survival after aBL treatment.

Methods: Mixtures of *L. monocytogenes* and *P. aeruginosa* strains were cultured as single or dual species to grow biofilms on stainless steel (SS) coupons using a drip flow biofilm reactor (DFBR). The coupons were placed in the DFBR chambers containing tryptic soy broth (TSB), inoculated with 9 log CFU/ mL of cultures, and after 6-h static incubation, TSB was pumped for 48-h biofilm growth. Coupons were exposed to aBL using a high intensity lamp at different doses. Coupons were sonicated in buffer, and suspensions were diluted and plated on tryptic soy agar for single species biofilms and on *Pseudomonas* isolation agar and modified oxford agar for dual species. Plates were incubated at 37°C for 24-48 h and colony forming units were calculated. Independent experiments were conducted at least twice, and

statistical analyses were performed using ANOVA.

Results: The viable counts of *L. monocytogenes* and *P. aeruginosa* single species biofilms were reduced by 2.2 and 2.6 log CFU/ coupon (p>0.05) after aBL exposure to 543 and 581 J/cm^2, respectively. At twice these doses, the inactivation of *L. monocytogenes* remained at about the same value, but the count of *P. aeruginosa* declined by 4.23 log CFU/ coupon (p<0.05). The presence of *P. aeruginosa* in dual species biofilms with *L. monocytogenes* resulted in at least 3.5 log CFU/ coupon reduction of *Listeria* when exposed to aBL dose of 581 J/cm^2.

Significance: The presence of *P. aeruginosa* combined biofilms with *L. monocytogenes* may have reduced the tolerance of the latter to aBL.

Award Application: Developing Scientist Award

P2-209: NON-ANTIBIOTIC ANTIMICROBIAL PRESSURE SHAPES EFFLUX AND STRESS-RESPONSE PATHWAYS IN FOOD-ASSOCIATED AND ENVIRONMENTAL ESCHERICHIA COLI

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Non-antibiotic antimicrobials such as triclosan are extensively employed in food processing and sanitation practices. Their extensive use raises concerns about bacterial adaptation, persistence and survival in food-associated environments. Although efflux-mediated resistance mechanisms have been well documented in clinical isolates, resistance and tolerance mechanisms operating in non-clinical settings remain poorly understood.

Purpose: To understand the genetic variation in regulatory; efflux and stress-associated pathways linked to triclosan tolerance in *Escherichia coli* isolated from retail meat and wildlife sources.

Methods: Whole-genome sequencing data from triclosan-resistant *E. coli* isolates (n = 78) collected from retail meat and wildlife were analyzed using GeneiousPrime. Amino acid substitutions in the oxidative stress regulator gene (*soxS*), the multidrug efflux transporter (*mdfA*), and the stress-associated gene (*ydcU*) were assessed relative to the reference ATCC triclosan-susceptible strain.

Results: Multiple substitutions were identified in *soxS* (K5Q, A12S, D52E, Q95E**)**, along with a recurrent mutation E16K, potentially altering oxidative stress regulation. Extensive sequence variation was observed in *mdfA*, including recurrent substitutions at conserved positions (V94I, R190Q, M296I, and L379Q). In addition, multiple substitutions were identified in *ydcU* (I297V, R23H, I295V, and A168T), indicating possible involvement of envelope- or stress-associated pathways in triclosan tolerance.

Significance: These findings demonstrate that triclosan tolerance in food-associated and environmental *E. coli* is associated with coordinated genetic variation across regulatory, efflux, and stress-response pathways beyond classical resistance determinants. Such adaptations may contribute to bacterial persistence in food-related environments and have important implications for food protection strategies and public health risk assessment.

Award Application: Developing Scientist Award

P2-210: ANTIMICROBIAL RESISTANCE OF ESCHERICHIA COLI AND ENTEROCOCCUS SPP. IN PASTURED POULTRY FLOCKS: IMPLICATIONS FOR ONE HEALTH AND FOOD SAFETY

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Category: Antimicrobials

Abstract:

Introduction: Pastured poultry production is on the rise, and the persistence of antimicrobial-resistant (AMR) pathogens such as *E. coli* and *Enterococcus* spp*.* remain a global public health threat. Periodic assessment of flock status can help farmers understand microbial ecology and devise mitigation plans.

Purpose: This study characterized the prevalence and antimicrobial resistance of *E. coli* and *Enterococcus* spp. in pastured poultry across multiple farms.

Methods: Boot swabs (n=10) from eight flocks, hot-rehang (n=20), and post-chill (n=20) carcasses from processing plant were collected and homogenized in buffered peptone water. The samples were enriched in tryptic soy broth and incubated at 37°C for 24 h. *E. coli* (EC) was enumerated on Petrifilm™ and confirmed on EMB agar. *E. faecalis* (EF) and *E. faecium* (EM) were isolated on *Enterococcus* differential agar after 48 h at 37°C. AMR profiles of 72 pre-harvest isolates were analyzed for EC, EF, and EM (n=24 each) using the Sensititre system.

Data were analyzed using Chi-square in JMP (p□0.05).

Results: There were 100% prevalence of EC and EF from all the flocks, while EM was 98%. This reduced significantly (p<0.05) from boot swabs to carcass rinses, decreasing to 42% and 46% for EC, 78% and 84% for EF, and 60% and 50% for EM at respective pre- and post-chill. EC loads varied across farms, from 5.5 to 7.6 $\log_{10}$ CFU/mL. AMR profiles of farm isolates showed 67% EC, 100% of EF, and EM were resistant. Multidrug resistance (MDR) was observed in 2 (8%) EC, 20 (83%) EF, and 17 (71%) EM isolates. Overall, EC showed lower AMR with resistance mainly to sulfisoxazole (33%) and tetracycline (38%). In contrast, over 50% of EF and EM isolates exhibited resistance to most of the antibiotics tested, including vancomycin, while resistance to daptomycin, quinupristin/dalfopristin, and tetracycline was the highest.

Significance: Pastured poultry harbored high MDR *Enterococcus* spp., highlighting potential public health risks.

Award Application: Developing Scientist Award

P2-211: ANTIMICROBIAL EFFICACY OF PEROXYACETIC ACID SPRAY CABINET ON ESCHERICHIA COLI AND SALMONELLA SURROGATES INOCULATED ON LAMB CARCASSES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Reducing the bacterial load transferred prior to carcass opening can minimize meat safety risks associated with pathogens such as pathogenic *E. coli* and *Salmonella*. Peroxyacetic acid (PAA) has become a widely used antimicrobial intervention to achieve this in meat processing facilities.

Purpose: This study aimed to evaluate the antimicrobial efficacy of a PAA spray cabinet on *E. coli* and *Salmonella* surrogates inoculated on lamb carcasses at a commercial lamb processing plant.

Methods: Over three days, a total of 18 eviscerated hot carcasses were sampled before and after passing through PAA spray cabinet (537ppm, 9 psi) to evaluate bacterial reductions (APC: aerobic bacterial count; EB: *Enterobacteriaceae* count) for inoculated *Escherichia coli* and *Salmonella* surrogates. Samples were collected at the posterior of the lamb carcasses. Two post-PAA samples were collected either from the same side of carcasses as the corresponding inoculated pre-PAA sample (S1) or from the opposite side of the inoculated pre-PAA sample (S2). Two-way ANOVA with Tukey-adjusted marginal means (α

= 0.05) was performed in R to assess the fixed effect of PAA treatment and the block effect of day.

Results: The results showed that the surrogate reductions ($P < 0.05$) for inoculated samples were 0.84 ± 0.08 log CFU/cm^2 for APC and 0.73 ± 0.09 log CFU/cm^2 for EB on S1. For S2, the reductions ($P < 0.05$) were 0.94 ± 0.065 log CFU/cm^2 for APC and 0.91 ± 0.067 log CFU/cm^2 for EB. No significant differences were observed for either APC ($P = 0.46$) or EB ($P = 0.16$) when comparing the S1 and S2 samples. A significant block effect ($P < 0.05$) of day was also detected for the study.

Significance: These findings indicate that the PAA spray cabinet can effectively reduce inoculated surrogates for *E. coli* and *Salmonella* inoculated on whole lamb carcasses. However, the magnitude of reduction may vary depending on operational conditions, PAA concentration, and the initial levels of natural bacteria.

P2-212: ANTIMICROBIAL EFFECT OF SORGHUM LEAVES EXTRACT AGAINST FOODBORNE PATHOGENS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: The increasing incidence of foodborne illnesses has intensified the demand for natural alternatives to synthetic preservatives. *Sorghum bicolor*, a drought-resistant cereal crop, is known for its rich phytochemical composition, particularly its leaves.

Purpose: This study aimed to evaluate the antimicrobial properties of sorghum leaf extract against selected foodborne pathogens as well as assess how extraction time and solid-to-solvent ratio affect its phytochemical content, antioxidant activity, and microbial inhibition.

Methods: Sorghum leaves were dried, ground, and extracted using a 70:30 water-ethanol mixture at different ratios (1:5, 1:10, 1:15) and extraction times (0, 24, 48, and 72 hours). Phytochemical screening was conducted to identify bioactive compounds, while total phenolic and flavonoid contents were measured using standard colorimetric methods. Antioxidant activity was assessed using the DPPH assay. Antimicrobial activity was tested against *E. coli*, *S. aureus*, *Salmonella* spp., *Bacillus* spp., *Aspergillus niger*, and yeast using the disc diffusion and broth dilution methods.

Results: The results revealed that phenols, flavonoids, glycosides, saponins, and terpenoids were present in all the extracts. The highest phenolic content (4.05±0.09 mg GAE/g) was recorded for the 1:5 ratio after 24 hours, while the highest flavonoid content (2.33 mg QE/g)

was observed consistently at the 1:15 ratio. The greatest antioxidant activity (72.46%) was found at 48 hours for the 1:15 ratio. The antimicrobial tests showed effective inhibition of the pathogens by the extracts most especially against gram-positive bacteria.

Significance: The sorghum leaves extracts demonstrated significant antimicrobial and antioxidant capacity. This study highlight its promise as a natural food preservative, offering a safer and more sustainable approach to food safety.

P2-213: EFFECTIVENESS OF HOUSEHOLD ACID WASH TREATMENTS FOR REDUCING ESCHERICHIA COLI ON APPLES INTENDED FOR AT-HOME FREEZE-DRYING

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: At-home freeze-drying is increasingly used for long-term food preservation; however, this process lacks a microbial-killing step, allowing the preservation of foodborne pathogens. Raw fruits are frequently implicated in foodborne outbreaks and may pose an increased risk when freeze-dried without effective pre-treatment.

Purpose: This study evaluated the effectiveness of household-accessible acid wash treatments, alone and in combination with sodium chloride, for reducing generic *Escherichia coli* on foods intended for at-home freeze-drying.

Methods: Apple slices were inoculated with 6 log CFU/g of generic *E. coli* and air-dried under a biosafety cabinet prior to treatment. Samples were immersed for 1–2 minutes with gentle agitation in the following treatments: 3% acetic acid, 5% citric acid, and each acid combined with sodium chloride at 0 or 1200 ppm. Untreated inoculated samples served as controls. Following treatment, samples were air-dried, and *E. coli* was enumerated using Sorbitol MacConkey Agar. Data were analyzed using two-way ANOVA with Tukey's HSD post hoc test ($p<0.05$).

Results: All citric acid treatments (5%), regardless of sodium chloride inclusion (0 or 1200 ppm), reduced *E. coli* to below the limit of detection, corresponding to >3.0 log CFU/g

reductions relative to controls (p<0.05). Acetic acid at 3% without sodium chloride similarly reduced levels below the limit of detection (>3.0 log CFU/g; p<0.05). In contrast, the combination of 3% acetic acid with 1200 ppm sodium chloride resulted in a 2.1 log reduction in CFU/g of *E. coli*.

Significance: This study provides practical, evidence-based data on the antimicrobial efficacy of household-accessible acid wash treatments for foods intended for home freeze-drying. The findings will help identify effective pre-treatment strategies to reduce pathogen risk in high-risk foods and support the development of consumer-focused food safety guidance.

Award Application: Undergraduate Student Award

P2-214: INVESTIGATION OF THE ANTIMICROBIAL ACTION OF CAPRYLIC ACID AND LAURIC ACID AGAINST ESCHERICHIA COLI O157:H7 UNDER ACIDIC AND ALKALINE CONDITIONS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Although medium-chain fatty acids (MCFAs) are promising antimicrobial agents that primarily act through membrane disruption, their membrane-disruptive mechanisms against foodborne pathogens under alkaline conditions remain insufficiently understood due to the predominant focus of previous studies on acidic or near-neutral environments.

Purpose: This study aimed to elucidate the membrane-disruptive effects of MCFAs on *Escherichia coli* O157:H7 ATCC 35150 under acidic (pH 4) and alkaline (pH 10) conditions.

Methods: *E. coli* O157:H7 was treated with 1% MCFAs in Luria–Bertani broth without NaCl (LBWS) at pH 4 or pH 10. Sublethal injury and membrane destabilization were assessed by growth recovery in the presence of Mg^{2+} or Ca^{2+}. Intracellular reactive oxygen species (ROS) levels, membrane permeability, and membrane potential dissipation were measured using H_2DCFDA, propidium iodide, and $DiBAC_4(3)$, respectively. Ultrastructural membrane damage

was examined by transmission electron microscopy.

Results: At pH 4, MCFA-treated cells showed no significant growth recovery with Mg^{2+} or Ca^{2+}, whereas both cations significantly enhanced recovery at pH 10 ($p < 0.05$), with Ca^{2+} providing greater protection. ROS levels increased approximately twofold at pH 4 and further increased at pH 10, with lauric acid (LUA)-treated cells exhibiting 4–5-fold higher fluorescence than the control ($p < 0.05$). Caprylic acid (CLA)-treated cells exhibited higher membrane permeability at pH 4 than at pH 10 ($p < 0.05$), while LUA- and phytic acid-treated cells showed minimal permeability at pH 4 but a marked increase at pH 10 ($p < 0.05$). These trends were consistent with membrane potential dissipation and were confirmed by transmission electron microscopy.

Significance: These findings highlight the importance of environmental pH in modulating MCFA-membrane interactions and provide a basis for optimizing MCFA-based antimicrobial strategies against foodborne pathogens.

Award Application: Developing Scientist Award

P2-215: SYNERGISTIC INACTIVATION OF ESCHERICHIA COLI AND SALMONELLA IN ORANGE JUICE BY HIGH-PRESSURE PROCESSING, MILD HEAT, AND TEN NATURAL ANTIMICROBIAL COMPOUNDS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Foodborne illnesses from *Escherichia coli* and *Salmonella* remain critical public health challenges worldwide. High-pressure processing (HPP) combined with natural antimicrobials offers a promising non-thermal intervention strategy to enhance microbial safety while maintaining food quality.

Purpose: This study investigated the synergistic efficacy of HPP combined with mild heat and ten natural bactericidal compounds for inactivating *E. coli* and *Salmonella* in orange juice.

Methods: Hub880 Barocycler Units applied 400 MPa elevated hydrostatic pressure combined with mild heat (25°C and 37°C) to orange juice inoculated at 10^7 CFU/ml for 0 (control), 3, and 5 minutes. Ten antimicrobial compounds (0.01% each) were tested: Citrospet, Citricidal, caprylic acid, carvacrol, lactic acid, benzoic acid, thymol, citric acid, DL-malic acid, and sorbic acid. Results were statistically analyzed using Tukey- and Dunnett's-adjusted ANOVA.

Results: HPP at 400 MPa with mild heat (25°C) achieved >99.99% (□4 log CFU/mL) reduction of *E. coli*, while *Salmonella* was reduced by □5 log CFU/mL using six compounds. Sorbic acid, malic acid, carvacrol, caprylic acid, lactic acid, and thymol demonstrated optimal antimicrobial synergy, while benzoic acid and citric acid derivatives showed moderate effects.

Significance: These findings provide a data-driven foundation for developing tailored, product-specific HPP protocols that maximize microbial safety without compromising food quality, ultimately enhancing consumer protection and public health outcomes.

P2-216: ANTIFUNGAL INGREDIENT SYSTEMS AGAINST SPOILAGE MICROORGANISMS ISOLATED FROM REFRIGERATED BAKERY

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Refrigerated bakery products are prone to quality loss driven by surface-colonizing molds and yeasts, necessitating robust antifungal strategies to preserve shelf life and sensory quality.

Purpose: To evaluate the antifungal efficacy of Upgrade Pro WS (fermented wheat flour) combined with Flavoset 8300L (natural flavor) against mold and yeast isolates recovered from a refrigerated baked matrix.

Methods: Antimicrobial solutions were prepared in potato dextrose broth (PDB) for yeasts and semisolid PDB (PDB + agar) for molds with the following treatments: no antimicrobial control, combination of fermented wheat flour (0.5 and 0.75%) with natural flavor (0.3, 0.5 and 1%). All formulations were adjusted to pH 6.5, dispensed into 100 well plates, and

inoculated separately with mold or yeast isolates to ~6 log CFU/mL. Plates were incubated at 25°C on an accelerated data generation platform for 7–14 days, with OD600 monitoring. Growth curves were fit to a modified Gompertz model to estimate maximum growth rate (μ_max; d^{-1}) and lag time (days). Data were analysed using one-way ANOVA ($p < 0.05$).

Results: Across all tested treatments, lag time was significantly extended and μ_max reduced compared with the control ($p < 0.05$). For mold isolates, the treatment with 0.75% fermented wheat flour and 1% natural flavor combination extended lag time to ~20 days and reduced growth rate to μ_max ~ 0.96 d^{-1}. For yeast isolates, treatments increased lag by ~8 days and reduced growth rate to μ_max ~ 0.24 d^{-1}.

Significance: The fermented wheat flour and natural flavor ingredient combinations demonstrated robust antifungal activity against yeasts and molds isolated from a refrigerated bakery, significantly delaying growth onset and reducing growth rates. These findings support their use as formulation hurdles to mitigate fungal spoilage and extend refrigerated shelf life in bakery applications.

P2-217: CLEAN LABEL ANTIMICROBIAL SYSTEMS AS REPLACEMENTS FOR POTASSIUM SORBATE AND SODIUM BENZOATE IN DELI SALAD

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Deli salad is a refrigerated ready-to-eat product that is prone to spoilage and post-process contamination by *Listeria monocytogenes*, necessitating effective antimicrobial control throughout refrigerated storage.

Purpose: This study evaluated clean-label antimicrobial systems as alternatives to potassium sorbate and sodium benzoate for shelf-life extension and *Listeria* control in deli salad.

Methods: Three formulations were tested: (1) control with 0.2% potassium sorbate and sodium benzoate; (2) clean-label with 0.5% IsoAge D100 and 0.5% Durafresh 5970; (3) clean-label with 0.2% IsoAge D100, 0.5% Durafresh 5970, and 0.3% Flavoset 8300L. Products were stored at 4°C for 20 days. Uninoculated samples were analyzed for aerobic plate counts, lactic acid bacteria, psychrotrophs, *Pseudomonas*, and yeasts. Samples inoculated with *Listeria*

monocytogenes (~2.0 log CFU/g) were monitored throughout storage. Spoilage was defined at 7.0 log CFU/g. Data were analyzed by one-way ANOVA ($p < 0.05$).

Results: The control showed rapid microbial growth, with spoilage by day 11 as psychrotrophs, *Pseudomonas*, and yeasts reached ~8.0, 5.0, and 6.5 log CFU/g respectively. In contrast, clean-label treatments significantly limited the growth of all spoilage microorganisms ($p < 0.05$). Aerobic and lactic acid bacteria populations in clean-label samples remained below 3.0 log CFU/g through day 20. Yeast populations increased to approximately 6.5 log CFU/g by day 20 in clean-label treatments, resulting in product spoilage and representing a 9 day shelf-life extension relative to the control. In *Listeria* inoculated samples, control exhibited pathogen growth >1.5–2.0 log CFU/g by day 20, whereas, clean-label treatments restricted increases to <0.5 log CFU/g.

Significance: Clean-label antimicrobials effectively controlled spoilage and *Listeria*, performing better on extending shelf life, supporting their use as alternatives to benzoate/sorbate replacement in deli salad.

P2-218: APPLICATION OF ANTIMICROBIAL SYSTEMS TO EXTEND SHELF LIFE AND CONTROL PATHOGENS IN ROOM-TEMPERATURE STORED FOCACCIA BREAD

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Presentation type/Format: Technical

Category: Antimicrobials

Abstract:

Introduction: Focaccia bread stored at ambient temperature is susceptible to spoilage and growth of pathogens such as *Listeria monocytogenes* and *Bacillus cereus*, necessitating effective antimicrobial strategies.

Purpose: To evaluate an antimicrobial system applied for extending shelf life and controlling pathogen outgrowth in focaccia bread stored at room temperature.

Methods: Bread was prepared with no antimicrobials and a formulation with antimicrobials in base (0.1% calcium propionate) and topping (0.1% Flavoset 5400L, 0.3% calcium propionate). Products were baked, packaged and stored at 25°C for 20 days. Samples were analyzed for APC, LAB, and yeasts. Challenge studies conducted by surface inoculating *L. monocytogenes* or *B. cereus* (3.0 log CFU/g). pH, water activity, and moisture were monitored

throughout. Qualitative bench sensory evaluations were conducted relative to reference. Data were analyzed using one way ANOVA ($p < 0.05$).

Results: Physicochemical properties remained stable across treatments (a_w 0.95–0.96; moisture 33–39%; pH ~4.5 in control and ~4.8 in treated samples). Relative to control, the antimicrobial treated bread maintained consistently lower spoilage counts. APC were reduced by approximately 0.6–0.9 log CFU/g, while LAB were suppressed by 0.6–1.6 log CFU/g throughout storage (20 days). Yeasts in treated samples was below the limit of detection. In challenge studies, *L. monocytogenes* populations declined and remained below the 1 log threshold in treated bread, while control samples showed higher levels (~2 log). *B. cereus* populations decreased or remained stable, with treated samples measuring ~2.0–2.2 log CFU/g by day 20 compared to 2.7–3.0 log CFU/g in control. Sensory evaluations did not indicate adverse differences relative to the reference.

Significance: Applying antimicrobials to bread base and topping effectively reduced spoilage and controlled key pathogens, maintaining quality and shelf life for ambient-stored specialty breads.

P2-219: CIDAL EFFECT OF CLEAN-LABEL ANTIMICROBIALS AGAINST LISTERIA MONOCYTOGENES IN REFRIGERATED BEEF BROTH

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* remains a critical food safety concern in refrigerated products, including liquid systems like beef broth. Clean-label antimicrobials offer potential for controlling pathogen outgrowth.

Purpose: This study evaluated the efficacy of natural flavor-fermentate system in preventing *L. monocytogenes* outgrowth in refrigerated beef broth.

Methods: Beef broth was prepared and aliquoted into sterile containers. Two treatments were tested: (i) no-antimicrobial control, (ii) 1.5% Flavoset 5400L + 1.3% BC 59 (natural flavor-fermentate). Antimicrobials were added to broth and mixed for 2 min to ensure homogeneity. Five-strain *Listeria monocytogenes* cocktail was inoculated to the broth to achieve 3.5 log CFU/g. After inoculation, samples were stored under refrigeration at 4°C. Enumeration was performed at 30 min, 24 h, 48 h, Day 4, and Day 14 post-inoculation by

plating onto Modified Oxford Agar (MOX) and incubated at 35°C for 24 h. Failure criteria was defined at 2 log outgrowth. Data were analyzed by ANOVA ($p<0.05$).

Results: The antimicrobial treatment showed a rapid reduction in *Listeria monocytogenes*, with an immediate ~1.0-log decrease by 30 min post-inoculation. In the no-antimicrobial control, *L. monocytogenes* increased significantly ($P>0.05$) over time, reaching ~4 log CFU/g by Day 4 and and increasing to 5 log CFU/g through Day 12. In contrast, broth treated with natural flavor-fermentate fell below the limit of detection by 24 h and remained unrecoverable through Day 12, demonstrating a pronounced cidal effect over 24 h. Overall, the clean-label system achieved elimination of detectable *L. monocytogenes* under refrigeration, while the control met the predefined failure criterion (2.0-log outgrowth) by Day 8.

Significance: Natural flavor-fermentate antimicrobial system effectively controls pathogens such as *Listeria monocytogenes* in refrigerated beef broth.

P2-220: VALIDATING CLEAN-LABEL ANTIMICROBIAL AND ANTIOXIDANT SOLUTIONS TO EXTEND THE SHELF LIFE OF BUFFALO REUBEN DIP

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Refrigerated ready-to-eat dips are prone to microbial spoilage and oxidative degradation, and the current Buffalo Reuben Dip preservative system supports only 24–28 days of shelf life. To meet supply-chain demands, clean-label solutions are needed to improve refrigerated stability.

Purpose: This study evaluated a clean-label antimicrobial and antioxidant system to replace the current formulation and extend Buffalo Reuben Dip shelf life beyond 60 days under refrigerated storage.

Methods: Control (T1) contained market formulation with dry vinegar and rosemary extract. Clean-label treatment (T2; Nourishield D4040, Flavoset 5400L, Durafresh 5924L, and Flavoset 5080) was prepared at sensory-validated use levels. Treatments (25 g; duplicates/timepoint)

were stored at 4°C for 63 days and sampled on days 0, 5, 16, 31, 41, 45, 55, and 63. Samples were plated on tryptic soy agar for aerobic plate count (APC), de Man–Rogosa–Sharpe agar for lactic acid bacteria (LAB), and potato dextrose agar for yeast and mold. Oxidative stability was assessed using the Thiobarbituric Acid Reactive Substances (TBARS) assay to monitor secondary lipid oxidation. Data were analyzed using oneway ANOVA (P 0.05).

Results: By day 63, LAB decreased from 5.5 log CFU/g to less than 2 log CFU/g, indicating cidal effect. Whereas APC remained below 6 log CFU/g for all treatments, with significant differences at days 41 and 56 (P<0.05), where control had significantly higher counts than clean label treatment. LAB, yeast, and mold showed no significant differences across treatments, with yeast and mold consistently near detection limits. TBARS results indicated no significant oxidative changes, confirming antioxidant performance from natural flavoring.

Significance: The clean label system extended Buffalo Reuben Dip shelf life to more than 60 days under refrigerated conditions while maintaining microbial and oxidative stability, offering a viable replacement for the current preservative system.

P2-221: SCREENING OF CLEAN-LABEL ANTIMICROBIAL SOLUTIONS FOR YEAST AND MOLD CONTROL IN PLANT-BASED EGG BASE USING ACCELERATED GROWTH MODELING

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Plant-based egg bases are prone to yeast and mold spoilage due to neutral pH and refrigerated storage, creating a need for clean label antimicrobial systems to extend shelf life.

Purpose: This study characterized spoilage organisms in a plant-based egg base and evaluated clean label antimicrobial solutions using an accelerated growth screening platform to guide recommendations.

Methods: Egg base samples were tested for microbiological and physicochemical analysis, including pH (6.41), water activity (0.93), moisture (78.7%), salt (0.54%), fat (8.54%), and citric acid (0.18%). Microbial testing included aerobic plate count (APC), lactic acid bacteria (LAB), yeast, mold, *Pseudomonas*, and psychrotrophs. Spoilage isolates showed APC up to 2.18 log

CFU/g and mold up to 6.3 log CFU/g. Flavoset 8300L (natural flavor) was tested at 0.1–1.5% in broth (pH 6) using the Accelerated Data Generation Platform (ADGP). Optical density at 600 nm was recorded every 30 min for 14 days at 25°C. Growth rates, lag times, and minimum inhibitory concentrations (MICs) were modelled using the modified Gompertz equation. ANOVA and Tukey's test (P □ 0.05) compared treatments; percent growth rate reduction and lag extension were calculated.

Results: Natural flavor showed strong antimicrobial activity. For APC, control growth rate was 0.895 (h^{-1}) with a 9.01(h) lag; natural flavor achieved complete inhibition (MIC □ 0.5%). Mold control exhibited a growth rate of 0.095 (h^{-1}) and lag of 1.23 (h), with no growth at □0.5% 8300L. Intermediate concentrations reduced growth rates by 40–70% and extended lag phases by more than 3 days versus control (P<0.001).

Significance: Clean-label antimicrobial systems effectively inhibited spoilage organisms in plant-based egg base, supporting their use for extending refrigerated shelf life under neutral pH conditions. ADGP screening provides a statistically validated, data-driven foundation for prototype development and shelf-life study design.

P2-222: VALIDATION OF CLEAN-LABEL ANTIMICROBIAL SOLUTIONS FOR CONTROLLING LISTERIA MONOCYTOGENES AND SPOILAGE MICROORGANISMS IN REFRIGERATED, PLANT-BASED, READY-TO-EAT SALADS

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Category: Antimicrobials

Abstract:

Introduction: Regulatory limits on potassium sorbate and recent recalls highlight the need for clean-label antimicrobials to maintain safety and shelf life in refrigerated ready-to-eat (RTE) salads, which are vulnerable to *Listeria monocytogenes* during extended cold storage.

Purpose: This study evaluated clean label antimicrobial solutions as replacements for potassium sorbate by validating 45 days of shelf life and assessing their ability to inhibit *L. monocytogenes* outgrowth in plant-based RTE salads.

Methods: Three salad types—Couscous, Legume, and Quinoa—were prepared with and without potassium sorbate and treated with either no antimicrobial (control;T1), 0.7% IsoAge D100 + 0.1% Flavoset 5400L + 0.5% Durafresh 5924L (T2), or T2 instead being at 1% DF5924L (T3). Salads (25 g; duplicates/timepoint) were stored at 4°C. Uninoculated samples were analyzed for aerobic plate count (APC), lactic acid bacteria (LAB), yeast, and mold on tryptic

soy agar, de Man–Rogosa–Sharpe agar, and potato dextrose agar. Couscous and Legume salads were inoculated with 2.0–2.5 log CFU/g *L. monocytogenes* (five strain cocktail). Sampling days were 0, 16, 28, 45, and 56. Data were analyzed using one-way ANOVA (P 0.05).

Results: Aerobic plate count, LAB, yeast, and mold remained below 6 log CFU/g across treatments. In inoculated salads, *Listeria* populations remained stable at 2.0–2.5 log CFU/g or declined to nondetectable levels. At day 56, clean-label treatments showed no significant differences from control for APC, LAB, or yeast, indicating equal effectiveness. Additional early significant differences (P 0.05) occurred for APC, LAB, and yeast in Couscous, Legume, and Quinoa, but none impacted final-day stability.

Significance: Clean-label antimicrobial solutions effectively replaced potassium sorbate, prevented *L. monocytogenes* outgrowth, and maintained microbiological stability in plant-based salads for more than 45 days, supporting safer formulations.

P2-223: VALIDATION OF CLEAN-LABEL ANTIMICROBIAL SOLUTIONS FOR CONTROLLING LISTERIA MONOCYTOGENES AND SPOILAGE MICROORGANISMS IN HUMMUS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* poses a major risk in ready-to-eat (RTE) hummus, especially during large-batch steps like bag piercing before packaging. Recent outbreaks highlight the need for validated controls to ensure safety during extended refrigerated storage.

Purpose: This study validated clean-label antimicrobial solutions to inhibit *L. monocytogenes* over 140 days in refrigerated hummus while monitoring background microflora and physicochemical changes.

Methods: Traditional and Roasted Red Pepper hummus were treated with either no preservative (control;T1), 0.7% IsoAge D100 + 0.1% Flavoset 5400L + 0.5% Durafresh 5924L (T2), or 0.7% IsoAge D100 + 0.1% Flavoset 5400L + 1.0% Durafresh 5924L (T3). Hummus (25 g;

duplicates/timepoint) was modified atmosphere packaged (70% N_2/30% CO_2) and stored at 4°C. Inoculated samples were challenged with 2.0–2.5 log CFU/g *L. monocytogenes* (five-strain cocktail). aerobic plate count (APC), lactic acid bacteria (LAB), yeast, and mold on tryptic soy agar, de Man–Rogosa–Sharpe agar, and potato dextrose agar. Sampling days were 0, 14, 30, 60, 90, 100, 144. Data were analyzed by one-way ANOVA (P □ 0.05).

Results: Across all treatments, APC and LAB remained well below 6 log CFU/g, and yeast/mold counts were undetectable throughout. In the *Listeria* challenge study, populations remained stable (2.0–2.5 log CFU/g) in Traditional hummus and declined to non-detectable levels in Roasted Red Pepper hummus by day 144, with no treatment showing outgrowth above 2 log CFU/g. Significant treatment effects were observed for APC in Roasted Red Pepper hummus at day 14, where T1 had significantly higher counts than T2 and T3 (P<0.05) and for LAB at day 31 and day 60 (P<0.001), and in Traditional hummus at day 90 (P<0.01).

Significance: Clean-label antimicrobials effectively prevent *Listeria* outgrowth and maintain microbiological stability in hummus up to 144 days, supporting their use to enhance safety and shelf life of RTE dips.

P2-224: VALIDATION OF CLEAN-LABEL VINEGAR SOLUTIONS FOR CONTROL OF LISTERIA MONOCYTOGENES AND SPOILAGE MICROORGANISMS IN REFRIGERATED FRENCH FRIES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Retail's shift from frozen to refrigerated storage has moved French fries into chilled spaces, increasing risk of *Listeria monocytogenes* and spoilage. Regulatory needs to replace sorbate with clean-label further drives the need for validated antimicrobials.

Purpose: This study evaluated IsoAge buffered vinegar solutions for controlling *L. monocytogenes* and spoilage microorganisms in refrigerated French fries for more than 20 days at 4°C and 7°C compared with conventional.

Methods: French fries were prepared with a conventional (0.09% potassium sorbate and 0.05% sodium benzoate), and clean label treatments (0.3%, 0.4%, 0.5%, or 0.6% vinegar). Eleven-gram samples (duplicates/timepoint) were stored aerobically at 4°C and 7°C. Uninoculated samples were monitored for visible mold and enumerated for aerobic plate count (APC), lactic acid bacteria (LAB), yeast, and mold on tryptic soy agar, de Man–Rogosa–Sharpe agar, and potato dextrose agar, respectively. For the challenge study, fries were

inoculated with 2–2.5 log CFU/g *L. monocytogenes*. Outgrowth above 1 log CFU/g for *Listeria* or spoilage counts above 6 log CFU/g was failure. Data were analyzed using one-way ANOVA ($P \leq 0.05$).

Results: At 4°C, *Listeria* increased by more than 1 log CFU/g in the conventional control by day 20, while vinegar at ≥0.4% prevented measurable outgrowth. At 7°C, the control exceeded 1 log CFU/g growth by day 7. Vinegar treatments limited growth to less than 1 log CFU/g through day 25. Across uninoculated samples, APC, LAB, and yeast remained below two log CFU/g for all clean label treatments, and no visible mold was observed. No significant treatment effects were detected for APC, LAB, or yeast in uninoculated samples ($P > 0.05$), with counts near detection limits.

Significance: Buffered vinegar at ≥0.4% effectively prevented *Listeria* outgrowth and maintained spoilage control in refrigerated French fries for more than 25 days at both 4°C and 7°C supporting its use as a clean label solution.

P2-225: VALIDATION OF CLEAN-LABEL VINEGAR SOLUTIONS TO CONTROL SPOILAGE MICROORGANISMS IN FRESH MOZZARELLA CHEESE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Fresh mozzarella is prone to mold contamination in products with and without brine, leading to early product spoilage. Clean-label buffered vinegar (IsoAge) is known to improve microbial control and extend shelf life.

Purpose: This study evaluated vinegar for controlling spoilage microorganisms in water-packed and vacuum-packed fresh mozzarella and its ability to extend refrigerated shelf life.

Methods: Mozzarella curds were produced with either 0.3% vinegar or no antimicrobial (control). Samples (11 g; duplicates/timepoint) were stored at 4°C and plated on selective media for aerobic plate count (APC), lactic acid bacteria (LAB), yeast, mold, psychrotrophs,

and *Pseudomonas*. Plating ceased if counts exceeded 6 log CFU/g, visible mold developed, or objectionable odor occurred. Data were analyzed by one-way ANOVA (P □ 0.05).

Results: Buffered vinegar kept all microbial groups below 6 log CFU/g for the full test duration in both mozzarella types. Controls reached spoilage at multiple points. In vacuum-packed control, APC and LAB exceeded 6 log CFU/g by day 30, *Pseudomonas* by day 25, and psychrotrophs by day 60. In water-packed control, APC reached spoilage by day 52, LAB by day 45, *Pseudomonas* by day 30, and psychrotrophs by day 25. Yeast remained below 6 log CFU/g in all treatments, and no mold was detected in the vinegar treated samples. At the final sampling day, all organisms except mold showed significant treatment differences (P<0.05) with control counts being significantly higher than vinegar treatment.

Significance: Buffered vinegar effectively controlled spoilage organisms and extended the shelf life of water-packed mozzarella from 37 to 52 days and vacuum-packed mozzarella from 60 to 100 days. These results demonstrate its potential as a clean-label solution for improving microbial stability in fresh mozzarella cheese.

P2-226: VALIDATION OF CLEAN-LABEL ANTIMICROBIAL SOLUTIONS FOR SHELF-LIFE EXTENSION OF REFRIGERATED INDIAN-INFLUENCED MEALS USING ISOLATED SPOILAGE MICROORGANISMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Prepared meals rely on frozen storage for year-long shelf life, but rising demand for refrigerated options requires 30–40 days of chilled shelf-life. Microbial spoilage and package bloating remain key challenges, prompting evaluation of clean-label preservation strategies.

Purpose: This study identified spoilage organisms in expired refrigerated meals and evaluated clean-label antimicrobial solutions using an accelerated growth screening platform to support shelf-life extension recommendations.

Methods: Spoilage microorganisms were isolated from bloated chicken tikka masala and butter chicken, including aerobic plate count (APC) organisms, lactic acid bacteria (LAB), yeast, *Pseudomonas*, and psychrotrophs (12 isolates; 1.9–9.5 log CFU/g). Antimicrobial

solutions (Nourishield D4040, Flavoset 5400L, Durafresh 5924L, IsoAge D100 (vinegar), and combinations) were prepared up to 1.5% at pH 5.5. Broth treatments were inoculated at 6 log CFU/g. Growth was measured using the Accelerated Data Generation Platform (ADGP) (optical density: 600 nm) every 30 minutes for 7 days at 25°C or 30°C. Growth rates, lag times, and minimum inhibitory concentrations (MICs) were modelled using the modified Gompertz equation. Oneway ANOVA and Tukey's post-hoc test (P□0.05) were used. Reduction in growth rate and lag time versus control were calculated.

Results: Vinegar reduced APC growth rate by 74% and extended lag phase by 2.1 days compared to control (P<0.001). Combination treatments completely inhibited APC, LAB, and yeast growth (MIC□0.75%) and extended lag phase over 3 days. LAB growth rate decreased by 54% with vinegar alone and was fully inhibited in combination treatments. Yeast growth was suppressed by 34% with vinegar and eliminated in combination treatments.

Significance: Clean-label antimicrobials demonstrated effectiveness against key spoilage organisms, achieving full inhibition at optimized use levels and supporting refrigerated shelf-life extension to 30–40 days.

P2-227: UVC INACTIVATION OF LISTERIA MONOCYTOGENES IN SALMON JUICE ON FOOD CONTACT SURFACES USING THE CONTAMINATION SANITATION INSPECTION AND DISINFECTION (CSI-D+) DEVICE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Listeria monocytogenes* is a severe foodborne illness-causing pathogen in the United States, resulting in 1600 cases of listeriosis per year, with a 94% hospitalization rate according to the Centers for Disease Control. The Contamination Sanitization Inspection and Disinfection (CSI-D+) device is a UVC-emitting handheld system that represents a novel approach to detecting residue and disinfecting food contact surfaces.

Purpose: This study aimed to evaluate the disinfection module of the CSI-D+ device targeting *L. monocytogenes* within salmon residue on food contact surfaces.

Methods: The food contact surfaces tested included stainless steel (304) and polyethylene. Raw salmon juice was inoculated with *L. monocytogenes* strains 1/2a, 1/2b, and 4b on the food contact surfaces and then exposed to the 275 nm germicidal wavelength of the CSI-D+ disinfection module for 30 and 45 seconds. The samples were then plated on the selective *Listeria* Oxford Agar. Additionally, the total number of aerobic bacteria was assessed using

APC Petrifilm™.

Results: The average *L. monocytogenes* percent reduction for all strains, surfaces, and time combinations was 49.6%, with log reductions ranging from 0.2 to 0.4 log CFU/ml. The highest log reduction of 56.4% (0.4 log CFU/ml) was observed on the polyethylene surface following the 45-second treatment for *L. monocytogenes* 1/2b. Additionally, the lowest reduction was 41.2% (0.2 log CFU/ml) for the 1/2a strain on polyethylene for 45 seconds. Statistical analysis using two- and three-way ANOVA was performed in RStudio, but no variables were found to be statistically significant ($p < 0.05$).

Significance: The CSI-D+ device effectively reduced *L. monocytogenes* strains 1/2a, 1/2b, and 4b on the contact surfaces of stainless steel and polyethylene within salmon juice residue. These findings suggest that the CSI-D+ has potential as an additional method for controlling microbial contamination in food processing areas.

P2-228: PREVALENCE AND ANTIMICROBIAL RESISTANCE GENE PROFILES OF ESCHERICHIA COLI ISOLATED FROM RETAIL GROUND BEEF IN CALIFORNIA

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial resistance (AMR) is a growing public health concern, and foodborne bacteria in the food supply chain can serve as reservoirs of AMR genes. *Escherichia coli* (*E. coli*) is one of the most commonly detected organisms in retail meats.

Purpose: To determine the prevalence of *E. coli* and characterize AMR genes in isolates from retail ground beef collected in California.

Methods: Retail ground beef samples were collected monthly from randomly selected grocery stores in California by the FDA National Antimicrobial Resistance Monitoring System (NARMS) program. Samples were tested for *E. coli* using the NARMS Retail Meat Isolation Protocol. Confirmed *E. coli* isolates were subjected to whole-genome sequencing to identify AMR genes targeting major antimicrobial classes, including aminoglycosides, beta-lactams, colistin, fosfomycin, macrolides, phenicol, quinolones, sulfonamides, tetracyclines, and trimethoprim.

Results: In total, 201 retail ground beef samples were collected in 2018, 2020, and 2021. Among the 197 tested samples, 22.3% (44/197) were positive for *E. coli*. At least one AMR gene was detected in 54.5% (24/44) of *E. coli* isolates. The most frequently detected AMR gene was *mdf(A)*, an intrinsic multidrug resistance gene, identified in 47.7% (21/44) of *E. coli* isolates, followed by *tet(A)* and *tet(B)*, tetracycline resistance genes, and *sul2*, a sulfonamide resistance gene, each detected in 6.8% (3/44) of *E. coli* isolates. AMR genes conferring colistin and fosfomycin resistance were not detected in this study.

Significance: The detection of AMR genes in *E. coli* indicates the continued presence of AMR determinants in the retail ground beef supply in California. Findings of lack of resistance to several critically important drug classes are encouraging. These results highlight that ongoing surveillance of *E. coli* and its resistance gene profiles in retail beef is essential for monitoring AMR trends in retail foods.

Award Application: Developing Scientist Award

P2-229: ADVANCING LISTERIA CONTROL IN PLANT-BASED DELI PRODUCTS THROUGH CLEAN-LABEL ANTIMICROBIALS

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Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* (LM) employs adaptive stress response mechanisms that enable it to withstand osmotic stress and grow under psychrotrophic conditions, including refrigeration. Its resilience, combined with the high-moisture content, nutrient availability, and near-neutral pH of plant-based products, makes LM a significant food safety hazard, particularly due to the risk of post-processing contamination in ready-to-eat (RTE) foods. These challenges underscore the critical need for effective antimicrobial interventions to inhibit LM growth.

Purpose: The study evaluated the effectiveness of a clean-label antimicrobial Fermand™ Fermented Garlic (FG) in combination with buffered vinegar in controlling LM in RTE plant-based deli meat.

Methods: Plant-based deli slices (soy protein base) were prepared with two clean-label antimicrobial treatments: FG + buffered vinegar at 50:50 (T1) and 90:10 (T2), each at an inclusion rate of 1% (w/w) in the final formulation. Positive control without antimicrobials was also included. All samples were surface-inoculated with a cocktail of LM at 2.5 log CFU/g,

vacuum-packaged and stored at 4°C for 103 days. LM growth was monitored at regular intervals. The study was repeated twice with duplicate samples (n=4).

Results: In the positive control, LM increased to 8 log CFU/g from an initial 2.5 log CFU/g by day 38 and maintained at 7 log CFU/g for 103 days at 4^{o}C. FG + buffered vinegar treatments (T1&T2) were highly effective in controlling LM in plant-based deli meat and did not allow LM to grow >1 log CFU/g from the initial inoculum. Treatments kept LM growth below 3.5 log CFU/g throughout the storage period (p<0.05) and resulted in 4–5 log LM growth inhibition compared to control (p<0.05).

Significance: The use of FG combined with buffered vinegar as a natural antimicrobial offers an effective solution for controlling LM in plant-based deli meat, reducing reliance on synthetic preservatives.

P2-230: A CLEAN-LABEL CASEIN FERMENTATE CONTROLS LISTERIA MONOCYTOGENES IN A HIGH-MOISTURE SOFT CHEESE MODEL

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: High-moisture soft cheeses are susceptible to post-process contamination of *Listeria monocytogenes*. As the dairy industry moves away from synthetic preservatives, clean-label, fermentation-derived antimicrobials are needed to ensure product safety without compromising label simplicity.

Purpose: This study evaluated the antimicrobial efficacy of a clean-label casein fermentate (NPc), produced using the GRAS organism *Aspergillus oryzae*, by determining its minimum inhibitory concentration (MIC) and assessing its ability to control the growth of *L. monocytogenes* in a high-moisture soft cheese model during refrigerated storage.

Methods: NPc was produced by fermenting a 2% casein peptone substrate with *A. oryzae* for six days, followed by concentration by extraction or freeze-drying. MIC was determined using an agar dilution assay against *L. monocytogenes* 10403S. For food-model validation, high-moisture soft cheese was prepared with three treatments: no antimicrobial, NPc at full fermentate strength (1×), or 0.1% potassium sorbate. Cheese samples were inoculated with a five-strain *L. monocytogenes* cocktail (4 log CFU/g) and stored at 4°C for two weeks.

Populations were enumerated on Modified Oxford agar and analyzed statistically.

Results: NPc completely inhibited *L. monocytogenes* at 0.25× fermentate strength in vitro. In the cheese model, untreated controls increased by ~4.0 log CFU/g, and sorbate-treated samples increased by ~2.8 log CFU/g during storage. In contrast, NPc-treated samples showed no significant increase relative to initial levels ($p = 0.55$) and had significantly lower counts than both control ($p = 0.0023$) and sorbate-treated samples ($p = 0.0075$). RNAseq additionally indicated a change in amino acid synthesis and iron acquisition.

Significance: These findings demonstrate that NPc effectively controls the growth of *L. monocytogenes* in a high-moisture soft cheese system, supporting its potential as an antimicrobial for food safety applications.

P2-231: CHANGES IN GENETIC EXPRESSION AND VIABILITY OF LISTERIA MONOCYTOGENES ASSOCIATED WITH THE PRESENCE OF PHAGE A511

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* can persist in food processing environments, and although bacteriophages are promising biocontrol agents, bacterial regrowth following phage exposure suggests the presence of tolerance mechanisms.

Purpose: To evaluate the changes in expression and viability in *Listeria monocytogenes* associated with exposure to phage A511 in vitro.

Methods: Four strains with different phage susceptibility and biofilm formation capacity were exposed to phage A511 (MOI 1) and incubated at 30°C for 24 h or 4°C for 15 days. Expression of *agrA, luxS, flaA, Lmo2550,* and *rmlB* was analyzed by RT-qPCR using gene-specific standard curves. Bacterial viability was evaluated using the PrestoBlue assay at MOI 0.01, 0.1, and 1 under the same conditions. Untreated cultures served as controls. Experiments were conducted in biological triplicate. Data were expressed as mean ±SD and

analyzed by one-way ANOVA with Tukey's test ($p < 0.05$).

Results: Phage exposure significantly downregulated *flaA* and *agrA*, with stronger effects at 4°C ($p < 0.05$). Expression of *luxS, Lmo2550,* and *rmlB* showed no significant differences ($p > 0.05$). All strains showed reduced viability compared with controls, particularly phage-susceptible strains. At 30°C and MOI 0.01, strains LM4b and LM11 showed ~95% viability reduction within 10 h ($p < 0.05$), followed by regrowth after 16 h.

Significance: *L. monocytogenes* adapts to phage predation by modulating motility and quorum sensing related genes, promoting survival and regrowth. These insights inform the development of more robust phage-based strategies for food safety and public health.

Award Application: Developing Scientist Award

P2-232: DECONTAMINATION OF LISTERIA MONOCYTOGENES AND SALMONELLA TYPHIMURIUM IN VITRO AND ON POST-HARVEST SEED (ALFALFA, ALMOND, MUNG BEAN, SOYBEAN) BY COTTONSEED PROTEIN ISOLATES

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Pathogen contamination of produce is a food safety concern. The use of biorational procedures to minimize food contamination can enhance consumer safety.

Purpose: The objective of this study was to evaluate the decontamination efficacy of cottonseed protein isolates (CSPI, underutilized and abundant agricultural byproduct), a GRAS sanitizer, and their combination against *Listeria monocytogenes* and *Salmonella* Typhimurium in vitro and on seed types.

Methods: Glanded (CSPI-Gd), glandless (CSPI-Gl) protein isolates, organic acid and organic acid sanitizer (100 µL) were evaluated for *L. monocytogenes* and *S*. Typhimurium (6-log) reduction on TSA medium and liquid substrate by optical density and spread plating. CSPI-Gd, CSPI-Gl and sanitizer antimicrobial effects were assessed in co-inoculation assays and on post-harvest seeds (alfalfa, almond, mung bean, and soybean). Substrate homogenates were

prepared by stomaching in peptone water. Pathogen populations were quantified by plating 100 µL of dilutions on MOX (*Listeria*) and XLT-4 (*Salmonella*) selective media at 37°C. Experiments were conducted in triplicates and data analyzed by ANOVA to assess significance of treatments by SAS.

Results: Significant antimicrobial activity of CSPI-Gl and CSPI-Gd against both pathogens ($p<0.05$) were recorded. CSPI demonstrated comparable biocidal effects to GRAS sanitizer. CSPI treatments at 500 ppm inactivated >5.0 log *Salmonella* and *Listeria* in vitro. CSPI-Gl and CSPI-Gd plus sanitizer, resulted in significant ($p<0.05$) pathogen reductions in co-inoculations. S*almonella* and *Listeria* reductions on pathogen-inoculated seed ranged from 0.50–2.68 log, with bacterial inactivation enhanced by combining treatments with sanitizer.

Significance: The antimicrobial efficacy of CSPI byproduct in vitro and on seed was significant. The CSPI bactericidal properties, in addition to the GRAS sanitizer, can enhance fresh produce safety.

P2-233: EFFICACY OF PERACETIC ACID (PAA) IN COMBINATION WITH A PAA BOOSTER AGAINST DRY BIOFILMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Dry biofilms are gaining interest in the food industry as a potential source of contamination in food production and over concern that they may be more resistant to commonly used sanitizers and disinfectants.

Purpose: A study was conducted to examine the efficacy of PAA with and without a PAA booster against single and multi-species wet biofilms and their dry counterparts.

Methods: The CDC biofilm reactor was used to grow single species *Pseudomonas aeruginosa* and dual species *P. aeruginosa* – *Listeria monocytogenes* wet biofilms on 304 stainless steel carriers. Dry biofilms were established by removing the carriers from the reactor and incubating them at 25°C and 50% relative humidity (RH) for 24h. The Single Tube method was used to evaluate the efficacy of PAA alone and in combination with PAA boosters against both biofilm types. Treatments were applied at 20°C for 10 min followed by neutralization. Bacterial viability was determined by serial dilution plating. The Games-Howell test was used to compare treatment means for statistical significance.

Results: After 24h at 25°C and 50% RH the biofilms were visibly dry. Bacterial viability for *P. aeruginosa* remained relatively high (> 8.5 $\log_{10}$ CFU/carrier) with < 1 log difference between the wet and dry biofilms. PAA concentrations of 200–600 ppm resulted in log reduction (LR) values of 1.2–2.8 for the dry biofilms and 0.7–3.9 for the wet biofilms. A PAA booster consisting of a mixture of organic acids, chelants, and surfactants when combined with 200 ppm PAA was highly effective (> 6.0 LR) against both biofilm types. For *P. aeruginosa*, PAA with and without booster performed similarly against wet biofilms and their dry counterparts.

Significance: In food production systems where cycles of wet and dry conditions may exist it is important to understand how that may impact the microorganisms present and their relative susceptibility to sanitizers and disinfectants.

P2-234: SHELF LIFE AND CHALLENGE STUDY IN MAYONNAISE: PRESERVATION SYSTEMS FOR YEAST AND LAB CONTROL UNDER AMBIENT STORAGE

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Spoilage microorganisms pose significant challenges to the shelf life of mayonnaise. Clean-label antimicrobial systems offer a natural alternative to conventional preservatives.

Purpose: To evaluate natural antimicrobial systems (NAS – Nourishield D4040) for controlling spoilage yeast and lactic acid bacteria (LAB) in mayonnaise.

Methods: Mayonnaise samples were prepared and inoculated with a yeast cocktail (*Saccharomyces cerevisiae, Candida* spp.*, Pichia membranifaciens*) and LAB cocktail (*L. plantarum, L. buchneri, L. fermentum, L. brevis*) to achieve 2–3 log CFU/g. Treatments included: T1: Blank (preservative-free), T2: 0.1% sorbic acid, T3: 0.5% NAS, T4: 0.5% NAS + 0.1% sorbic acid and T5: 0.75% NAS/ Samples were stored at 25°C for up to 160 days. Enumeration was performed for LAB (MRS agar, anaerobic, 37°C, 48 h) and yeast (PDA, aerobic, 25°C, 5 days). Spoilage thresholds were defined as 6 log CFU/g. Data was analyzed using one-way ANOVA vs control ($P < 0.05$). The limit of quantification (LOQ) was defined as 0.6 log CFU/g.

Results: LAB counts increased from 3.08 to 7.19 log CFU/g by Day 56 in control samples, exceeding spoilage. Sorbic acid alone delayed growth but reached 7.12 log CFU/g by Day 70. T3 slowed LAB but exceeded spoilage after Day 56. T4 achieved complete inhibition by Day 21 and maintained counts below LOQ through Day 160. T5 suppressed LAB by Day 42 and remained undetectable thereafter. Yeast counts showed growth in Control from 2.71 to 7.67 log CFU/g by Day 56. Sorbic acid eliminated yeast by Day 4 and maintained counts below LOD through Day 160. T3, T4, and T5 achieved full yeast inhibition by Day 4 and sustained counts throughout storage. ANOVA confirmed significant differences for all treatments vs. control ($P<0.05$).

Significance: Preservation systems combining Nourishield D4040 and sorbic acid provided rapid and sustained inhibition of yeast and LAB, extending mayonnaise shelf life beyond 160 days under ambient conditions.

P2-235: EVALUATION OF NATURAL SYSTEMS AGAINST SPOILAGE YEAST AND MOLD IN YOGURT

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Spoilage microorganisms pose significant challenges to the shelf life of dairy products.

Purpose: Evaluate natural preservation systems for controlling spoilage yeast and mold in yogurt during refrigerated storage while maintaining viable lactic cultures (LAB).

Methods: Yogurt was inoculated with mold (*Aspergillus niger*) and a yeast cocktail (*Saccharomyces cerevisiae, Candida parapsilosis,* and *Candida lusitaniae*). Initial yeast and mold levels were ~2.0 log CFU/g; LAB started at 7.5 log CFU/g. Treatments were: T1: Control (No Preservative), T2: 0.5% Cloud S6, T3: 0.7% Cloud S6 and T4: 0.4% DF5001 + 0.5% IsoAge D100. Samples were stored at 4°C for 70 days. LAB, yeast, and mold were enumerated at multiple time points on selective media. Spoilage threshold for yeast/mold was >6 log CFU/g or until visible growth was present. Data were analysed by one-way ANOVA comparing each treatment to the control ($P < 0.05$). Limit of quantification (LOQ) was defined as 0.6 log CFU/g.

Results: All treatments showed similar LAB near baseline across 70 days (7.3–7.6 log CFU/g), comparable to control, indicating no adverse impact on live cultures. In yeast, control showed an increase from 1.77 to 5.26 log CFU/g by Day 70, with visible mold at day 56. T2 and T3 decreased yeast to <LOQ by Day 56 and held undetectable through Day 70. T4 achieved complete inhibition by Day 35 and remained at undetectable levels thereafter. In mold, control increased from 1.61 to 3.11 log CFU/g by Day 70. T2 and T3 inhibit mold growth to 1.61 log CFU/g at Day 70. T4 below LOQ by Day 35 and stayed undetectable through Day 70. ANOVA confirmed significant differences vs. control for all treatments ($P < 0.05$).

Significance: Natural preservation systems effectively suppressed spoilage yeast and mold during 70-day refrigerated storage without compromising LAB viability. Among them, T4 produced the fastest and most complete inhibition, supporting its use to extend shelf life in cultured dairy products.

P2-236: EVALUATION OF SHELF LIFE AND LISTERIA CONTROL IN PORK RINDS USING CONVENTIONAL PRESERVATION SYSTEMS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Purpose: To evaluate the effectiveness of a conventional antimicrobial system in extending shelf life and controlling *Listeria* spp. in pork rinds (chicharrón prensado) during ambient storage.

Methods: Pork rind samples were treated with T1: 0.75% Provian N + 0.2% Sodium Propionate and compared to a preservative-free Control. Products were vacuum-packed and stored at 25°C for 84 days to simulate export conditions. Initial inoculation of *Listeria* cocktail was ~3.5 log CFU/g. Enumeration was performed at multiple time points using MOX agar. Spoilage threshold was defined as 6.0 log CFU/g. Data were analysed by one-way ANOVA comparing treatment vs. Control ($P < 0.05$).

Results: The Control (preservative-free) exhibited continuous *Listeria* growth, increasing from 3.12 log CFU/g (Day 0) to 6.64 log CFU/g (Day 84), surpassing the spoilage threshold by Day

28. In contrast, the treated samples with T1 maintained counts below 1.32 log CFU/g from Day 0 and reached LOQ by Day 14, sustaining undetectable levels through Day 84. ANOVA showed significant differences between the treatment group and control ($P < 0.05$). Visual evaluation of quality characteristics (humidity, color, texture) and mold yeast growth showed significant differences between treatments and control after 60 days of storage.

Significance: The T1 provided robust *Listeria* control and extended shelf life beyond 90 days under ambient conditions, meeting export requirements and ensuring microbial safety for pork rind products.

P2-237: EFFICACY OF NATURAL SYSTEMS AGAINST SPOILAGE AND PATHOGENIC MICROORGANISMS IN TURKEY DELI MEAT

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Spoilage and pathogenic microorganisms such as *Leuconostoc mesenteroides* and *Listeria* spp. pose significant challenges to the safety and shelf life of ready-to-eat meat products. Clean-label antimicrobial systems offer a natural alternative to conventional preservatives.

Purpose: To evaluate the antimicrobial efficacy of natural, clean-label agents against *L. mesenteroides* and *Listeria* spp. in turkey deli meat stored at 4°C.

Methods: Turkey deli meat was treated with four antimicrobial systems and a negative control, combining in formula applications and surface spray:

T1: Negative Control
T2: 0.5% Smoke-1 + 0.3% Cultured Sugar in formulation; 0.05% Smoke-2 applied as

surface spray
T3: 0.5% Smoke-1 + 0.5% Cultured Sugar incorporated in formulation

Samples were inoculated with *L. mesenteroides* (1.5–2.0 log CFU/g) and *Listeria* spp. cocktails (2.0–2.5 log CFU/g), packed, and stored at 4°C for 28 days. Enumeration was performed on de Man, Rogosa, Sharpe agar for *L. mesenteroides* and MOX agar for *Listeria*. Spoilage was defined as 6 log CFU/g. Data were analysed using one-way ANOVA ($P < 0.05$). Limit of Quantification (LOQ) was defined as 0.4 log CFU/g.

Results: All treatments significantly reduced microbial growth compared with T1 (Negative Control) during refrigerated storage ($p < 0.05$). T2 and T3 exhibited inhibition effect on *Listeria* growth (2.5 □ 1.5 log CFU/g) over 21 days of storage, successfully controlling outgrowth. For L*. mesenteroides*, T2 and T3 showed complete inhibition (<0.4 log CFU/g) by Day 28.

Significance: This study demonstrates the effectiveness of clean-label antimicrobial systems in controlling spoilage and pathogenic microorganisms in turkey deli meat, offering to industry a natural solution for improved safety and shelf life.

P2-238: ANTIMICROBIAL EFFICACY OF NATURAL SYSTEMS AGAINST SPOILAGE AND PATHOGENIC MICROORGANISMS IN ACCELERATED DATA GENERATION PLATFORM (ADGP)

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Spoilage and pathogenic microorganisms such as Lactic Acid Bacteria and *Listeria* spp. pose significant challenges to the safety and shelf life of food products. The use of Accelerated Data Generation Platforms is necessary to achieve accurate and faster results.

Purpose: Evaluate natural and conventional systems against lactic acid bacteria (LAB) and *Listeria* spp. in selective broths in accelerated conditions.

Methods: Treatments: T1-Negative Control, T2: 0.4% AM5 + 0.3% Fermented Sugar (Clean label) and T3: 0.4% Provian N + 0.3% Fermented Sugar (Conventional System). LAB cocktail was inoculated into MRS broth (MRSb) (3.0–3.5 log CFU/mL) and incubated 14 days at 35°C. *Listeria* cocktail was inoculated into BHI broth (3.5–3.8 log CFU/mL initial) and incubated 14 days at 35°C. Enumeration was performed at each time point; spoilage threshold was set at 6.0 log CFU/mL, data were analyzed by one-way ANOVA versus the positive control ($P < 0.05$).

Limit of quantification (LOQ) was defined as 0.4 log CFU/mL.

Results: Positive control showed an increase from 3.55 to 7.91 log CFU/mL in LAB samples by Day 1 and remained >5 log thereafter (5.45 at Day 4; 3.20 at Day 14). T2 and T3 showed LAB suppression by Day 1 through Day 14 ($P < 0.05$). Positive control increased from 3.78 to 6.64 log CFU/mL in *Listeria* samples by Day 4, indicating >2-log outgrowth and reaching the spoilage threshold. T2 limited growth to 3.45 (Day 1), 4.33 (Day 14); T3 to 4.03 (Day 1), 4.56 (Day 7), both presents statistically difference compared with control and threshold 6.0 log CFU/mL ($P < 0.05$).

Significance: Both conventional and natural solutions produced rapid, sustained LAB suppression in MRSb and effectively constrained *Listeria* growth in BHI, preventing spoilage level outgrowth under invitro conditions.

P2-239: EVALUATION OF AN IN VITRO METHOD TO DETERMINE THE VIRULENCE POTENTIAL OF LISTERIA MONOCYTOGENES BY ASSESSING GUT BARRIER TIGHT JUNCTION PERMEABILITY

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Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* (Lm) in fresh produce and food supply chains show varying virulence levels. Lm causes listeriosis and compromises gut barrier function by crossing the intestinal epithelium through intracellular invasion and disrupting cell-to-cell junctions. This phenomenon by Lm can be used to develop a method for determining the virulence potential of Lm strains.

Purpose: To develop a quantitative method to compare Lm strain virulence by measuring gut barrier tight junction permeability.

Methods: Human intestinal epithelial cells (Caco-2/TC7) were used to develop an in vitro model of gut barrier tight junctions. Polarized Caco-2/TC7 monolayers were treated with certain Lm strains and purified cell wall proteins of Lm, following which trans-epithelial electrical resistance (TEER) and lucifer yellow flux were measured.

Results: TEER values of monolayers decreased markedly when exposed to Lm strains.

However, the degree of change varied among strains. Using purified cell wall proteins, we demonstrated that both *Listeria* adhesion protein and internalin B can compromise the paracellular permeability of monolayers. The proteins had a delayed effect compared to whole cells of Lm. An alternative method using lucifer yellow penetration confirmed that these treatments disrupted tight junctions in the cell monolayers.

Significance: Preliminary data indicates that this in vitro method could allow the determination of virulence levels of different Lm strains.

P2-240: EVALUATING THE GROWTH KINETICS OF PLANKTONICALLY GROWN AND BIOFILM-ORIGINATING LISTERIA MONOCYTOGENES IN DELI-TURKEY FORMULATED WITH ANTIMICROBIALS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Antimicrobial control of *Listeria monocytogenes* (*Lm)* has been validated using cells grown in a planktonic state. Less is known about the antimicrobial control of biofilm-originating *Lm* in products.

Purpose: Evaluate the growth inhibition of planktonically grown *Lm* and biofilm-originating, sublethally stressed *Lm* inoculated on deli turkey slices formulated with common antimicrobials.

Methods: Deli turkey was prepared without antimicrobials (negative control; NC), 3.5% sodium lactate/diacetate (SLD), or 1% vinegar powder (VP). Samples were inoculated with a planktonically grown *Lm* strain (ca 2 log CFU/g) or with *Lm* cells harvested from sublethally stressed biofilms. *Lm* was inoculated onto Buna-N coupons (1x1 cm, in 10 mL tryptic soy broth (TSB)) and incubated (37°C; 72 h), with TSB refreshment every 24 h. TSB was decanted, and coupons were rinsed with phosphate-buffered saline prior to 14-day storage at 10 °C. Coupons were then submerged in 200 ppm chlorine solution (10 sec). Coupons were placed in a neutralizing broth and scrubbed to collect biofilm cells. Samples were vacuum-packaged

and stored at 4°C. Upon enumeration, a 1:2 weight of buffered peptone water was added to the samples. After stomaching, serial dilutions were performed and spread plated on modified Oxford agar (37°C; 48 h). Lactic acid bacteria for non-inoculated samples were enumerated on De Man, Rogosa, and Sharpe agar (30°C; 72 h).

Results: Samples were analyzed for 175 days. All treatments had similar (P>0.05) starting Lm populations of ca. 2 log CFU/g. Planktonic *Lm* reached 2-log outgrowth (TTG2) at d 16 for NC, d 71 for SLD, and d 87 for VP. TTG2 for biofilm-originating *Lm* was 19 d for NC, 78 d for SLD, and 167 d for VP. Biofilm origination significantly (p<0.05) slowed the growth rate of *Lm* in VP-treated turkey.

Significance: Despite the relevance of inoculating with biofilm-originating *Lm*, the currently accepted approach of using planktonic cell growth kinetics is affirmed.

P2-241: MAXIMUM SPECIFIC GROWTH RATE (MMAX) DRIVEN MECHANISTIC MODELING TO QUANTIFY DRY VINEGAR CLEAN LABEL ANTIMICROBIAL IMPACT IN LISTERIA RISK PREDICTION FOR RTE TURKEY

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Presentation type/Format: Poster

Category: Modeling and Risk Assessment

Abstract:

Introduction: RTE meats remain highly vulnerable to *Listeria monocytogenes* (LM), making formulation-based controls an essential part of modern food safety. Dry vinegar, a widely used natural antimicrobial, offers promising inhibition yet its effects must be translated into parameters like maximum specific growth rate which drive quantitative microbial risk assessments.

Purpose: This study integrated challenge study data with Baranyi primary modeling and dry vinegar-dependent secondary models to demonstrate how the maximum specific growth rate (μ_{max}) functions as the key quantitative link between antimicrobial concentration, LM growth, and shelf-life safety decisions in RTE turkey.

Method: Challenge study with *Listeria monocytogenes* were conducted on RTE turkey formulated with varying concentrations of dry vinegar (0.6–1.0%) stored at 38±1.5°F & enumerated up to 90 days. Growth curves for each dry vinegar level were fitted using Baranyi primary model to estimate Y_0, Y_{max}, μ_{max}, and h_0. Lag time (λ) was derived from fitted parameters. Dry Vinegar-μ_{max}- λ dependent growth kinetics secondary model was developed.

Primary and secondary models were integrated to predict time-dependent LM growth and evaluate dry vinegar requirements for safety targets.

Results: Modeling results showed, increasing dry vinegar concentration produced a strong reduction in the μ_{max} from 0.052 to 0.009 and increase in lag time (λ) from 18-34 days, indicating enhanced physiological suppression at higher usage rate of antimicrobial. μ_{max}–dry vinegar secondary model accurately interpolated growth kinetics at intermediate concentration (0.85%) with R^2>0.80 (*p*-value 0.05).

Significance: This work demonstrates how μ_{max} based secondary modeling can translate clean-label antimicrobial effects into quantitative, shelf-life predictions for LM growth in RTE turkey. By linking dry vinegar concentration directly to growth kinetics, it is possible to design safe, and long shelf life for RTE meat formulations.

P2-242: EFFECTIVENESS OF LIQUID-BUFFERED VINEGAR IN ENHANCING THE SHELF LIFE OF FRESH POULTRY PRODUCTS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: While adherence to USDA-FSIS food safety requirements is essential, antimicrobial interventions remain critical components of modern poultry processing. Traditional interventions like PAA remain essential, but they provide limited protection against spoilage and shelf-life loss during release delays. Clean-label antimicrobials offer new intervention options, but their effectiveness is strongly influenced by how they are applied.

Purpose: This study evaluates the potential contribution of liquid buffered vinegar as a clean-label intervention to extend the shelf life of fresh poultry under prolonged storage conditions associated with regulatory hold times.

Method: Fresh poultry portions (breast fillets and wings) were treated with peracetic acid (PAA) as positive control followed by buffered vinegar applied at 20% and 40% levels. Breast samples received a PAA dip and subsequent buffered vinegar spray on a conveyor to target approximately 2% uptake, whereas wings were treated with inline PAA spray and buffered vinegar to increase surface coverage. Samples were packaged and stored under refrigeration for extended hold times. Shelf life was assessed by Total Aerobic, Lactic Acid Bacteria, Yeast,

and Mold counts using AOAC methods.

Results: Buffered vinegar applied at 40% spray concentration significantly extended the shelf life of fresh chicken breast ($p<0.05$, *t*-test), maintaining APC levels below 3 log CFU/g for up to 18 days compared with PAA alone and PAA followed by a 20% buffered vinegar spray. In contrast, chicken wings showed no significant differences ($p>0.05$) among the control, and both the treatments in shelf-life extension.

Significance: The significant improvement observed in chicken breast treated with a 40% buffered vinegar suggests that clean-label interventions can reduce spoilage during prolonged regulatory hold times, helping processors maintain product quality and reduce economic loss.

P2-243: EVALUATING THE EFFICACY OF CLEAN-LABEL ANTIMICROBIALS IN CONTROLLING LISTERIA MONOCYTOGENES IN HOT DOGS STORED AT AN ABUSIVE TEMPERATURE OF 48.5° ± 1.5°F

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* (LM) remains a leading pathogen of concern in ready-to-eat (RTE) meat products due to their ability to survive and grow under refrigeration. Temperature abuse during distribution or retail storage further increases the risk of post-lethality growth, challenging processors to validate robust antimicrobial systems.

Purpose: This study aimed to determine the ability of novel clean-label antimicrobials in inhibiting *Listeria monocytogenes* and key spoilage organisms growth in hot dogs over 60 days at temperature abusive storage conditions.

Methods: Hot dog samples were prepared with cultured dextrose (T1), dry vinegar (T2), and cultured dextrose-dry vinegar (T3) along with an untreated negative control. All the samples were surface inoculated with a five-strain (ATCC 19111, 19112, 19115, 19118, 13932) cold-adapted cocktail of *Listeria monocytogenes* at a target inoculum rate of 2-3 log CFU/g. Samples were vacuum packaged and stored at 48.5 ± 1.5°F to simulate abusive refrigeration. Samples were enumerated for *L. monocytogenes* using the FDA-BAM method. Shelf-life testing was performed on uninoculated samples, for Total Aerobic, Lactic Acid Bacteria, and

Yeast & Mold Counts using respective AOAC standard methods.

Results: Both T2 and T3 significantly suppressed *L. monocytogenes* growth relative to the T1 and control. However dry vinegar, T2 provided the strongest inhibition, limited the LM growth < 2 log CFU/g through day 60 and demonstrating statistically significant reductions ($p < 0.05$) at an abusive storage temperature compared with T3. Microbial counts for shelf-life testing remained below 6 log CFU/g for all the treatments.

Significance: Dry vinegar provides robust, multitarget microbial control in RTE hot dogs, offering processors enhanced safety margins and extended shelf life without reliance on traditional antimicrobial systems, and positioning it as a promising next generation antimicrobial solution for RTE meat applications.

P2-244: ELECTROSTATIC ORGANIC ACID TREATMENTS AS A POSTHARVEST INTERVENTION TO REDUCE LISTERIA MONOCYTOGENES AND E. COLI O157:H7 ON CUCUMBERS

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: Postharvest treatments help control foodborne pathogens on fresh produce, and organic acids like propionic and lactic are common lower-risk antimicrobials, with favorable human and environmental safety profiles.

Purpose: This study evaluated the antimicrobial effectiveness of propionic acid (PA) alone and in combination with lactic acid (LA) against *Listeria monocytogenes* and *Escherichia coli* O157:H7 on cucumbers.

Methods: Fresh cucumbers (~120 g) were surface inoculated separately with 1 mL of a three-strains cocktail of GFP-tagged, ampicillin-resistant *E. coli* O157:H7 (F4546, ATCC 43895, H1730) to ~8 log CFU/g, or a four-strains cocktail of nalidixic acid-resistant *L. monocytogenes* (V7, LCDC, Scott A, 101M) to ~7.5 log CFU/g. Samples were sprayed with PA (0.5, 1, or 2% v/v), alone or combined with LA (1:1), using an electrostatic sprayer for 30 s at 25 cm, followed by a 5-min drying period. Spread plate count and scanning electron microscopy (SEM) were

used to quantify log reductions and observe morphological changes respectively after organic acid treatment. Data were analyzed using one-way ANOVA with Tukey's post hoc test.

Results: Statistically significant differences were observed among treatment concentrations (p<0.05). The greatest reductions with PA alone occurred at 2% PA, reducing log counts to 7.49 ± 0.16 log CFU/g for *E. coli* O157:H7 and 6.37 ± 0.29 log CFU/g for *L. monocytogenes*. Whereas for the combination treatments, 2%PA+2%LA treatment showed the highest log reductions, reducing counts to 6.76 ± 0.47 log CFU/g for *E. coli* O157:H7 and 5.40 ± 0.33 log CFU/g for *L. monocytogenes*. SEM images showed clear cell damage after organic acid treatment, including surface pitting and irregular cell shape.

Significance: These findings support organic acid combinations as effective postharvest interventions to improve the microbial safety of fresh produce.

Award Application: Developing Scientist Award

P2-245: GROWTH ON PLATES OF BACTERIOCIN-TREATED LISTERIA MONOCYTOGENES ON INFECTED CILANTRO LEAVES IS INFLUENCED BY THE GROWTH MEDIA

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: New methods are needed to control and mitigate bacterial pathogens on agricultural products. The use of bacteriocins, antimicrobial peptides produced by microbes, is one such approach. The beneficial bacteria *Lactobacillus plantarum* produces bacteriocins that have antibacterial activity against *Listeria monocytogenes* (Lm) and we have earlier shown that these bacteriocins can kill Lm on cilantro leaves.

Purpose: To evaluate two different media on the recovery of Lm treated with bacteriocins on cilantro leaves.

Methods: The leaves of coriander plants were spotted as follows: Plant 1 with 5 µl of Lm and Plant 2 with 5 µl of bacteriocin and 5 µl of Lm. These plants were placed in the growth chamber to mimic agricultural settings for 0, 1, 2, 4, and 24 h. Three leaves of each plant were collected at each time point, and each leaf was placed in 5 ml saline and serial dilutions were plated on Oxford agar and Brain Heart Infusion (BHI) agar and incubated at 37°C for 48 h.

Results: The plating results from three independent experiments of bacteriocin treated Lm showed 5 log order of killing in Oxford agar but 2–3 log order of killing in BHI agar. Furthermore, at each time point more 2–3 log order more Lm colonies grew on BHI than on Oxford agar. Thus, in all experiments, BHI Rif enabled better recovery of bacteriocin treated

Lm compared to Oxford Rif.

Significance: Although both BHI and Oxford media are routinely used for Lm growth, here we show that growth of bacteriocin treated Lm on plates varies depending upon the media used in that BHI media shows higher number of colonies than Oxford media. This is important to note because if the right media is not used in studying bacteriocin mediated Lm killing, an erroneous conclusion would be reached.

P2-246: EXPERIMENTAL EVOLUTION OF DDAC TOLERANCE IN LISTERIA MONOCYTOGENES AND ITS RELEVANCE TO FOOD SANITATION

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* persists in food production environments despite routine sanitation due to its ability to adapt to commonly used quaternary ammonium compounds (QACs). Understanding these adaptive mechanisms is critical for improving sanitation strategies and reducing persistence in high-risk food systems and adaptive laboratory evolution provides a controlled framework for characterizing sanitizer tolerance in *L. monocytogenes.*

Purpose: To assess the adaptation of *L. monocytogenes* to didecyldimethylammonium chloride (DDAC) after repeated sublethal exposure and determine whether this induces cross-tolerance to other QACs and antibiotics.

Methods: Adaptive lab evolution experiments were initiated with three parent strains (LM10, LM16, LM20) cultured in TSB at 37°C under sub-MIC DDAC stress or negative controls cultured in the absence of DDAC. Cultures were serially passaged every 24 h for 35 passages with growth kinetics recorded at each transfer. Minimum inhibitory concentrations (MICs) were quantified at 7-day intervals to assess changes in DDAC tolerance. The genomes of the evolved lineages were sequenced along with the parent strains to identify mutations underlying DDAC tolerance.

Results: The parent strains (LM10, LM16 and LM20) had a starting MIC of 1µg/ml, 2µg/ml and 1 µg/ml, respectively. The DDAC-evolved lineage for LM20 resulted in a 2-fold increase in MIC, from 1 µg/mL to 2 µg/mL, after 35 passages. The evolved lineages for LM10 and LM16 remain constant across passages. The sequenced genomes of DDAC evolved lineages identified mutations in candidate genes likely responsible for increased tolerance.

Significance: This study demonstrates the adaptive mechanisms of *L. monocytogenes* to DDAC and the sequences revealed shared mechanisms underlying persistence and potential cross-tolerance among QACs. These findings provide insights that can help inform evidence-based sanitation and risk mitigation strategies in food production environments.

Award Application: Developing Scientist Award

P2-247: INFLUENCE OF POTASSIUM FERRATE CONCENTRATION AND CONTACT TIME ON THE REMOVAL OF LISTERIA MONOCYTOGENES ON PLASTIC CUTTING BOARD

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Presentation type/Format: Poster

Category: Antimicrobials

Abstract:

Introduction: *Listeria monocytogenes* is a dangerous foodborne bacterium that persists in the food processing environment even after sanitation cycles.

Purpose: Ferrate ($FeVIO_4^{2-}$) is a potent oxidizing agent and known to effectively eliminate a variety of micropollutants and microbes during wastewater remediation. However, its efficacy against foodborne pathogens such as *Listeria monocytogenes* is not known.

Methods: This study investigated the antimicrobial efficacy of potassium ferrate (K_2FeO_4) against *L. monocytogenes* on plastic cutting board surfaces. Plastic cutting board pieces (CBP) were inoculated with *L. monocytogenes* Bug600 (serotype 1/2a) and ScottA (serotype 4b), at 10^7 CFU/ml. CBP were exposed to potassium ferrate (PF) concentrations of 0 (control) to 1–8 mg/mL for 5 or 10 min. After PF treatment, the CBP were vortexed in BPW to recover the surviving *L. monocytogenes* cells, which were then quantified by spread plating on non-selective agar containing esculin.

Results: The results demonstrated a PF concentration-dependent reduction in *L. monocytogenes* on CBP. At 2 mg/ml of PF, *L. monocytogenes* counts decreased by 3 log CFU/ml on CBP within 5–10 min. At 4 mg/ml of PF, there was 4-log reduction of *L. monocytogenes* counts on CBP within 5–10 min. The next highest concentration of 8 mg/ml of PF resulted in a consistent 5-log CFU/ml reduction of *L. monocytogenes* on CBP within 5 or 10 min. Both *L. monocytogenes* strains Bug600 and ScottA showed similar responses.

Significance: PF is effective at high concentrations for the removal of *L. monocytogenes* cells from the plastic surface. Further research is necessary to optimize disinfection protocols using PF to effectively eliminate *L. monocytogenes* hotspots if surviving on both food-contact and non-food contact surfaces in the food processing environment.

P3-001: TRAINING AND OUTREACH IMPACTS FOR THE SOUTHERN CENTER FOR FSMA TRAINING, 2018–2024

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Presentation type/Format: Poster

Category: Communication, Outreach and Education

Abstract:

Introduction: The Food Safety Modernization Act's Produce Safety and Preventive Controls for Human Foods Rules require training, outreach, and technical assistance for impacted stakeholders.

Purpose: The Southern Center (SC) for FSMA Training, a consortium of 22–26 institutions in 14 states and 2 territories, goal is to enhance produce safety through a variety of educational means, including conducting and evaluating standardized courses (Produce Safety Alliance (PSA) grower training and Food Safety Preventive Controls Alliance Preventive Controls Qualified Individual (PCQI) training).

Methods: Validated pre-/post-tests were given at PSA and PCQI courses to assess short-term knowledge gains. A 4-month post training qualitative evaluation was conducted to evaluate behavior change related to food safety practices. PSA (*n* = 347, 5,654 participants) and PCQI (N=33, 501 participants) courses were held from 10/18–09/24.

Results: Average PSA post-test scores (21.42/25, *n* = 3,267) were significantly higher than the

average pre-test scores (14.25/25, $t = 14.24$, $p < 0.01$), indicating a significant increase in knowledge. Of 13 practices included in the PSA behavior change surveys, the most adopted practices were: creating or modifying food safety record-keeping systems (53%, 196/370); writing or modifying farm food safety plans (54%; 192/357); and implementing new or different practices for monitoring on-farm facilities (48%, 183/380). Of seven practices included in the PCQI behavior change surveys ($n = 149$) the most frequent behavior changes were: recommended the training to others ($n = 56$); fine-tuning existing food safety plans ($n = 46$); training employees on FSPCA rules ($n = 40$); and implementing new food safety plans ($n = 36$).

Significance: Members of the SC have continued to create resources and provide opportunities for stakeholders. The educational outcomes of SC's education priorities show significant knowledge gain and behavior change amongst training participants.

P3-002: UNDERSTANDING FARM STOP OPERATIONS: INSIGHTS FROM A STATEWIDE NEEDS ASSESSMENT IN ARKANSAS

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Presentation type/Format: Technical

Abstract:

Introduction: Farm stops and small, locally focused markets are emerging as important retail models within regional food systems, offering consumers access to local products while creating new market opportunities for small-scale producers. A statewide needs assessment was conducted to capture real-world experiences from farm stop operators and inform future technical assistance and resource development.

Purpose: The purpose of this assessment was to identify operational challenges, common practices, and priority areas for support among farm stops and similar local food retail businesses in Arkansas to inform future programming.

Methods: Informal, conversational interviews were conducted with farm stop owners, market operators, and small-scale producers between 2024 and 2025. Discussions centered on day-to-day operations, sourcing practices, infrastructure, marketing, labor, financial sustainability, and future goals. Responses were reviewed and organized into broad themes.

Results: Interviews revealed several recurring themes that shaped the success and sustainability of farm stops in Arkansas. Operators consistently pointed to challenges in sourcing, infrastructure, marketing, labor, and financing, issues that are deeply interconnected and often managed through informal systems. These constraints, particularly around capacity and cold storage, emerged as critical factors limiting growth despite strong

consumer demand. Cross-cutting insights revealed that challenges are interconnected, informal systems carry much of the operational burden, and capacity—not demand—often limits growth.

Significance: Findings highlight the resilience and creativity of farm stop operators while underscoring structural challenges that affect long-term sustainability. These insights will inform Extension efforts to provide practical guidance, foster peer learning, and develop coordinated resources that reduce duplication of work and strengthen local food systems.

P3-003: ADVANCING PRODUCE SAFETY EDUCATION THROUGH STUDENT-DEVELOPED, PRODUCE SAFETY RULE-ALIGNED INSTRUCTIONAL MEDIA

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Presentation type/Format: Poster

Abstract:

Introduction: Strengthening the food safety workforce requires educational approaches that build technical knowledge and science-based communication skills. The Produce Safety Rule (PSR) establishes critical standards for minimizing microbial risks in covered produce production.

Purpose: This project evaluated an experiential learning model in which students developed produce educational material aligned with PSR requirements.

Methods: Undergraduate students enrolled in a food microbiology course participated in a

structured assignment that focused on the development of produce safety related material. Students were divided into five teams consisting of 2-3 individuals and were assigned PSR topic areas, including worker health and hygiene, farm food safety plan, wildlife, and post-harvest handling. Students were provided material where they were able to review regulatory language, consult resources, and translate requirements into clear, accurate messaging for stakeholder audiences (e.g., growers, farm workers, extension personnel). In addition, students received several rounds of feedback to improve developed material.

Results: This work resulted in five finalized video scripts and visual storyboards designed to support future video production. Through this experience, students demonstrated improved comprehension of PSR concepts and increased confidence in communicating technical food safety information. Students who had taken the food regulations course were allowed to use what they learned regarding the PSR material. The project generated a collection of PSR-aligned educational material suitable for adaptation into extension programming, classroom instruction, and stakeholder training.

Significance: Integrating experiential-focused assignments into food safety curricula supports workforce development while expanding the availability of science-based outreach tools. This model contributes to advancing food protection through education and capacity building.

P3-004: ENHANCING FOOD SAFETY KNOWLEDGE AMONG MINORITY FARMERS THROUGH CULTURALLY TAILORED FSMA TRAINING

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Presentation type/Format: Poster

Abstract:

Introduction: The Food Safety Modernization Act (FSMA) aims to prevent foodborne illness through science-based standards; however, minority farmers often face cultural, linguistic, and structural barriers that limit effective implementation. This study evaluates the effectiveness of a bilingual, culturally responsive food safety training program designed to improve understanding of FSMA principles among minority farmers.

Purpose: To assess pre- and post-training differences in farmers' knowledge related to FSMA principles, personal hygiene, water testing, soil amendments, and contamination prevention.

Methods: Pre- and post-tests containing 14 knowledge questions were administered to minority farmers (N = 203). Questions covered FSMA objectives, handwashing duration, microbial contamination, water safety, soil amendments, and hygiene practices. Data were analyzed using paired *t*-tests and bootstrapped confidence intervals.

Results: Among respondents who reported age (n=63), 54% were between 35 and 54 years old, 13% were female, and most had 1–15 years of farming experience (28%). The majority of participants identified as Asian (18%). The overall food safety knowledge score improved significantly from 8.15 (SD = 2.63) to 9.82 (SD = 2.13), $t(149) = -4.30$, $p < .001$, Cohen's d = 0.70. Significant improvements were observed in understanding of handwashing ($M_1 = 0.78$, $M_2 =$

0.96, $t(149) = -4.15$, $p < .001$, d = 0.68), water testing ($M_1 = 0.62$, $M_2 = 0.87$, $t(149) = -3.65$, $p < .001$, d = 0.59), and soil amendment intervals ($M_1 = 0.70$, $M_2 = 0.96$, $t(149) = -3.96$, $p < .001$, d = 0.65).

Significance: Findings highlight the effectiveness of culturally tailored FSMA education in improving food safety knowledge among minority farmers, particularly in hygiene and contamination prevention.

P3-005: EVALUATION OF PRODUCE SAFETY ALLIANCE GROWER TRAINING LEARNING OUTCOMES AND GROWER SELF-REPORTED COVERAGE BY THE FOOD SAFETY MODERNIZATION ACT PRODUCE SAFETY RULE IN CALIFORNIA

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Presentation type/Format: Poster

Abstract:

Introduction: California is the leading agricultural producing state in the nation. Produce farms covered under the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) must have at least one supervisor or responsible party complete food safety training equivalent to the standardized curriculum.

Purpose: Determine FSMA PSR farm coverage status for California-based growers attending Produce Safety Alliance (PSA) Grower Training and evaluate learning and behavior change resulting from course attendance.

Methods: A total of 27 PSA Grower Training courses were conducted from Oct 2024–Sep 2025, with 279 participants. Standard PSA course evaluation data was obtained from participants covering worker health, hygiene, and training; soil amendments; animals and

land use; agricultural water; post-harvest handling and sanitation; and farm food safety plan. Demographics questions and farm coverage status were included in the data set.

Results: A total of 232 out of 279 participants completed the evaluation. Across all content areas an average of 60% of participants “strongly agreed” and 32% “agreed” that training increased their knowledge. Similarly, an average of 60% of participants strongly agreed and 32% agreed that they were confident in implementing practices to reduce food safety risks. Over half of respondents (n=125) were either not covered or classified as very small businesses under the FSMA PSR. Additionally, 104 participants reported 0–5 years of farming experience and 85 reported 0–10 acres in fruit and vegetable production. Nearly half of participants (n=115) did not have a food safety plan; but 68% (n=78) plan on developing one within the year.

Significance: Post-course evaluations demonstrated increased knowledge and confidence in implementing produce safety practices among growers. Half of participants indicated farms being not covered or qualified exempt from the FSMA PSR, highlighting the value of training outside of regulatory compliance.

P3-006: FLORIDA'S EXTENSION PROGRAMS PREPARE PRODUCE GROWERS FOR PRODUCE SAFETY RULE INSPECTION

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Abstract:

Introduction: The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) was the first legislation to establish minimum standards for growing, harvesting, packing, and holding fresh produce.

Purpose: Florida produce growers require impactful education and technical assistance to meet the requirements of the new rule.

Methods: The University of Florida Institute of Food and Agricultural Sciences and the Florida Department of Agriculture and Consumer Services have collaborated since 2017 to provide education and outreach through Produce Safety Alliance (PSA) Grower Training Courses and On-Farm Readiness Reviews (OFRR) to assist growers in meeting the requirements of the PSR. Pre- and post-tests are given at PSA Grower Trainings (n=1,924) to measure knowledge increase during the training. A follow up survey to determine practices changed or

implemented is sent four months post training. Following the completion of each OFRR (n=124), surveys (n=91) are completed to evaluate farm readiness for rule implementation and highlight areas where more education was needed to meet minimum requirements.

Results: Mean post-test scores (21.12) were significantly higher ($P < 0.05$) than pre-test (17.25), demonstrating an increase in knowledge after training participation (t=-12.83, $P < 0.05$). Follow-up surveys highlight the creation or modification of record keeping systems was the most common action taken based on the knowledge gained in the training. OFRR surveys indicated that 48% of farms met, 41% needed minor improvements, and 11% needed significant improvements to meet the FSMA PSR requirements. Sanitation (36%), pre-harvest water (13%), and worker training (25%) were the areas that required the most improvements.

Significance: Training and technical support have improved Florida farms' knowledge and practices regarding the FSMA PSR. As inspections continue in Florida, education and outreach are ongoing to ensure Florida farms are prepared to implement the FSMA PSR.

P3-007: FOOD SAFETY COMPLIANCE GAPS IN NORTHEASTERN PRODUCE PRODUCTION

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Presentation type/Format: Poster

Abstract:

Introduction: This study aims to enhance understanding of FSMA (Food Safety Modernization Act) compliance within the northeastern fresh produce industry. The goal is to identify areas in need of improvement and provide guidance for developing strategic, practical educational materials.

Purpose: The purpose of this study is to support farmers in improving the safety of their farm production by identifying potential problem areas in current practices.

Methods: In 2024, an in-person survey addressing FSMA requirements was administered across 12 farms in the northeastern United States. The survey included questions related to hygiene, worker health, training, water handling, soil and land use, traceability, record keeping, animal management, sanitation, handling, and transport. For analytical purposes, these topics were consolidated into three main categories: Record Keeping, Training, and Water Handling. Statistical analyses were performed using R-Studio.

Results: The average score percentages across all twelve farms for Record Keeping, Training, and Water Handling were 78%, 81%, and 64.62%, respectively, with Water Handling receiving the lowest average score. Pearson's correlation analysis revealed significant positive relationships between farm acreage and water handling score ($r = 0.73$, $p = 0.0069$), and training and record-keeping scores ($r = 0.69$, $p = 0.13$).

Significance: This study indicates that water handling is the weakest area of FSMA

compliance among northeastern produce farmers, representing a critical vulnerability in food safety practices. The strong positive relationships suggest that smaller farms face greater challenges in meeting FSMA water safety requirements. These findings highlight the need for directed educational efforts focused on improving agricultural water safety, particularly for small- and mid-sized farms.

P3-008: MODERNIZING RISK COMMUNICATION: ALIGNING FOOD SAFETY MESSAGING WITH CONSUMER PREFERENCES AND PERCEPTIONS

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Presentation type/Format: Poster

Abstract:

Introduction: For decades, USDA-FSIS has communicated food safety risks to consumers, yet behavior change remains limited. Research and feedback reveal that outdated, prescriptive messaging often fails to resonate, especially when cultural practices and consumer preferences are ignored.

Purpose: To evaluate the effectiveness of updated risk communication strategies that incorporate consumer research, behavioral insights, and flexible messaging formats to improve engagement and adherence to safe food-handling practices.

Methods: FSIS analyzed five years of consumer research, including observational studies (n = 94 control, 10 treatment) and web surveys (n > 2,400), to identify barriers to effective communication. Adjustments included:

Shifting tone from prescriptive (“Don’t wash poultry”) to explanatory (“Here’s why it’s risky and how to mitigate it”).
Providing options alongside the safest recommendation.
Tailoring message length and format for different platforms (e.g., short, visual content for social media; detailed factsheets for websites).
Effectiveness was measured through engagement metrics, consumer feedback, and behavioral observations.

Results:

Risk Perception Gap: 84% agreed foodborne illness is serious, but only 18% felt personally at risk.

Message Impact: After updating poultry washing communications, negative consumer responses decreased significantly, and engagement with FSIS content increased across social media and traditional channels.

Behavioral Influence: 66% of participants in intervention studies reported that revised messaging influenced their kitchen practices; 40% cited learning new information as the reason.

Significance: Modernizing risk communication by meeting consumers “where they are”—through culturally sensitive, option-based messaging and platform-specific formats—improves trust, engagement, and the likelihood of behavior change. These strategies offer a replicable model for public health professionals seeking to enhance food safety education.

P3-009: MIND THE GAP: BEHAVIORAL STRATEGIES TO IMPROVE CONSUMER FOOD SAFETY PRACTICES

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Presentation type/Format: Poster

Abstract:

Introduction: Consumers often believe they know safe food-handling practices, yet USDA Food Safety and Inspection Service (FSIS) research shows knowledge does not consistently translate into action. This gap increases risk for foodborne illness and undermines traditional awareness campaigns.

Purpose: To evaluate interventions that address the knowledge–behavior gap using behavioral science principles and measure their impact on high-risk food safety behaviors.

Methods: FSIS analyzed five years of consumer survey data (n = 1,500 annually) to identify gaps between knowledge and practice. Targeted interventions included visual cues, social norm messaging, and food safety campaigns deployed via digital media and community partnerships. Effectiveness was measured through pre- and post-intervention surveys and engagement metrics.

Results: Key gaps identified:

Date Labels: 87% claimed understanding, but only 53% identified correctly.
Behavior After Illness: 61% made no changes post-illness.
Risk Perception: 84% agreed foodborne illness is serious, yet only 18% felt personally at risk. Post-intervention, poultry washing decreased by 26%, thermometer use increased by 19%, and correct date label interpretation improved by 15% ($p < 0.05$). Social norm messaging and visual cues were most effective in shifting behaviors.

Significance: Addressing optimism bias and behavioral inertia through behavioral science

tools significantly improves compliance with safe food handling practices. Findings provide a roadmap for public health professionals to design interventions that move beyond awareness to sustained behavior change.

P3-010: MULTIDISCIPLINARY EDUCATION FOR HOME-BASED PROCESSORS: LONGITUDINAL EVALUATION OF KNOWLEDGE GAIN AND ECONOMIC GROWTH UNDER OKLAHOMA'S HOME FOOD FREEDOM ACT

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Presentation type/Format: Poster

Abstract:

Introduction: The 2021 Oklahoma Home Food Freedom Act (HFFA) expanded the types of foods permitted for home production, including select Time/Temperature Control for Safety (TCS) foods. This regulatory change created a need for targeted education to help cottage food producers understand state requirements and manage food safety risks in home-based operations.

Purpose: This program was developed to provide Oklahoma producers with the regulatory knowledge and technical food safety training required by the Oklahoma Department of Agriculture, Food, and Forestry (ODAFF) for TCS food production under the HFFA.

Methods: Over three years, 23 training sessions were delivered to 465 participants across Oklahoma. Training covered ODAFF regulations, foodborne hazards, allergen labeling, Good Manufacturing Practices (GMP), and specialized topics including "Is My Food Business a Hobby or a Business?" and "Food Safety in Canning." Knowledge gains were evaluated using pre- and post-tests and analyzed with paired *t*-tests ($\alpha = 0.05$).

Results: Participants demonstrated a significant mean knowledge increase of 21% ($P < 0.05$). The program is recognized by ODAFF as a preferred training for producers seeking to sell TCS

foods. Participant satisfaction was high, with 98.7% rating the training as good or excellent. Financial data from 66 participants indicated $2.3 million in annual sales and 92 jobs created; extrapolated estimates suggest over $16 million in annual sales and more than 640 jobs statewide.

Significance: This program fills a critical food safety and economic need by providing a validated educational pathway for safe TCS food production while supporting growth of Oklahoma’s home-based food businesses under the HFFA.

P3-011: THE EFFECT OF MILKFAT ON THE INACTIVATION OF LISTERIA SPP. AFTER HIGH PRESSURE PROCESSING (HPP

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Category: Food Processing Technologies

Abstract:

Introduction: High-pressure processing (HPP) is a nonthermal intervention that inactivates *Listeria* spp., without the heat-induced changes of conventional milk pasteurization. However, fat content may protect microorganisms from pressure-induced injury.

Purpose: To evaluate the impact of milkfat on HPP inactivation of *Listeria monocytogenes* 104 serotype 4B Scott A and *Listeria innocua* in milk.

Methods: Mixtures of milk were formulated to obtain four fat contents: 0.5, 17, 25, and 32.5%, and inoculated with 10^8-10^9 CFU/mL of *L. monocytogenes* 104 serotype 4B Scott A (ATCC 148^{TM}) and *Listeria innocua* (ATCC 57742^{TM}). Using three biological and technical replications, samples were treated at 450 MPa for 3 min and enumerated post-treatment.

Results: For *L. monocytogenes*, no significant differences ($p > 0.05$) were observed among reductions for 0.5, 17, and 25% milkfat. Overall, reductions ranged from 4.59 ± 0.57 to 4.77 ± 0.57 CFU/mL. However, significantly greater reductions ($p < 0.05$) were observed at 32.5% (5.33 ± 0.57 log) compared to 25% (4.59 ± 0.57 log). Similarly, for *L. innocua*, reductions across milkfat concentrations 0.5, 17, 25, and 32.5% were not significantly different ($p > 0.05$), ranging from 1.71 ± 0.57 to 1.79 ± 0.58 CFU/mL. These results also showed that the chosen *L. monocytogenes strain* was more sensitive to high-pressure treatment than *L. innocua,* which has often been used as a surrogate.

Significance: This study provides new insights into how milkfat content influences the efficacy of high-pressure processing (HPP), highlighting the importance of considering food matrix composition when applying HPP treatments to ensure the safety of milk products.

P3-012: SYSTEMATIC CONTENT ANALYSIS OF YOUTUBE VIDEOS ON FREEZE-DRYING FOODS AT HOME

Primary Author:
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Persons:
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Abstract:

Introduction: Freeze drying is a preservation method increasingly used in homes for the stabilization of high-quality food. Social media platforms such as YouTube are frequently consulted by consumers to gather information and guidance on food handling, processing, and the proper operation of freeze-drying equipment. However, the accuracy of the information collected on this platform remains uncertain.

Purpose: To evaluate the information disseminated through YouTube related to home freeze drying, with particular emphasis on deficiencies in food safety practices and the proper use of food and equipment.

Methods: A standardized rubric was developed to select 10 YouTube videos across nine food categories, resulting in a total of 90 videos. Information from each video was collected using a structured evaluation checklist. Subsequently, a category list and coding system were developed to assess the parameters evaluated for each video. Additionally, metadata were collected for each video, including the URL, number of views, number of likes, video duration, and number of subscribers.

Results: Across most food categories, home freeze-drying videos demonstrated poor (74% ± 18.78) and mediocre (34% ± 22.97) performance, presenting information related to food handling, hygiene practices, food safety practices, freeze-drying fundamentals, and post-treatment practices. Overall category evaluations ranged from 50% to 100% in the poor performance category.

Significance: This study highlights the accuracy and reliability of food safety information related to home freeze drying shared through social media platforms.

P3-013: FOOD SAFETY BEHAVIORS AND AIR-FRYER USE: INSIGHTS FROM SOCIAL MEDIA CONTENT

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Presentation type/Format: Poster

Category: Communication, Outreach and Education

Abstract:

Introduction: In 2022, 62% of air fryer (AF) owners reported weekly or daily use; by 2024 more than half of British households (51–58%) reportedly owned an air fryer. Despite popularity, little attention has been given to potential food safety risks associated with foodborne illness in relation to AF use.

Purpose: Identify and analyze social media content for information related to AF use focusing on key food safety behaviors.

Method: A predefined search strategy was applied to search Reddit, Facebook, TikTok, and Instagram for content related to domestic AF use over a 12-month period. A content analysis approach was utilized to ascertain AF consumer perceptions, awareness and reported practices with 13 search terms used to explore domestic practices to include cleaning (e.g. ‘scrub’) cooking (e.g. ‘temperature’) and preparatory behaviors (e.g. ‘defrost’). Qualitative data was extracted and coded for themes.

Results: Overall, 995 posts, 778 videos, 200 reels, 92 discussion threads and approximately 86,233 comments were analyzed. Potentially dangerous viral trends associated with food safety practices were identified e.g., cleaning AF with a *“dishwasher tablet, cover with water and bake on 160 for 15 mins. Works a treat”* (Tiktok), cooking whole chicken in a small AF *“I*

don't have a probe and always do chicken in AF" (Facebook), and cooking meat quickly without defrosting. Five themes were identified why social media is utilized in reference to AF use which were seeking reassurance, advice, inspiration, confidence in ability and sharing perceived positive sentiments.

Significance: Social media content can influence home cooking/preparation practices, highlighting the need to share accurate food safety information. Incorrect advice on cooking, cleaning and preparing food in an AF may increase the risk of foodborne illness, making it essential for trusted organisations to counter misinformation.

P3-014: UNDERSTANDING CONSUMER FOOD SAFETY PRACTICES ASSOCIATED WITH DOMESTIC AIR FRYER USE: QUALITATIVE INSIGHTS FROM THE ISLAND OF IRELAND

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Presentation type/Format: Poster

Category: Communication, Outreach and Education

Abstract:

Introduction: It’s now estimated that 61% of UK households own an air fryer (AF) with 29% of owners using it daily. Key food safety practices and knowledge when using an AF are required to reduce the risk of foodborne disease. To date, no comprehensive research has been undertaken to explore consumer use and attitudes of AF cooking.

Purpose: Explore and determine consumer food safety knowledge, attitudes, and self-reported practices concerning AF use in the island of Ireland.

Methods: Online and in-person focus groups (n=16) were conducted with consumers from the island of Ireland (n=76) to explore domestic AF food safety practices. Key topics covered included cooking and cleaning practices, food safety, thermometer use and trusted information sources. Transcripts were coded and thematically analysed using Nvivo.

Results: Cumulatively, all consumers described positive sentiment towards their AF, referencing quickness and ease of use as most favorable reasons for use. Negative sentiments only included cleaning or requiring countertop space. Older consumers more

often relied on food safety knowledge and experience *“I'm 60 years cooking meat. So I kind of know by looking at meat when it's cooked”.* Younger consumers were more likely to rely on social media, AI, and google for recipe inspiration and food safety advice *“I'd go straight to Google, to AI, one or the other, and ask the question”*. The most prominent theme to facilitate AF use was social influence. Parents used AF most frequently, often relying on frozen convenience foods *“I don't use the thermometer for the smaller things for the kids because they tend to be frozen food”.* Women more commonly cleaned AF after every use, motivated by responsibility for other family members food safety.

Significance: Understanding consumer perceptions and practices will aid the development of official guidelines to improve key food safety practices among AF users.

P3-015: OPTIMIZING FOOD SAFETY MESSAGE DESIGN ON FLOUR PACKAGE: AN EYE-TRACKING STUDY

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Presentation type/Format: Poster

Abstract:

Introduction: Despite several multistate outbreaks and recalls of raw wheat flour, many consumers remain unaware of the associated food safety risk. Although flour manufacturers have incorporated food safety messages on packaging, studies show these messages often go unnoticed, and consumers continue to engage in improper handling practices.

Purpose: This study aims to develop and optimize food safety message designs on flour packages to improve their accessibility and effectiveness for consumers.

Methods: Twelve mock flour packages (four message designs × three placement locations) were developed based on results from a national consumer survey. Participants were randomly assigned to examine one flour package as they would in a grocery store, wearing eye-tracking glasses. They were not informed that the food safety message was the study focus. Following the viewing task, participants completed a semi-structured interview and a post-survey.

Results: A total of 144 consumers participated, and 52% recalled seeing a food safety message after viewing the package. Message design features, including the presence of shape and warning title, did not significantly influence visual attention; however, message placement did ($p = 0.015$). Messages positioned on the front-top of the package received significantly longer fixation durations (median = 1.80 s) compared with front-bottom (median = 0.99 s) and back placements (median = 1.08 s). Fixation durations also differed significantly across package elements ($p < 0.001$), with the Nutrition Facts panel receiving the greatest

visual attention, followed by recipe, food safety message, ingredient list, and best-by date. Most participants (72%) were likely to purchase a product displaying the food safety message they viewed.

Significance: This study offers valuable insights into consumers' perceptions and visual attention to flour safety message designs, guiding industry efforts to enhance message accessibility and effectiveness.

Award Application: Developing Scientist Award

P3-016: FOOD SAFETY LITERACY AT SCALE: AN INNOVATIVE FRAMEWORK FOR ASSESSING COMPETENCE AMONG ENVIRONMENTAL HEALTH OFFICERS

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Presentation type/Format: Poster

Abstract:

Introduction: Food Safety Literacy (FSL) extends health literacy to food safety, reflecting the ability to access, understand, appraise, and apply food safety information in professional practice.

Purpose: This study applies the Food Safety Literacy Questionnaire – Brazil (FSLQ-Br) to assess Environmental Health Officers (EHOs) nationwide.

Methods: A cross-sectional survey was conducted with 567 EHOs, proportionally distributed by population density and representing all 27 Brazilian states, thus constituting one of the most comprehensive datasets on this topic worldwide. The FSLQ-Br was previously validated and comprises four factors (access, understand, appraise, and apply) and 24 indicators, each rated using a four-point scale. The overall FSL Index was calculated using the formula “(Average□1)×(50/3)” and categorized as “Inadequate” (0–22), “Problematic” (23–32),

“Sufficient” (33–39), and “Excellent” (40–50).

Results: Results revealed that 63.84% (n=362) of EHOs demonstrated limited literacy—21.16% (n=120) “Inadequate” and 42.68% (n=242) “Problematic”—while 36.16% (n=205) achieved “Sufficient” (23.28%; n=132) or “Excellent” (12.87%; n=73) levels. Mean competence scores differed significantly ($p<0.05$, Bonferroni test - different letters indicate significant differences), with “understand” (34.2A) ranking highest, followed by “apply” (30.4B), “appraise” (27.5C), and “access” (23.6D).

Significance: The classification thresholds proposed enable evidence-based managerial decisions concerning workforce training, supervision, and allocation. For instance, EHOs with “Problematic” literacy may benefit from targeted capacity-building programs, whereas those with “Excellent” literacy could act as peer mentors. By operationalizing the FSL construct through a validated, scalable tool, this study provides an innovative framework to strengthen food safety governance, promote competency-based management, and foster more standardized regulatory practices nationwide.

Award Application: Developing Scientist Award

P3-017: MICROLEARNING AS A FOOD SAFETY TRAINING STRATEGY IN HOSPITAL CATERING

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Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: In hospital catering, inadequate food-handling practices pose serious risks to immunocompromised patients, highlighting the need for innovative and effective food safety training strategies.

Purpose: This study evaluated the effect of a microlearning-based training intervention on food safety knowledge and Theory of Planned Behavior (TPB) constructs among hospital food handlers.

Methods: A quasi-experimental pre–post intervention study was conducted in a Brazilian hospital food and nutrition unit. Seventy-one food handlers completed the baseline assessment, and 50 participated in the intervention and post test. The intervention consisted of an eight-module microlearning program delivered through short instructional videos integrated into the work environment. Data were collected using a structured questionnaire assessing food safety knowledge (16 items) and TPB constructs (attitude, subjective norms,

perceived behavioral control, and intention; 5-point Likert scale). Pre- and post-intervention differences were analyzed using paired Student's *t*-tests (α=0.05), and effect sizes were calculated using Cohen's d.

Results: Food-safety knowledge significantly improved after the intervention, with mean scores increasing from 11.14±2.08 to 12.18±1.89 ($p<0.001$), corresponding to a 6.52% increase and a medium effect size (d=0.62). No statistically significant changes were observed for TPB constructs, including attitude ($p=0.706$), subjective norms ($p=0.921$), perceived behavioral control ($p=0.502$), or intention ($p=0.753$). After training, 72% of participants (n=36) reported that none of the evaluated hygiene practices were difficult to implement.

Significance: Microlearning is an effective strategy to improve food safety knowledge in hospital catering; however, knowledge gains alone were insufficient to modify behavioral predictors, underscoring the need for multimodal training approaches to promote sustained behavior change in high-risk healthcare settings.

P3-018: THE SCIENCE BEHIND SAFE FOOD HANDLING AND PREPARATION: RESOURCES FOR EXTENSION EDUCATORS AND VOLUNTEERS

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Abstract:

Introduction: Food science and food safety are important public health topics linking scientific principles to everyday food practices. However, instruction in these areas has declined in 6–12 education due to reductions in Family and Consumer Sciences programs, increasing the importance of nonformal education. Research has found that nonformal programs, such as 4-H, are well-position for this work and can improve youth food safety knowledge and behaviors.

Purpose: We developed an Extension-based food science and food safety curriculum designed to prepare 4-H volunteers to teach key concepts to youth in 6–12 and above. The curriculum emphasizes scientific foundations of food safety, safe food-handling practices, and compliance with 4-H food competition requirements.

Methods: A multidisciplinary team created four modules: 1) *Food Labeling and Nutrition,* 2) *Basic Cooking Skills and Kitchen Safety*, build fundamental background knowledge using curated resources; 3) *Safe Food Handling and Storage Practices,* and 4) *Food Preparation and Preservation*, include hands-on activities aligned with the 4-H experiential learning model (“Do, Reflect, Apply”). Activities address cross-contamination, cooking temperatures, food

storage, and safe versus unsafe practices, with supplemental materials for volunteers.

Results: Evaluation tools were developed after pilot testing. Trainers completed a retrospective survey measuring food safety knowledge, teaching self-efficacy, and perceptions of curriculum structure and support. Youth learning was assessed through performance-based activities (e.g., skits, infographics, podcasts) using a standardized rubric to evaluate participation, accuracy, and application of food safety concepts.

Significance: The curriculum will be available through UF/IFAS Extension EDIS, increasing access to evidence-based food safety resources, reducing implementation barriers, and supporting consistent delivery of practical food safety education in 4-H programs.

P3-019: COMMUNITY MEALS AND DISASTER FOOD SAFETY TRAINING

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Presentation type/Format: Marketplace

Abstract:

Introduction: Volunteer groups frequently provide food to communities during fundraisers, religious organization functions, and during disasters. Unlike professional food service providers, these volunteers typically have limited food safety knowledge. To address this knowledge gap, the North Carolina Department of Health and Human Services, Division of Public Health's Environmental Health Section partnered with a local university to develop a plain-language food safety course tailored for volunteer and emergency food service providers. Supplemental course materials were also developed, such as stickers and magnets.

Purpose: The purpose of the two-hour training was to improve food safety literacy among nontraditional food handlers.

Methods: Evaluation surveys with pre- and post-training responses were collected anonymously (n = 49). Participants rated their knowledge across six food safety topics using a 5-point Likert scale (1 = Poor, 5 = Excellent). Paired *t*-tests were used to determine if change in knowledge was statistically significant.

Results: Paired *t*-tests revealed statistically significant improvements in all categories, with *p*-values less than 0.001. Mean scores for food safety importance increased from 3.57 to 4.41, which is an increase of 23.5%. Knowledge of cross-contact and cross-contamination prevention rose from 3.65 to 4.53. This represents an increase of 24%.

Significance: Beyond education, the course fostered stronger connections between volunteer food providers and local health departments, highlights the value of community partnerships in food safety, and offers a replicable model for developing targeted, accessible food safety education.

P3-020: DEVELOPMENT AND IMPLEMENTATION OF 'ON-DEMAND' HARVEST WORKER FOOD SAFETY TRAINING IN ARIZONA

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Presentation type/Format: Poster

Abstract:

Introduction: A critical produce safety education and training gap lies with harvest foremen/supervisors and harvest workers. These stakeholders may receive initial food safety training from their companies at the start of the season, but there is a need for additional education and training.

Objective: To develop and deploy produce food safety education trainings to enhance knowledge and practices of harvest foreman/supervisors and workers across Arizona.

Methods: Specific education concepts that pertain to harvest foreman/supervisors and workers were modified from the Produce Safety Rule core curriculum and offered in an "on-demand" training format directly in-field or during transport to the field. These core curricula consisted of Hand Washing, Animal Intrusion and Cross-Contamination and ranged from 10 to 25-minute trainings. The presented material was provided in both English and Spanish using visual aids and hands on demonstration.

Results: A total of 3,944 harvest crew (n=3446) and supervisors (n=498) were trained

between 2023–2025. Commodities impacted included leafy greens, lemons/citrus, onions, and brassicas. A total of 36 firms in Arizona have been impacted by the worker training. Follow up communications with each firm indicted a positive change in knowledge and practices of workers trained in Hand Washing and Cross-Contamination of Tools and Surfaces. Additionally, each of the 36 firms that our team has worked with over the course of the project requested repeat and continued training in future seasons as workforce grows and changes over time (100% response rate).

Significance: The extensive number of stakeholders trained underscores industry commitment to improving food safety practices and workforce development by directly engaging those who have the most impact on food production and public health. This innovative “on-demand” training strategy is a model for successful food safety education targeting harvest workers and foreman.

P3-021: IMPACT OF MANAGERIAL FOOD SAFETY TRAINING ON HYGIENE COMPLIANCE IN UAE RESTAURANTS

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Abstract:

Introduction: Food safety is a major public health priority in the United Arab Emirates. (UAE). Regulatory authorities in Dubai and Sharjah have mandated certified food-hygiene training for restaurants' key personnel, such as the person in charge (PIC) and food handlers in Dubai, and Good Hygiene Practices (GHP) training for managers and food handlers in Sharjah, to ensure compliance with the UAE food code.

Methods: A mixed-methods study evaluated the effectiveness of training for GHP managers and PIC courses. Quantitative data included hygiene scorecard reviews from 100 restaurants across Dubai and Sharjah, assessed using an eight-section checklist. Managerial compliance was evaluated using a 19-item measuring checklist across five sections (max score 90). Qualitative data were collected via semi-structured interviews with restaurant managers on hygiene systems, staff training, monitoring, and regulatory compliance. Qualitative responses were coded thematically to enhance understanding of managerial impact. Quantitative data were analyzed using descriptive statistics and regression models to examine the relationship between training variables and hygiene outcomes.

Results: Restaurants supervised by certified managers exhibited higher compliance. The average hygiene management compliance rate was 89%, and the overall hygiene standard was 92% in restaurants with certified managers. Higher-level training correlated with a substantially higher score (p<0.05).The interview indicated improved employee awareness of hygiene practices, fewer noncompliance, and more consistent daily hygiene procedures following managers’ training.

Significance: These findings may useful to restaurant operations in efforts to improve food hygiene and be more productively directed toward individual restaurants. Findings also provide evidence-based insights for regulators and policymakers to enhance training/ certification policies and enforcement strategies at the national and international levels.

P3-022: WITHDRAWN

WITHDRAWN

P3-023: EMERGING ROLES OF COOPERATIVE EXTENSION SYSTEM IN FOOD RETAIL: RESULTS OF FSEN RETAIL COMMITTEE NEEDS ASSESSMENT FOR SOUTHEASTERN STATES

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Presentation type/Format: Technical

Abstract:

Introduction: The U.S. Cooperative Extension System (CES) is a resource for food safety education.

Purpose: To better tailor resource development efforts to meet the evolving needs of both the retail food industry and Extension educators in SE states and eventually throughout the U.S., the Food Safety Extension Network (FSEN) disseminated a needs assessment survey to CES educators to measure areas of strength and for improvement and innovation in delivering food safety education to retail business managers.

Methods: A 33-item questionnaire was distributed online using Qualtrics (UF, IRB#ET00046988). The survey was distributed to FSEN Network extension members via email from August to December 2025. Data was collected, and frequency data was analyzed with an Excel spreadsheet.

Results: Fifty-seven CES educators responded to the survey, representing statewide,

regional, and county-based roles; the majority (>60%) of respondents have been in their positions for 1–10 years. Respondents rated themselves very high in knowledge of local and state regulations, but lower in knowledge of federal regulations. Respondents reported a moderate level of technology access, but a higher level of competency; they rated the access and competency much lower for their clients. The majority of respondents (55%) indicated their most significant barrier is employee turnover or not having enough employees. The primary source of educational materials was from their own state's Extension resources (100%), followed by the federal or state government, food safety educational businesses, and nonprofits. Extension specialist input remains (38%) the most frequently used option when evaluating the credibility of educational materials. In-person mode of education remains a preferred method by the majority of respondents.

Significance: Enhancing CES's role in retail food protection education ensures evolving regulations are effectively translated into practice, directly safeguarding public health.

P3-024: SQUEAKY CLEAN OR SQUEAKING BY? PRESENCE AND ADEQUACY OF HANDWASHING ADVICE IN COMMERCIAL MEAL KITS

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Presentation type/Format: Poster

Abstract:

Introduction: Foodborne diseases cost approximately $75 billion each year. Domestic meal preparation accounts for an estimated 26% of all foodborne illnesses in the U.S., many of which are preventable using low-cost strategies. Despite food safety advances, handwashing remains critical to stop the spread of foodborne illness. However, consumers do not wash hands appropriately during meal preparation. Meal kits are boxes of pre-measured raw ingredients with easy-to-follow recipes for at-home preparation. Despite their potential to improve nutrition and cooking skills, the appropriate communication of meal-kit food safety practices (i.e. handwashing) is poorly understood.

Purpose: To evaluate the presence and quality of handwashing instructions in commercially available U.S. meal-kit recipes.

Methods: Recipe cards (n=1212) were collected from U.S. meal-kit suppliers (n=10, >90% of the market). A data extraction tool was designed to capture high-risk ingredients, suggested preparation practices, and instructions affecting recipe food safety. Handwashing advice (timing, frequency, methods) was extracted. Advice was evaluated and stratified by the inclusion of animal protein to assess cross-contamination risk.

Results: Only 8.9% of recipes (n=108) recommended washing hands before recipe preparation though 43.2% of recipes (n=524) directed consumers to use their hands during

meal preparation. Nearly 1/5 of recipes (19.2%, n=233) recommended handwashing during preparation. No cards included recommended hand drying. Only 182 (20.9%) of cards with animal protein (n=869) recommended handwashing during preparation. Seafood-containing recipes most frequently featured handwashing advice (12.7% before, 26.0% during preparation).

Significance: Commercial meal kits lack sufficient handwashing advice. While current deficiencies may increase the likelihood of foodborne infection, opportunities were identified to improve food safety advice through appropriate hand hygiene instructions.

P3-025: EFFECTIVE STRATEGIES FOR DEVELOPING CONSUMER-FOCUSED FOOD RECALL COMMUNICATION MATERIALS

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Vanessa Coffman, Stop Foodborne Illness

Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Effective communication during a food recall significantly affects whether consumers can avoid unsafe products in their possession, thereby reducing the risk of foodborne illness. However, there is limited evidence on effective strategies for designing and optimizing recall messages to achieve behavioral impact.

Purpose: To evaluate the most effective strategies for designing food recall communication that support informed food safety decision-making.

Methods: Novel simulated food recall messages were developed based on best risk communication practices using real-world examples and presented to study participants (n = 12). Following message exposure, participants were asked to prepare recipes using the provided ingredients, including the recalled product. Qualitative data on consumers'

experiences during message consumption and meal preparation were collected via semi-structured interviews and analyzed using thematic analysis.

Results: Salient visual cues (color contrast, bold headings, warning symbols) and contextual cues (brand names and health hazards) enhanced participants' attention and memory retention of recall information. Lot numbers, distribution areas, and contact numbers mostly went unnoticed. The majority (seven participants) consciously avoided the recalled food in the recipes due to safety concerns. All participants found the simulated recall message clear and well organized, with no reports of confusion, although three suggested rearranging the format to better highlight critical elements. One participant emphasized that avoiding unclear words like "potentially" could improve understanding. The resulting themes suggest that providing a summary of the main information increased participants' comprehension, and referencing credible sources enhanced their confidence.

Significance: These results provide a roadmap for regulatory agencies, public health officials, and industry members that can be used when modernizing recall message approaches.

P3-026: WITHDRAWN

WITHDRAWN

P3-027: BIOCONTROL OF LISTERIA MONOCYTOGENES AS A POST-PROCESSING CONTAMINANT IN SOFT CHEESE

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: *Listeria monocytogenes* (Lm) is a persistent food safety challenge in soft cheese due to its ability to grow during refrigerated storage.

Purpose: This study evaluated the efficacy of commercial protective cultures (PC) for controlling Lm as a post-processing contaminant in soft cheeses.

Methods: Ricotta (4% fat), Cottage (2% fat), and fresh mozzarella (4% fat) in brine were purchased at retail, inoculated with a six-strain cocktail of Lm at ~2 log CFU/g for all samples, and also with either LALCULT LC1, Lyofast LPAL, DVS BS-10, or HOLDBAC *Listeria* (HBL) at ~7 log CFU/g for treatment groups. Inoculants were prepared in 0.1% peptone and mixed into cheeses or added to brine for mozzarella. Cheeses were stored at 6±1°C for 36 days with samples (25 g) removed weekly for pH measurement and bacterial enumeration. Experiments were repeated at least three times and data were analyzed using repeated-measures ANOVA.

Results: PC counts remained stable throughout storage while mean Lm counts in controls reached 5.88, 2.38, 6.56, 6.85 log CFU/g in ricotta, cottage, mozzarella, and brine, respectively. HBL treatment in cottage cheese differed from all others (P□0.0015), reducing mean Lm counts by 1.68 log CFU/g at day 36. All PC treatments differed from control in mozzarella and brine (P□0.0025), with mean counts in HBL treatments 2.92 and 3.36 log CFU/

g lower than control at day 15, respectively ($P<0.0001$). Similarly, treatment effects were observed for all PCs in ricotta ($P \leq 0.0003$), with mean counts at least 1.62 log CFU/g lower than control at day 36. LPAL treatment reduced the pH of cottage cheese compared to control ($P<0.0001$), whereas the pH of ricotta differed from control in all treatments except LC1 ($P \leq 0.0369$). Differences in pH were also noted for BS-10 and HBL in mozzarella and brine compared to control ($P \leq 0.0068$).

Significance: These findings support the use of PCs as a promising approach to control Lm in different varieties of soft cheese.

Award Application: Developing Scientist Award

P3-028: BIOPROTECTIVE CAPABILITIES OF AN ARTISANAL STRAIN OF LACTOCOCCUS LACTIS AND CONSUMER'S SENSORY ACCEPTABILITY OF THE RESULTING COTTAGE CHEESE

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: An innovative method for improving safety and extending the shelf life of fermented dairy products without synthetic preservatives is to utilize bioprotective starter cultures (BPSC).

Purpose: Test the BPSC capabilities of *Lactococcus lactis* OSY-92 via antimicrobial gene transcription analysis, pathogen surrogate challenge studies in a cottage cheese model, and a sensory panel evaluation of the cheese made in a pilot-scale production facility.

Methods: Cultures of *L. lactis* OSY-92 in milk and rich media (control) were subjected to transcriptional analysis utilizing RT-qPCR, targeting the lactococcin B biosynthesis gene cluster. *L. lactis* OSY-92 was then utilized as a starter culture in a bench-scale cottage cheese production model in which milk was inoculated with *E. coli* K-12 or *E. faecium* NRRL b-2354. Pathogen surrogate counts were taken twice a week for three weeks and comparisons were made to cheese produced using a commercially available BPSC (control). Cottage cheese was prepared for sensory analysis in a dairy pilot plant using *L. lactis* OSY-92 as the starter culture. Panelists (n=103) rated various attributes of the experimental cheese and commercial cottage cheese (control).

Results: In the rich medium, lactococcin genes were upregulated in the early stationary phase of growth, and antimicrobial activity was observed in the late stationary phase. In the bench-scale model, cheese prepared with *L. lactis* OSY-92 as the starter culture decreased the population of *E. coli* K-12 by 2.9 log CFU/g over the 21-day storage period. The commercial BPSC displayed a 3.1 log CFU/g decrease in *E. coli* K-12 over the same period. Cheese prepared with *L. lactis* OSY-92 as the starter culture did not display any change in *E. faecium* NRRL b-2354 population. In the sensory analysis, panelists described the experimental cheese as fresh, firm, tangy and clean.

Significance: *L. lactis* OSY-92 has potential to be used as an anti-Gram-negative BPSC for soft, fresh cheese.

Award Application: Developing Scientist Award

P3-029: ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF POSTBIOTICS KE1 DERIVED FROM KEFIR AGAINST BOVINE MASTITIS CAUSATIVE PATHOGENS

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: Developing novel postbiotics is essential to mitigate bovine mastitis (BM) and improve dairy safety while reducing antibiotic reliance.

Purpose: This study aimed to screen *Lactiplantibacillus plantarum* strains from food sources and evaluate their derived postbiotics as alternative antibiotics by assessing antimicrobial and antibiofilm activities against BM-causing pathogens (BMP).

Methods: 15 *L. plantarum* were isolated from ginseng, kefir, and kimchi. Their safety was verified via hemolysin, gelatinase production, and antibiotic susceptibility tests in accordance with Ministry of Food and Drug Safety guidelines. Probiotic properties were assessed through auto-aggregation, hydrophobicity, biofilm formation, and VITEK ANC card biochemical analysis. Postbiotic cytotoxicity was evaluated using MAC-T cells, and organic acid profiles were analyzed via HPLC. Antimicrobial and antibiofilm activities against major BMP including *S. aureus*, *E. faecalis*, *E. coli*, and *Klebsiella pneumoniae* were tested at non-cytotoxic concentrations. Statistical differences among groups were evaluated using one-way

ANOVA followed by Tukey’s post hoc test.

Results: All isolates exhibited γ-hemolysis, no gelatinase, and antibiotic susceptibility. Kefir-derived strains KE1 and KE4 were selected for their superior physiological properties, including high biofilm formation (OD_{595} > 1.4), strong auto-aggregation ($p < 0.05$). At a 40% concentration, KE1 derived postbiotics (POS_KE1) was non-cytotoxic and showed significant antimicrobial and antibiofilm activities comparable to higher than lactic acid (86.6 mM) and ciprofloxacin (16 µg/mL) ($p < 0.05$). Notably, POS_KE1 contained 78.7 mM lactic acid indicating additional antibiotic factors.

Significance: These findings suggest that kefir-derived *L. plantarum* postbiotics can serve as safe and effective antimicrobial agents for the prevention of BM, thereby contributing to improved dairy product quality and public health.

Award Application: Developing Scientist Award

P3-030: COMPARATIVE ANALYSIS OF VIRULENCE FACTORS AND ANTIMICROBIAL SUSCEPTIBILITY IN CAUSATIVE PATHOGENS FROM BOVINE MASTITIS AND NORMAL RAW MILK

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Understanding virulence determinants of bovine mastitis (BM)-associated bacteria is critical for precision treatment, yet phenotypic differences between isolates from bovine mastitis milk (BMM) and normal raw milk (NRM) remain poorly understood.

Purpose: This study compared virulence phenotypes and antimicrobial susceptibility (AST) between BMM and NRM derived isolates.

Methods: Milk samples were collected from 18 dairy farms in Korea, including BMM (somatic cell count, SCC 200,000 cells/mL, n = 69) and NRM (SCC < 200,000 cells/mL, n = 44). Bacteria were isolated using selective mastitis media and identified by MALDI-TOF MS. Virulence phenotypes were assessed based on hemolysis, gelatinase, and biofilm formation, while AST was determined using Sensititre panels.

Results: A total of 253 isolates from BMM and 126 isolates from NRM were obtained. BMM

isolates showed significantly higher hemolytic activity (63.01% vs. 39.84%, $p < 0.05$) and strong biofilm formation (30.12% vs. 20.31%, $p < 0.05$) than NRM isolates, whereas gelatinase production was comparable (19.03% vs. 14.84%). Notably, major pathogens isolated from NRM, including *P. aeruginosa* and *E. faecalis*, exhibited high levels of multidrug resistance. In addition, *S. aureus* isolates from NRM showed higher resistance to oxacillin than those from BMM, indicating widespread antimicrobial resistance reservoirs across the dairy environment. Elevated β-hemolysis and strong biofilm formation in BMM isolates suggest a higher prevalence of clinically relevant BM pathogens in milk with increased SCC.

Significance: These findings support the utility of SCC as an indicator of udder health and milk quality. Integrating SCC with phenotypic virulence profiling may facilitate earlier diagnosis and risk stratification of bovine mastitis. Future studies should incorporate comparative metagenomic analyses to elucidate microbial community shifts and transmission reservoirs between BMM and NRM.

Award Application: Developing Scientist Award

P3-031: ASSESSING THE DEVELOPMENT OF ENVIRONMENTAL MONITORING PROGRAMS AMONG SMALL DAIRY PROCESSORS

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Small dairy processors can face unique challenges with controlling environmental pathogens, making it important to identify appropriate tools to assist these processors with the development of environmental monitoring programs (EMPs).

Purpose: To evaluate the impact of comprehensive training on EMP development by small dairy processors to advance future tools that support *Listeria* control practices in small dairy facilities.

Methods: Small dairy processors (n=10) were recruited for participation in 5 workshops focused on environmental monitoring related skills to improve *Listeria* control practices and develop EMPs. One participant per facility completed surveys pre- and post-training to determine attitudes and practices related to EMP associated activities. Following training, all processors received EMP template materials and developed a written EMP for their facility, which the research team then reviewed to provide feedback and identify areas for improvement.

Results: Prior to training all 10 processors found EMPs important, however only 3 processors

had EMPs in place. Post-training, 60% of processors were confident (n=5) or very confident (n=1) in their ability to develop an EMP; having an EMP prior to training was not associated with having high confidence in EMP development (p=0.2, Fisher's exact). Template EMP materials were used by 80% (n=8) of processors to develop their EMPs. The most common recommendations made by the research team to improve the developed EMPs included i) specifying record keeping practices, ii) developing sample site selection procedures, and iii) adding specific facility details to their programs. This suggests template usage may lead to designing plans that lack plant-specific details.

Significance: Understanding the use and impact of training and tools that support small dairy processors with environmental monitoring will strengthen future efforts to assist these processors with EMP development and *Listeria* control.

Award Application: Developing Scientist Award

P3-032: INVESTIGATING THE EFFECT OF LACTIC ACID BACTERIA ON FERMENTATION DYNAMICS OF SOY PROTEIN YOGURT

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Limited information exists on fermentation behavior and food safety controls in plant-based (PB) yogurt systems, creating uncertainty regarding the ability of lactic acid bacteria (LAB) cultures to consistently achieve critical acidification (pH<4.6) for microbial safety.

Purpose: This study evaluated the effect of different commercial LAB cultures on fermentation performance, acidification kinetics, and microbial viability in soy protein isolate (SPI) yogurt, with dairy yogurt used as a reference.

Methods: SPI yogurt was formulated using 6% SPI and sucrose. Three commercial LAB culture formulations containing *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were prepared according to manufacturers' instructions. The mixture was heated to 85°C, cooled to 45°C, inoculated, and homogenized for 1 min. Fermentation was conducted at 43°C for 24 h followed by refrigeration at 4°C for 24 h. Samples were analyzed at 0, 15, 24, and 48 h for pH, total titratable acidity (TTA), and viable LAB counts. Experiments were conducted in triplicate and analyzed using two-way ANOVA.

Results: LAB culture formulation influenced fermentation performance differently in SPI and dairy yogurts. In SPI yogurt, viable LAB populations at 24 h ranging 5.4–7.4 $\log_{10}$ CFU/mL depending on culture formulation, with higher effective inoculum concentrations maintaining LAB populations 1×10^6 CFU/mL. In dairy yogurt, greater culture-dependent LAB growth was observed, with 24 h counts ranging 5.6–9.7 $\log_{10}$ CFU/mL. Two-way ANOVA identified fermentation time as the primary determinant of pH in both matrices, accounting for > 99% of pH variation in SPI yogurt, while LAB culture formulation contributed < 1%.

Significance: These findings establish baseline fermentation and microbial performance data for SPI-yogurt systems and support future challenge studies evaluating the combined effects of heating and fermentation on *L. monocytogenes* and *E. coli* O157:H7 in PB-fermented foods.

Award Application: Developing Scientist Award

P3-033: INTEGRATING SWOT ANALYSIS AND ANALYTIC HIERARCHY PROCESS (AHP) FOR EVALUATING MILK SAFETY IN ADDIS ABABA, ETHIOPIA

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Presentation type/Format: Technical

Category: Dairy

Abstract:

Introduction: Informal milk marketing remains widespread in Ethiopia, posing potential foodborne and quality risks that threaten public health and consumer trust.

Purpose: This study aimed to systematically evaluate the strengths, weaknesses, opportunities, and threats (SWOT) affecting milk safety in Addis Ababa and to prioritize these factors using the Analytic Hierarchy Process (AHP).

Methods: A mixed-method design combined stakeholder interviews (n = 42; producers, processors, inspectors, and consumers) with quantitative AHP pairwise scoring. Twelve SWOT factors were identified through literature review and expert consultation. Pairwise comparisons were analyzed using Expert Choice 11.5 software, with a consistency ratio < 0.1 accepted. Weighted scores were computed to rank strategic priorities.

Results: The highest-ranked strengths were the growing demand for pasteurized milk (weight = 0.21) and increasing private investment (0.18). Weaknesses included poor cold-chain infrastructure (0.26) and limited regulatory enforcement (0.22). Key opportunities were emerging urban markets (0.29) and policy support for dairy modernization (0.23). Major

threats comprised informal vending (0.31) and feed-contamination risks (0.25). The overall composite priority indicated that addressing weaknesses and threats (total = 0.53) would yield greater improvement in milk safety than expanding existing strengths or opportunities.

Significance: Integrating SWOT with AHP provided a transparent, quantitative framework for prioritizing milk-safety interventions. The findings guide evidence-based resource allocation and policy action toward safer dairy value chains in Ethiopia and comparable urban settings.

P3-034: GENOMIC AND FUNCTIONAL CHARACTERIZATION OF POTENTIAL PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM LONG-LIVED INDIVIDUALS IN COSTA RICA

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: The exceptional health and longevity of elderly populations from the Nicoya Peninsula, Costa Rica, highlight their gut microbiota as a promising source of probiotics, including lactic acid bacteria (LAB).

Purpose: Two putative probiotic gut isolates *Lactobacillus gasseri* Nicoya-CR-0022 and *Bifidobacterium animalis* subsp. *lactis* Nicoya-CR-0253 were evaluated for genomic features and performance in yogurt.

Methods: Plain yogurt was independently inoculated with commercial probiotic strains (*Lactobacillus casei* or BB-12) or the autochthonous isolates and fermented at 38°C for 6 h. The pH and LAB counts were monitored over time. Probiotic viability and yogurt physicochemical properties (pH, syneresis, viscosity, titratable acidity, organic acid content) were determined during 46 days of storage at 5°C. All experiments were performed in

triplicate and analyzed statistically using ANOVA. Comparative genomic analyses were conducted on whole-genome sequences and included average nucleotide identity (ANI), functional annotation and screening for virulence and antimicrobial resistance genes.

Results: No significant differences (p>0.05) in pH or LAB counts were observed during fermentation regardless of strain, reaching a final pH of about 4.4 and probiotic populations up to 9 $\log_{10}$ CFU/g in all treatments. Isolate counts remained stable during storage with minimal physicochemical changes. ANI analyses confirmed species-level assignment against reference genomes (Nicoya-CR-0022: 98–99%; Nicoya-CR-0253: 99–100%). Functional annotation showed genes related to carbohydrate metabolism, membrane transport, and stress response. No virulence or antimicrobial resistance genes were detected.

Significance: Autochthonous Costa Rican LAB isolates showed a promising profile for industrial yogurt applications.

Award Application: Developing Scientist Award

P3-035: COMPARISON OF THE THERMAL RESISTANCE OF ENTEROCOCCUS FAECIUM NRRL B-2354 AND SALMONELLA SP. IN LOW-MOISTURE EXTRUDED TREAT PRODUCTS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Multiple outbreaks of salmonellosis have been associated with the consumption of low-moisture products. While it is known that *E. faecium* NRRL B-2354 can be a surrogate for *Salmonella* in extruded products, studies have not determined D-values in matrices with different a_w and pH.

Purpose: To determine D-values for *Salmonella* and *E. faecium* in low-moisture treat products with varying pH and water activities.

Methods: Low-moisture treat products (1: a_w 0.92, pH 6.43; 2: a_w 0.92, pH 6.17, 3: a_w 0.55, pH 5.11) were inoculated with 8-log CFU/g of *Salmonella* or *E. faecium* NRRL B-2354. One-gram samples were aliquoted into vacuum pouches, vacuum packaged and submerged in water baths heated at 68.3, 71.1, or 73.9°C. Samples were removed at specified time intervals and chilled. Populations of *Salmonella* and *E. faecium* were enumerated by buffering with peptone (pH 7.2) and plating onto Xylose Lysine Deoxycholate or *Enterococcus* agar overlayed with tryptic soy agar. Two trials were conducted, and average D-values were calculated from the inverse slope of the linear regression of the survival data (log CFU/g) over time.

Results: D-values for *E. faecium* were consistently longer than *Salmonella* in all treatments. Average D-values (n=2) for *E. faecium* at the reference temperature of 73.9°C were 8.30±0.05,

8.02±0.81, and 5.60±0.33 minutes for treatments 1-3 respectively, compared with values of 2.28±0.27, 1.90±0.05, and 2.99±0.46 minutes for *Salmonella* for treatments 1-3, respectively.

Significance: These data demonstrate *E. faecium* was more thermally resistant than *Salmonella* in all matrices tested, illustrating its capability as a surrogate for *Salmonella* in extruded products. Interestingly, *E. faecium* did not appear to follow *Salmonella*'s trend of increased resistance in lower a_w matrices. However, *E. faecium* resistance did increase with pH, demonstrating that both matrix pH and a_w impact the thermal inactivation rate of *E. faecium*, which is also observed in *Salmonella*.

P3-036: SURVEY OF COMMERCIAL POWDERED INFANT FORMULAS FOR SULFITE-REDUCING CLOSTRIDIA AND PRESUMPTIVE CLOSTRIDIUM BOTULINUM

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: From 2023–2025, the U.S. experienced an unprecedented outbreak of infant botulism, with 51 cases linked to a specific brand of powdered infant formula (PIF). Although current regulations do not require testing for the presence of *C. botulinum* in PIF, an indicator limit of 2 log/g sulfite-reducing clostridia (SRC) has been proposed.

Purpose: To screen PIF of varying organic classification, milk type (whole, non-fat, non-dairy), and species (cow, goat), for the presence of SRC, including botulinum neurotoxigenic (BoNT) Clostridia.

Methods: Eleven PIF (Organic Whole, Brands A, B; Non-Organic Whole, Brand C; Organic Non-Fat, Brands D, E; Non-Organic Non-Fat, Brands F, G; Non-Organic Non-Dairy Brands H, I; Non-Organic Goat Whole, Brand J; and Non-Organic, Non-Fat Goat, Brand K) were obtained from retailers. Two 100g samples of each formula were dissolved in 200 mL of Butterfield’s

diluent, centrifuged, and pellets resuspended in 12 mL Butterfield's. SRC were enumerated via 5-tube Most Probable Number method in Reinforced Clostridial Medium. Samples were enriched in Trypticase-Peptone-Glucose-Yeast extract broth (TPGY) and screened for BoNT/A and B via DIG-ELISA.

Results: SRC were detected in 6/11 (55%) PIF tested, with counts ranging from 0.73 log CFU/g (LOD) to 2.59 log CFU/g. SRC were undetectable (LOD 0.73 log CFU/g) in Brands A, B, D, G and H. SRC counts were highest in Brand C (ave 2.38 log CFU/g) when compared with Brand E (0.78 log CFU/g) or I (1.13 log CFU/g). Similarly, Brand J had higher SRC counts (2.17 log CFU/g) than K (0.78 CFU/g). Enrichments of Brands C, F and J were presumptive positive for BoNT/A-producing spores, with C also positive for BoNT/B. Notably, C and J contained ~2-log CFU/g SRC, while F had an average of 1.31 log CFU/g.

Significance: SRC were recovered regardless of organic label and milk type. Additional investigation is required to establish the quantitative relationship between SRC levels and BoNT-producing Clostridia.

Award Application: Developing Scientist Award

P3-037: ANALYSIS OF ESCHERICHIA COLI ISOLATES FROM DAIRY CATTLE IN PUERTO RICO AND THE INFLUENCE OF THEIR ANTIMICROBIAL RESISTANCE PROFILE

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: As an island, Puerto Rico (PR) plays an important role in the agricultural sector, particularly through its dairy industry. Grade A milk production must follow bacteriological parameters and industry regulations. However, environmental factors such as the season can induce environmental stress that promotes bacterial dissemination. Certainly, the prevalence of the environmental pathogenic *Escherichia coli* (*E. coli*) contributes to the emergence of clinical mastitis, foodborne outbreaks, and the selection of antimicrobial resistant (AMR) strains in raw milk.

Purpose: The aim of this study was to assess the impact of the season (summer-autumn & winter-spring) on AMR genes and the transmission of *E. coli* from dairy farms in PR.

Methods: This study included 265 raw cow milk samples collected from multiple regions during the summer-autumn & winter-spring with previously elevated somatic cell count.

After collection, milk samples were pre-enriched, cultured in selective and differential media, and confirmed with biochemical tests. Confirmed *E. coli* isolates were then analyzed by whole genome sequencing and bioinformatically characterized to determine AMR gene profiles.

Results: *E. coli* was identified in 41.1% (43/129) of the samples collected during summer-autumn, and 39% (53/136) during winter-spring. The chi-square test showed no significant differences between seasons in *E. coli* prevalence (χ^2= 0.051, *p*-value= 0.821). Raw milk isolates showed differences between phenotypic and genotypic resistance through the bioinformatic analysis, with β-lactamase showing the highest detection in both seasons. In addition, 10% (9/86) raw milk isolates exhibited multi-drug resistance and a diversity of serogroups and sequence types.

Significance: These results suggest that the season together with farm practices may influence the prevalence of *E. coli* in dairy cattle. Continuous monitoring is therefore essential to mitigate AMR transmission within the dairy supply chain.

Award Application: Developing Scientist Award

P3-038: SEASONAL PATTERNS OF ANTIMICROBIAL RESISTANCE AND FOOD SAFETY RELEVANT VIRULENCE IN STAPHYLOCOCCUS AUREUS FROM SUBCLINICAL MASTITIS IN DAIRY HERDS IN PUERTO RICO

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: *S. aureus* is a common cause of subclinical mastitis in dairy cattle and is often associated with antimicrobial resistance, posing food safety concerns. Data on its prevalence and resistance patterns in Puerto Rico remain limited.

Purpose: This study estimated the prevalence of *S. aureus* in dairy cattle with subclinical mastitis and characterized antimicrobial resistance across seasons.

Methods: A total of 267 milk samples were collected from dairy farms in Puerto Rico during summer–fall (n = 129) and winter–spring (n = 136). Samples were cultured on selective media, and *S. aureus* was identified using Gram staining and biochemical tests. Methicillin-resistant *S. aureus* (MRSA) was differentiated using CHROMagar™, antimicrobial susceptibility was determined using the VITEK® 2 system, and whole-genome sequencing was performed using

the Illumina MiSeq platform with bioinformatic analyses using Galaxy Trakr.

Results: Preliminary results show, no significant seasonal differences in *S. aureus* prevalence between summer–fall and winter–spring ($\chi^2(1) = 0.67$, $p = 0.41$). All isolates were resistant to benzylpenicillin. During summer–fall, *S. aureus* was detected in 17% of farms, with 2.3% of isolates classified as MRSA. Resistance to oxacillin and erythromycin was observed in 13% of isolates, tetracycline in 10%, and clindamycin in 3%. In winter–spring, *S. aureus* was detected in 20% of farms, with 4.4% classified as MRSA; 21% of isolates exhibited resistance to oxacillin, erythromycin, and tetracycline. Genomic analyses revealed antimicrobial resistance genes associated with aminoglycosides (*lmrS, aph*), β-lactams (*mecA, blaZ*), lincosamides (*ermT*), macrolides (*mphC*), tetracyclines (*tet(38), mepA*), lipopeptides (*mprF*), fluoroquinolones (*norA, norB, arlS*), and fosfomycin (*fosB*).

Significance: These findings demonstrate antimicrobial resistant *S. aureus* in the dairy production chain in Puerto Rico, highlighting food safety risks associated with raw milk contamination.

Award Application: Developing Scientist Award

P3-039: BULK TANK MILK IN PUERTO RICO HARBORS GENETICALLY DIVERSE ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS WITH ANTIMICROBIAL RESISTANCE GENES

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Bulk tank milk represents a critical interface between dairy production and food supply, serving as a potential reservoir for antimicrobial resistant bacteria. Bacteria can enter the dairy supply chain from inadequate farm hygiene practices or dairy cows with intramammary bacterial infections. In Puerto Rico (PR), this concern is important since dairy industry is one of the island's largest agricultural sectors. Whole-genome sequencing (WGS) enables high-resolution characterization of bacterial populations and their associated antimicrobial resistance (AMR) genes, but genomic data describing AMR in bacteria from bulk tank milk in PR remain limited.

Purpose: To characterize *Escherichia coli* and *Staphylococcus aureus* isolates from bulk tank milk using WGS and assess their AMR gene profiles.

Methods: Bulk tank milk samples were collected from dairy farms in PR in 2024 and 2025. *E. coli* and *S. aureus* samples were isolated using selective culture methods, and gDNA was sequenced using the Illumina MiSeq. Genome assemblies were analyzed for genetic diversity through multilocus sequence typing, AMR genes, plasmid content, and virulence factors. To date, over 140 *E. coli* and *S. aureus* isolates have been recovered from bulk tank milk samples, with 121 sequenced.

Results: Genomic analysis identified AMR genes associated with clinical antimicrobials. *β*-lactam and tetracycline resistance genes detected most frequently, then aminoglycoside and sulfonamide resistance genes. Of the sequenced isolates, 27 met the definition of multidrug resistance, containing AMR genes to at least three antimicrobial classes.

Significance: These findings highlight bulk tank milk as a reservoir of genetically diverse bacteria carrying AMR genes. The use of WGS for the characterization of dairy-associated bacteria provides insight into the ecology of AMR and will be used to inform the development of an evidence-based extension program aimed at reducing AMR risks at the farm level.

Award Application: Developing Scientist Award

P3-040: THE EFFECT OF AGING TEMPERATURE ON FOODBORNE PATHOGENS IN RAW MILK HARD CHEESE: A COMPARATIVE ANALYSIS

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Cheese manufacture with raw milk may harbor pathogens if proper processing and hygienic conditions aren't followed. U.S. regulations require aging for ≥60 days at >1.6°C for microbial control.

Purpose: Evaluate the effect of aging temperature on foodborne pathogens in raw milk cheese.

Methods: Milk was inoculated with *S. aureus*, *L. monocytogenes* (LM), and *E. coli* O157:H7 at 3.5log. Cheese was prepared in the lab and aged at 32°C and 12°C for 70 days. Samples were collected at pre-aging and weekly during aging for microbial and compositional analysis. bioMerieux's Tempo was used to enumerate *S. aureus*, lactic acid bacteria (LAB), aerobic count (AC), Enterobacteriaceae (EB), and *E. coli* (EC); spread plating on SMAC and MOX agar for *E. coli* O157:H7 and LM. Data (mean of 3 replicates) was analyzed with ANOVA, mixed-effect models, Tukey's, and Sidak's tests ($p<0.05$).

Results: At pre-aging, *S. aureus* increased significantly (1.5log) in curds. *L. monocytogenes* and *E. coli* O157:H7 decreased slightly after curd salting ($p>0.05$). During aging, *S. aureus* decreased significantly at 32°C, from 3.6 to <1.0log by day 40 ($p<0.05$), while at 12°C the reduction by day 70 was 1.5log. At 32°C, LM declined significantly and became undetectable

by day 20 (p<0.05), while at 12°C remained at an average of 1.8log. *E. coli* O157:H7 became undetectable by day 1 at both temperatures (p<0.05). LAB averaged 2.2log at both conditions, with no significant changes (p>0.05). AC remained stable at an average of 4.4log in both conditions (p>0.05), while EB and EC were undetectable at both temperatures. The effects of time and temperature were significant for *S. aureus* and LM during aging (p<0.05). Moisture, a_w, and pH (41%,0.7,6.5) were consistent at both conditions (p>0.05).

Significance: Aging at 32°C controlled pathogens significantly versus 12°C. Salt and a_w may have influenced microbial behavior, supporting the need for product-specific aging parameters.

Award Application: Developing Scientist Award

P3-041: HPP REPLACEMENT USING CLEAN-LABEL ANTIMICROBIAL SYSTEMS FOR SHELF-LIFE EXTENSION AND LISTERIA CONTROL IN LOBSTER SPREAD

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Introduction: High-pressure processing (HPP) extends seafood spread shelf life but requires high energy, specialized equipment, and packaging. With growing demand for sustainability and clean-label products, the industry seeks natural, resource-efficient alternatives.

Purpose: Evaluated a clean-label antimicrobial system as an HPP replacement in lobster spread.

Methods: Three treatments were prepared: (1) HPP lobster spread (2) non-HPP lobster spread without antimicrobials, and (3) non-HPP lobster spread with clean-label antimicrobials-0.95% NouriShield D4010, 0.2% Flavoset 5400L, 1.03% DuraFresh 5924L, 0.4% Flavoset 5080 (treatment). Samples (n= 36) were stored at 4°C for 60 days and analyzed for aerobic plate count (APC), lactic acid bacteria (LAB), and yeasts/molds. Spoilage limit was set at 7 log outgrowth of tested microorganisms. A challenge study was done by inoculating

Listeria monocytogenes at 1 log CFU/g, with failure defined as □2 log CFU/g. Data were analyzed using ANOVA ($p < 0.05$).

Results: Non-HPP control exceeded 7 log APC by Day 45, while HPP and clean-label treatments showed only moderate APC increases 2–4 log through Day 60. LAB in non-HPP controls rose >5.5 log by Day 45, whereas HPP and clean-label treatments stayed undetectable until Day 45 and reached ~4 log by Day 60. Yeast in the non-HPP reached the spoilage threshold (□7 log CFU/g) by Day 38, whereas the HPP remained below detection throughout and the clean-label treatment increased gradually, reaching ~6 log only by Day 60. In challenge studies, *Listeria* in non-HPP controls increased by ~0.7 log, while HPP samples rose >1 log by Day 30 before declining to below detection by Day 60. Clean-label treatments-maintained *Listeria* below 1 log throughout storage.

Significance: These findings demonstrate that a clean label antimicrobial can effectively replace HPP and extend Maine Lobster Spread shelf life to 60 days under refrigerated conditions.

P3-042: CLEAN-LABEL STRATEGIES FOR LISTERIA MONOCYTOGENES CONTROL AND SHELF-LIFE EXTENSION IN YOGURT-BASED DIP

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Yogurt-based dips pose food safety challenges due to high moisture, low salt, and moderately acidic pH, which can support *Listeria* and spoilage.

Purpose: Evaluate the efficacy of clean-label technologies in controlling *Listeria monocytogenes* and enhancing the safety and shelf life of yogurt-based dip.

Methods: Yogurt-based dips were prepared with: No antimicrobial control, two antimicrobial systems: Treatment 1: 0.75% D4040, 0.1% Flavoset 5400L, 1.5% Flavoset 8300L, 0.5% DF5970) and Treatment 2 (0.1% Flavoset 5400L, 1.5% Flavoset 8300L, 0.5% DF5970. All treatments were prepared in triplicate**.** Samples were stored at 7°C for 21 days. Spoilage evaluation included Aerobic Plate Count (APC), Lactic Acid Bacteria (LAB), yeasts/molds and *Pseudomonas* spp. Sampling was performed periodically over 3 weeks (days 0, 2, 14, and 21). In a separate trial, dips were inoculated with 3.0 log CFU/g *Listeria monocytogenes* and

sampled for 21 days, and statistical significance of *Listeria* reductions was determined using one-way ANOVA ($p < 0.05$).

Results: Treatment 1 achieved a 3-log reduction of *L. monocytogenes* within 48 hours and maintained levels below detection. Treatment 2 also significantly reduced *Listeria* to undetectable levels by Day 14, while control remained at 2–3 log CFU/g. APC in control exceeded 5 log CFU/g by Day 21, whereas both treatments remained 3 log CFU/g. LAB counts showed distinct trends: control and Treatment 2 supported LAB growth, increasing from ~4.5 and 4 log CFU/g at Day 0 to 7.5 and 6.5 log CFU/g by Day 21, respectively, while LAB in Treatment 1 remained below detection. Treatment 2 preserved beneficial LAB populations typical of yogurt-based products while reducing pathogens and spoilage organisms. No visible mold growth or gassing occurred in treated samples.

Significance: Both clean-label systems-controlled *Listeria* and spoilage in yogurt dip. Treatment 1 offers maximum suppression, while Treatment 2 preserves LAB viability.

P3-043: REDUCING MICROBIAL GROWTH AND EXTENDING SHELF LIFE OF FRESH HISPANIC CHEESES

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Category: Dairy

Abstract:

Introduction: Fresh Hispanic-style cheeses are highly perishable due to their high moisture content and neutral pH, creating an environment conducive to microbial growth. Spoilage during storage often results in bloating and textural changes, leading to reduced shelf life and economic losses. This study focuses on isolating spoilage organisms and evaluating antimicrobial treatments to mitigate their growth in fresh Hispanic-style cheeses.

Purpose: The purpose of this study was to identify spoilage microorganisms associated with fresh Hispanic-style cheeses and assess the efficacy of selected antimicrobial solutions in inhibiting their growth under controlled conditions.

Methods: Spoiled product lots were sampled and enumerated for aerobic plate count, lactic acid bacteria, yeast, and mold using selective media. Isolates were treated with Flavoset 5400L, IsoAge DV100, and DF5015, alongside untreated controls. Growth kinetics were monitored for 14 days at 25°C using an automated microplate reader, and lag time and maximum growth rate were calculated using the modified Gompertz model. The samples were prepared in triplicate for analysis of growth kinetics.

Results: Flavoset 5400L completely inhibited growth of all yeasts and molds, including *Candida zeylanoides* and *Penicillium glabrum*, with no growth over 14 days. IsoAge DV100 delayed onset and reduced growth rates, e.g., *Streptococcus thermophilus* dropped from 3.34 h^{-1} to 0.46 h^{-1}, with lag phases extended up to 4 days. DF5015 provided uniform inhibition, increasing yeast lag phases from 4.5 to 9.8 hours and mold lag phases from 17.9 to 23.6 hours. Combination treatments of DF5015 with Flavoset 8300L achieved complete suppression of all spoilage organisms, including *Pseudomonas*, throughout the assay.

Significance: This project demonstrates that targeted antimicrobial strategies can effectively control spoilage microorganisms, extending shelf life of fresh Hispanic style cheeses.

P3-044: NATURAL ANTIMICROBIAL SOLUTIONS COMPARED TO NATAMYCIN FOR MOLD INHIBITION IN SHREDDED MOZZARELLA AND CHEDDAR

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Category: Dairy

Abstract:

Introduction: Natamycin is widely applied to shredded cheese as a surface-active antifungal to reduce spoilage molds during refrigerated storage and distribution. Shredded mozzarella and cheddar cheese present an elevated risk of fungal growth due to increased surface area, post-shred handling, and physiochemical conditions favorable for mold growth. However, the use of natamycin is being challenged due to increase in demand for natural and clean-label ingredients.

Purpose: To quantify antifungal effects of clean-label ingredients in shredded mozzarella and cheddar cheese.

Methods: Commercial shredded Cheddar and Mozzarella were inoculated with a three-strain mold cocktail – *Aspergillus niger*, *Penicillium commune*, *Cladosporium cladosporioides* and evaluated for visible mold. Samples were nitrogen flushed and stored at 4°C, they were moved to storage at 7°C at 42 days. The cheese samples were subjected to multiple

antimicrobial treatments at 0.5%, 0.6%, 0.8%, 1% and 1.25%, along with natamycin at 5 ppm, which are commercially relevant levels. Samples were stored under refrigerated conditions, and mold growth was monitored visually over time. Physiochemical properties and microbiological assays were performed to assess product characteristics. Samples were prepared in triplicate, and days to visible mold were compared using one-way ANOVA with Tukey post-hoc testing. All antimicrobial treatments significantly extended shelf life compared to the control ($p < 0.05$).

Results: Untreated shredded mozzarella and cheddar cheese developed visible mold by day 93, while natamycin and all natural antimicrobial treatments maintained no visible mold for >200 days. Physiochemical properties remained stable across treatments, confirming the extended shelf life was due to antimicrobial ingredients.

Significance: Natural antimicrobial systems effectively reduced mold growth in shredded Mozzarella and Cheddar extending time-to-visible spoilage under refrigerated storage.

P3-045: ANTIMICROBIAL TREATMENTS TO PREVENT YEAST AND MOLD GROWTH IN COTTAGE CHEESE

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Category: Dairy

Abstract:

Introduction: Cottage cheese is a high-moisture, high-protein dairy product that is particularly susceptible to microbial spoilage due to its nutrient-rich composition. Common spoilage organisms such as *Enterococcus*, and molds like *Penicillium* have been frequently associated with reduced shelf life and quality issues. To address these challenges, antimicrobial treatments were evaluated to identify effective solutions for controlling microbial growth and extending product shelf life.

Purpose: Identify effective antimicrobial solutions that completely inhibit spoilage microorganisms in cottage cheese.

Methods: Cottage cheese samples were analyzed for pH, moisture, water activity, salt, and organic acids and screened for spoilage microorganisms using selective media. Isolated strains, including yeasts, molds, and *Pseudomonas* spp., were subjected to Accelerated Data Generation Platform (ADGP) antimicrobial testing. Growth kinetics were monitored at 30°C for up to 72 hours using Bioscreen, and lag time and growth rate were calculated via the modified Gompertz model. A positive growth control-no antimicrobial treatment was

included.

Results: Clean-label antimicrobial treatments demonstrated significant inhibition of spoilage organisms. A broad spectrum clean-label antimicrobial completely inhibited lactic acid bacteria, aerobic bacteria, and *Pseudomonas*. Two additional clean-label antimicrobials significantly slowed growth, reducing rates from 3.34 h^{-1} to 0.46 h^{-1} and extending lag phases from 1.4 to 2.4 days for *Streptococcus thermophilus. Enterococcus faecium* showed similar trends, with growth rates dropping from 12.15 h^{-1} to 8.67 h^{-1} and lag phases increasing from 0.23 to 0.26 days. Complete inhibition was achieved for *Hafnia psychrotolerans* and *Kocuria varians* across all clean-label treatments.

Significance: Effective antimicrobial treatments can eliminate spoilage organisms in cottage cheese, extending shelf life, reducing waste, and ensuring consistent product quality.

P3-046: REPLACING POTASSIUM SORBATE IN FETA CHEESE WITH CLEAN-LABEL INGREDIENTS

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Category: Dairy

Abstract:

Introduction: Feta is a high-moisture, brined cheese that is highly susceptible to microbial spoilage, requiring effective preservation for quality and safety. Potassium sorbate is commonly used in brine to inhibit yeast and mold, even ~0.1% usage can extend shelf life of feta cheese to one year in brine. However, the need for clean-label ingredients drives the development of natural antimicrobial alternatives with similar efficacy.

Purpose: This research aimed to identify natural, clean-label alternatives to potassium sorbate for controlling spoilage microorganisms and extending shelf life in feta cheese.

Methods: Feta cheese samples were prepared under three conditions: no antimicrobials, potassium sorbate, and clean-label blends (DF5015, DF5970, Flavoset 8300L, IsoAge D100, NS D4040) in varying combinations and concentrations. Physicochemical properties-pH, moisture, water activity, salt, fat, organic acids and microbial counts for yeast and mold, aerobic plate count, lactic acid bacteria, psychrotrophs and *Pseudomonas* were determined. Growth kinetics were assessed using Bioscreen, and minimum inhibitory concentrations (MICs) were modeled via the modified Gompertz equation. The samples were prepared in

triplicate.

Results: Clean-label systems achieved complete inhibition or >4-log reductions for yeast, APC, and *Pseudomonas*. Combinations of DF5970 + DF5015 and Flavoset 8300L + DF5970 were most effective, extending lag phases up to 7 days and reducing growth rates by 90% compared to controls. The strongest formulation of IsoAge D100 + Flavoset 8300L prevented growth entirely for all tested spoilage organisms over 14 days.

Significance: Clean-label ingredients can effectively replace potassium sorbate in feta cheese, with antimicrobial effects and extended shelf life, meeting consumer need for natural products.

P3-047: NATURAL ANTIMICROBIAL SOLUTIONS FOR CHEESE BRINE PRESERVATION

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Category: Dairy

Abstract:

Introduction: Brine solutions are essential for cheese flavoring and preservation but prone to microbial contamination. Brine solution is often reused, which accumulates cheese particles and increasing microbial growth and posing threat to food safety. Chemical preservatives and bio-protective cultures can control spoilage, but increasing demand for clean-label products limits their use.

Purpose: This study aims to isolate microbial strains from brine solutions of feta, muenster, and dill cheeses to develop natural antimicrobial solutions.

Methods: Brine samples from dill, muenster, and feta were analyzed for pH, moisture, water activity, minerals, salt, fat, protein, and organic acids. Microbial testing was conducted for aerobic plate counts, lactic acid bacteria, yeast and mold; isolates were identified and screened. Each sample was repeated in triplicates. Growth of the microbes were monitored in Bioscreen inoculated at 6 log CFU/ml incubated at the required temperature. Optimal density (OD) at 600 nm was recorded every 30 minutes for 14 days and minimum inhibitory concentrations (MIC) were calculated using Gompertz equation.

Results: Bioscreen analysis confirmed that untreated controls exhibited rapid growth with higher OD increase and shorter lag phases (<1 day). Spoilage strains isolated from brine included *Debaryomyces hansenii*, *Candida sojae*, *Staphylococcus warneri*, *Bacillus subtilis*, and *Lactobacillus brevis*. Clean-label treatments significantly slowed growth; individual treatments extended lag phases up to 9 days and reduced growth rates up to 90%, while the combination of IsoAge D100 and Flavoset 5400L (labelled as vinegar, natural flavor) completely inhibited growth for all strains for over 14 days, demonstrating strong synergistic efficacy.

Significance: This study shows that clean-label antimicrobial ingredients effectively control spoilage microorganisms in cheese brine, extending shelf life, reducing waste, and enhancing food safety.

P3-048: EFFECTS OF MILK PRODUCTION SYSTEMS ON MICROBIAL COMMUNITIES AND FATTY ACID PROFILES IN RAW MILK

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Category: Dairy

Abstract:

Introduction: Raw milk composition and microbiota may vary depending on production practices, potentially influencing milk quality and nutritional value.

Purpose: This study evaluated how conventional, antibiotic-free, and organic milk production systems affect microbial communities, fatty acid profiles, and their associations in raw milk.

Methods: Raw milk samples were collected during winter in South Korea and classified as conventional, antibiotic-free, or organic. Microbial counts and somatic cell counts were determined. Microbial communities were analyzed by metagenomic sequencing with diversity assessment. Fatty acid composition was determined, and multivariate, correlation, and network analyses were performed to examine associations between dominant microbial genera and fatty acids.

Results: Total bacterial counts did not differ among milk types (3.83–3.97 log CFU/mL), whereas somatic cell counts were significantly higher in organic milk (2.68×10^6 cells/mL) than in conventional and antibiotic-free milk. Microbial diversity and overall community

structure showed no significant differences among groups (PERMANOVA, $p > 0.05$), with *Pseudomonas* dominating all samples (75.7%). Organic milk showed significantly higher relative abundances of *Lactococcus*, *Acinetobacter*, and *Carnobacterium*. Fatty acid composition differed significantly among milk types ($p = 0.004$), with organic milk containing higher levels of polyunsaturated fatty acids, including C18:2n-6, C18:3n-3, and C20:4n-6. Correlation and network analyses revealed positive associations between PUFAs and microbial genera enriched in organic milk, including *Lactococcus*-C18:2n-6 and *Serratia*-C18:2n-6 and C20:5n-3.

Significance: These findings demonstrate that milk production practices influence fatty acid composition and specific microbial populations, highlighting a potential role of milk microbiota in modulating nutritional quality and informing food quality and safety management strategies.

P3-049: IN-LINE SAMPLING REVEALS OPPORTUNITIES FOR IMPROVEMENTS IN BULK TANK MILK QUALITY

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Bulk tank sampling is the standard industry approach for evaluating raw milk quality, but a single composite sample may not capture deviations in milking practices that influence quality parameters throughout the day.

Purpose: To evaluate whether in-line sampling provides higher resolution for monitoring raw milk quality compared to bulk tank sampling.

Methods: Dairy farms (n=3) with 2□3 milking shifts per day were sampled for 7 consecutive days. Samples were collected each shift using QualiTru ports (n=44) and once daily from the bulk tank (n=25). All samples were tested in duplicate for somatic cell count, standard plate count (SPC), laboratory pasteurization count, coliforms, preliminary incubation count, Gram-negative bacteria, mesophilic spores, and psychrotolerant spores. Shift effects were evaluated using mixed effects models with day as the random effect. Results were examined across all farms and within individual farms.

Results: Across all farms, no significant differences were detected for any test (p>0.05), likely due to limited sample size. Nevertheless, microbial counts in in-line milk samples varied substantially by shift within a single day, with SPC ranging up to 2.43, 1.45, and 1.41 log CFU/mL on farms A, B, and C, respectively. Large shift to shift variability within individual days was

observed for other bacterial tests (e.g., Gram-negative bacteria, coliforms), while slight variability was observed for spore counts and laboratory pasteurization. Across pooled data from all farms, 5 of 8 microbial measures showed higher average variability in inline than in bulk tank samples.

Significance: In-line sampling revealed temporal variation and farm-specific patterns that bulk tank sampling masked. These microbial concentrations are driven by milking hygiene practices, suggesting that in-line sampling offers opportunities for milk quality improvement.

P3-050: RAPID DETECTION OF MICROORGANISMS IN ALL-PURPOSE CREAM USING HYGIENA'S ATP-BASED INNOVATE™ MICROBIAL SCREENING SYSTEM

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Commercial sterility testing is critical for validating the shelf-life stability of Ultra-High Temperature (UHT) processed and aseptically packaged all-purpose cream. Detection of microbial contaminants can be challenging due to low initial bioburden, matrix-induced inhibition and delayed microbial resuscitation caused by emulsifiers and stabilizers. This study evaluates the performance of the Hygiena® Innovate™ System, which utilizes RapiScreen™ ATP bioluminescence technology to accelerate microbial detection compared to conventional methods.

Purpose: This study was designed to evaluate and compare a modified and shorter enrichment method for rapid ATP-based screening with a traditional culture-based method for the detection of risk organisms such as *Bacillus cereus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* in all-purpose cream samples.

Methods: Three organisms of concern, including *Bacillus cereus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa,* were prepared to inoculate 250 mL packs of all-purpose cream. Organisms were added to triplicate sachets at a level of 1, 3 and 9 CFUs/pack, packs were

incubated at 30°C for 24 hours to allow recovery and initial growth from single-cell contamination), transferred 1 mL of the sample into BPW or BHI enrichment broth and incubated for an additional 18–24 hours to accelerate cell growth and reach approximately 10^4 CFU/mL and tested across a two-day period. Sample aliquots (50 µL) were aseptically removed each day and tested with the RapiScreen Dairy assay. At the same time, samples were plated for culture confirmation.

Results: Using an established threshold of 50 RLU to differentiate contaminated products, the ATP-based method detected all organisms and replicates after 42 hours of incubation. Culture results were in complete agreement.

P3-051: THERMAL INACTIVATION MODEL FOR THE HISTAMINE-PRODUCING SPECIES, LENTILACTOBACILLUS PARABUCHNERI, IN NON-HOMOGENOUS MILK

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: *Lentilactobacillus parabuchneri* (LP), a common contaminant in milk, is a primary producer of histamine via decarboxylation of histidine in cheeses causing histamine poisoning. Thermal treatments are necessary to eliminate this health concern in cheese. LP has been shown to survive pasteurization, but its thermal resistance has not been well characterized.

Purpose: To create a thermal inactivation model for the histamine-producing bacteria LP, in non-homogenous milk using D- and Z-values generated from thermal death time (TDT) studies.

Methods: Non-homogenous whole milk was inoculated with 8-log CFU/mL of LP, and portioned into water impermeable pouches, vacuum sealed, and submerged into a preheated water bath at temperatures: 130, 135, 140, 145, and 150°F. Triplicate samples were removed and immediately submerged in an ice bath at designated time intervals. Surviving populations were enumerated by serial dilution in buffered peptone, plated on De Man-Rogosa-Sharpe agar (MRS) with an MRS overlay, and anaerobic incubation at 30°C for 5 days. Three trials were conducted at each temperature and average D-values were calculated from

the inverse slope of the linear regression of surviving LP populations relative to time. The Z-value was calculated from the inverse slope of the log of the D-values relative to temperature.

Results: LP exhibited high average D-values at 130, 135, 140, 145, and 150°F of 13.36±2.55, 6.79±0.65, 4.16±1.14, 1.61±0.44, 0.55±0.09 minutes, respectively. The Z-value was calculated to be 14.66°F. Data were used to develop a thermal inactivation model to demonstrate time/temperature hold for sufficient inactivation of LP in milk during cheese making.

Significance: This study demonstrates thermostability of histamine-producing LP and will be used to generate a model that can be used by cheesemakers to ensure LP will be eliminated during cheese milk thermization, therefore reducing risk of future histamine poisoning-related illnesses.

P3-052: VIRULENCE POTENTIAL OF BACILLUS CEREUS ISOLATES ISOLATED FROM ITALIAN INFANT FORMULAE

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Several international studies have confirmed the sporadic presence of pathogenic bacteria and have enumerated spoilage organisms in commercial powdered formula products, including *Bacillus cereus*.

Purpose: This study aimed to investigate the prevalence and counts of *Bacillus cereus* in powdered formulae for infants marketed on the Italian market.

Methods: 83 samples, including 23 infant formulae (PIF), 42 follow-on formulae (FOF), and 18 formulae for special medical purposes (SMP) were analyzed, enumerating *B. cereus* by spreading aliquots onto PEMBA agar. Genomic DNA from *B. cereus* isolates was extracted. To determine the presence of toxin-encoding genes in *B. cereus*, conventional PCR was performed. Target genes included sphingomyelinase (sph), enterotoxin Bcet (bcet), enterotoxin FM (entFM), phosphatidylinositol-specific phospholipase C (plcA), cytotoxin K (cytK), and the non-hemolytic enterotoxin components (nheA, nheB, nheC) and cereulide-ces. *B. cereus* isolates were submitted to antimicrobial susceptibility testing by broth microdilution using the Sensititre™ automated system.

Results: *B. cereus* was detected in 45.8% of samples, but counts detectable (1 log CFU/g) in just three samples. 42 presumptive *B. cereus* were isolated. All the strains were able to produce proteases, 21 strains secreted PC-PLC and 28 produced the tripartite hemolysin HBL. Among toxin-encoding genes, the most prevalent was sph (97.7%), followed by nheA, nheB, nheC (76.2%), entFM (64.3%) and bcet (45.2%). Only 7.1% of the isolates carried cytK gene. ces was in 28.6% of the isolates. Antimicrobial resistance pattern of the isolates showed high resistance rates to β-lactams and a moderate resistance to erythromycin.

Significance: The prevalence, virulence potential, and antibiotic resistance profiles of *B. cereus* isolates raise important concerns especially considering the vulnerability of the target population.

P3-053: CARDOON RENNET AS A NATURAL ANTIBACTERIAL STRATEGY TO ENHANCE FOOD SAFETY IN TRADITIONAL CHEESE PRODUCTION

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Purpose: Here, we aimed at investigating the antibacterial activity of thistle-rennet against major foodborne pathogens, relevant to cheese production.

Methods: Cardoon was obtained from a Portuguese traditional cheese factory as dried plant material. Thistle rennet was prepared according to the cheese producer. Ethanolic extracts (1:5) were prepared from rennet and different dairy fractions, including whey cheese, whey, freshly coagulated curd, and matured cheeses. Antibacterial activity was assessed against several strains associated with foodborne diseases, including *Escherichia coli* O157 (NCTC 12900), *Listeria monocytogenes* (NCTC 11994), *Salmonella* spp. (NCTC 13175), using standard microdilution assays. Phenolic distribution was evaluated by FRAP. All experiments were performed in at least three independent replicates.

Results: Thistle-rennet exhibited dose-dependent inhibition of all tested bacteria, with growth reductions ranging from 40–75% for *E. coli*, 45–70% for *Listeria* spp., 50–72% for *Salmonella* spp., and 42–68% for *Staphylococcus aureus* at the highest tested concentrations, when compared to controls ($p < 0.01$). Higher antibacterial activities were observed in *requeijão*, whey, and cottage cheese, although phenolic compounds were more distributed in freshly coagulated and matured cheeses.

Significance: These results indicate that cardoon-derived rennet may effectively limit the survival of foodborne pathogens in cheese, enhancing product safety and mitigating zoonotic risks, while highlighting potential strategies to optimise antibacterial activity distribution in dairy products. Given its widespread use in the food industry and GRAS status, cardoon represents a promising natural intervention aligned with One Health principles.

P3-054: ANTIMICROBIAL PEPTIDES PRODUCED BY DAIRY FERMENTATION AS NATURAL PRESERVATIVES FOR PET FOODS

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Pet foods rely on thermal processing and synthetic preservatives to ensure microbiological safety. However, increasing consumer demand for “clean-label” products is accelerating the search for natural alternatives. Antimicrobial peptides (AMPs) have emerged as promising candidates due to their targeted activity, biodegradability, and safety profile.

Purpose: Antimicrobial peptides produced through controlled dairy fermentation were tested as natural preservatives and antimicrobial agents in pet foods.

Methods: Antimicrobial peptide-rich extracts were obtained through a proprietary fermentation process using whey and milk as substrates. Activities of the fermented fractions were first assessed in vitro against a panel of foodborne bacteria, including *Escherichia coli* O157, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus.*

Minimum inhibitory concentrations (MICs) were determined for each target microorganism using standard microdilution assays. Subsequently, the antimicrobial efficacy of the peptide preparations was evaluated at three different concentrations in pet food matrices artificially inoculated with 10^5 *E. coli* O157 CFU/mL. Bacterial growth was monitored over 24 and 48 hours of exposure.

Results: Fermentation-derived peptide extracts exhibited broad-spectrum antibacterial activity against all tested foodborne bacteria, with measurable MIC values confirming effective growth inhibition. The peptides induced a substantial reduction in *E. coli* O157 contaminated pet foods log 1.25 CFU/mL, when compared to the control group, log 8.5 CFU/mL, representing an almost 7-fold reduction. These significant inhibitory effects were observed at 24 h, demonstrating that peptide activity was maintained in complex food matrices.

Significance: Results support our peptides' potential use as a natural preservative in pet foods and as complementary antimicrobial agents for hygiene and biofilm control in processing environments.

P3-055: EFFECT OF OXIDATIVE STRESS ADAPTATION ON THE SURVIVAL OF BIFIDOBACTERIUM SPP. IN YOGURT

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Category: Dairy

Abstract:

Introduction: *Bifidobacterium* spp. are considered the most beneficial probiotic bacterial genera and species, with an extensive range of health benefits linked to their inclusion in foods. However, as strict anaerobes, *Bifidobacterium* spp. are highly sensitive to oxygen, making them susceptible to oxidative stress effects during yogurt production.

Purpose: This study investigated the impact of oxidative stress adaptation on *Bifidobacterium* spp. viability, fermentation characteristics, and physicochemical properties during yogurt fermentation and shelf life at 4˚C.

Methods: Oxidative (H_2O_2) stress-adapted and unadapted strains of *Bifidobacterium bifidum*, *B. breve* and *B. animalis* subsp*. animalis,* were incorporated at the onset of the yogurt fermentation at 6 to 7 log CFU/mL. Viability (plate counts and PMAxx-qPCR) and physicochemical properties were monitored over 28 days at 4°C. Statistical significance of differences in both probiotic cell counts and physicochemical characteristics between yogurts containing adapted or unadapted *Bifidobacterium* spp. was determined through ANOVA ($\alpha = 0.05$) (SPSS v29, IBM, USA) and principal component analysis (PCA) was performed (GraphPad Prism 10.0). Two independent experiments, each performed in duplicate, provided four replicates per treatment ($n = 4$).

Results: Stress-adaptation enhanced *B. bifidum* survival during fermentation by 25 1.0 log CFU/g compared to unadapted strains ($P < 0.0001$), with *B. breve* showing similar 26 improvement, though this advantage diminished throughout shelf life in both species. *B. animalis* remained stable regardless of the adaptation treatment. While the plate count method 28 showed a decline in viability of *B. bifidum* and *B. breve*, the PMAxx-qPCR method detected a 29 significantly higher level of viable cells ($P < 0.05$) in the yogurt.

Significance: Oxidative stress adaptation may enhance *Bifidobacterium* spp. viability and the functional value of probiotic yogurt.

P3-056: REDUCTION OF LISTERIA SPP. AND SALMONELLA SPP. ON CHEESE VARIETIES MANUFACTURED WITH REGULAR AND ULTRAFILTERED MILK

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Presentation type/Format: Poster

Category: Dairy

Abstract:

Introduction: Cross-contamination of ready-to-eat foods is a substantial concern in the transmission of pathogenic organisms such as *Salmonella* spp. and *Listeria monocytogenes*. During cheddar cheese curd production, lactose is converted to lactic acid by starter cultures, and lactic acid bacteria may produce antimicrobial compounds, including bacteriocins. In addition, milk naturally contains the antimicrobial protein lactoferrin.

Purpose: The objective of this study was to evaluate the survival of *Listeria* spp. and *Salmonella* spp. on different cheese varieties manufactured with regular or ultrafiltered milk and stored at either room or refrigeration temperature.

Methods: Cheese surfaces were inoculated with *Listeria* spp. or *Salmonella* spp. and monitored for pathogen reduction under the designated storage conditions.

Results: For *Listeria* spp., regular milk cheese curds and ultrafiltered milk cheese curds stored at room temperature showed the greatest statistically significant log reductions of 1.2 and 3.4, respectively. For *Salmonella* spp., the same treatments demonstrated the largest statistically significant reductions of 3.3 and 4.7 log CFU/g, respectively. Cheeses manufactured with ultrafiltered milk consistently demonstrated greater reductions of both pathogens compared to cheeses made with regular milk.

Significance: These findings suggest that ultrafiltration technology may enhance the antimicrobial properties of cheese, potentially reducing pathogen survival and the risk of product recalls. This technology could improve consumer confidence and provide economic benefits to the dairy industry. Further research is warranted to determine whether increased lactoferrin concentrations in ultrafiltered milk contribute to the observed antimicrobial effects.

P3-057: BLUE LIGHT INACTIVATION OF SALMONELLA ENTERICA IN GOAT MILK POWDER

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Dry foods, such as powdered milk, frequently pose possible food safety risks due to the presence of pathogens, such as *Salmonella*. As a result, effective treatment to inactivate *Salmonella* is critical. However, effective nonthermal strategies for reducing *Salmonella* in dry dairy matrices remain limited.

Purpose: The objective of this study was to evaluate the efficacy of 403.5 nm blue light for the inactivation of *Salmonella enterica* in whole and skim milk goat milk powders.

Methods: Two varieties (whole and skim milk) of commercial goat milk powder (GMP) were inoculated with a four strains rifampin resistant *Salmonella* (Enteritidis PT 9c, Montevideo, Saint Paul, and Newport) air dried for 30 min under the biosafety hood. The inoculated *Salmonella* population in the GMP was ~5 $\log_{10}$ CFU/g. After inoculation, two g of each sample were placed in a sterile petri dish and spread evenly in a thin layer and treated with blue light at 16.5 cm. *Salmonella* reduction in treated and control samples was measured after 8, 16, 24

and 48 h of exposure. Samples were plated on tryptic soy agar with rifamycin and counted. Experiments were conducted in duplicate with three replications (n=6). Results were analyzed using the GLM procedure of SAS and means were generated using LSM and separated with the Tukey-Kramer Test. Means were considered significantly different at p 0.05.

Results: *Salmonella* reductions ranged from 0.83–1.16 $\log_{10}$ CFU/g for non-fat GMP and 1.57–3.24 $\log_{10}$ CFU/g for whole fat GMP. In non-fat GMP, there was a significant difference in *Salmonella* reduction between 8 and 48 h treatment. In whole fat GMP, treatment time had a significant effect on *Salmonella* reduction.

Significance: The study's findings revealed that using blue light can reduce *Salmonella* in goat milk powder. Goat milk powder's microbiological safety can be efficiently enhanced by using blue light as an alternative to traditional thermal treatments.

P3-058: CHARACTERIZATION OF BACTERIAL SPORE INJURY INDUCED BY EMULSIFIER-COMBINED HIGH-PRESSURE PROCESSING USING FLOW CYTOMETRY AND QUANTITATIVE PCR

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Detailed characterization of spore injury induced by combined emulsifier and high-pressure processing (HPP) treatments remains limited.

Purpose: The objective of this study was to evaluate bacterial spore injury induced by emulsifier-combined HPP using flow cytometry and quantitative PCR.

Methods: *Bacillus spizizenii* NBRC 3134 was used as the test strain. Several food-grade emulsifiers were applied simultaneously with HPP to bacterial spores at 30–70 MPa. The ratio of injured spores after HPP was evaluated based on differences in Ct values (ΔCt) obtained from qPCR amplification curves of bacterial DNA following propidium monoazide (PMA) treatment. In addition, spore injury was assessed by flow cytometric analysis (FCM) of fluorescently stained spores.

Results: PMA-qPCR analysis showed that the emulsifier-combined HPP resulted in significantly higher ΔCt values at 50 MPa and 70 MPa. Furthermore, FCM revealed that, even

at 30 MPa, the ratio of injured cell populations increased with emulsifier combination, although it remained below 8% of the total bacterial population (1,224 of 15,000 events). At 50 MPa and 70 MPa, a marked increase in the ratio of injured bacterial populations was observed regardless of emulsifier combination. These results suggest that the injury characteristics of bacterial spores were markedly influenced at 30–50 MPa.

Significance: Bacterial spores were more effectively injured by emulsifier-combined treatment than by HPP alone. Notably, spore injury was enhanced at 30–50 MPa, suggesting that this pressure range may influence mechanisms weakening spore-heat resistance.

Award Application: Undergraduate Student Award

P3-059: REMOVAL AND SURVIVAL OF ESCHERICHIA COLI TVS 353 AND LISTERIA INNOCUA ON ROMAINE LETTUCE TREATED WITH HOME-BASED ULTRASOUND TECHNOLOGY

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Although there is research exploring the use of commercial ultrasound (US) to inactivate microorganisms on produce, the effectiveness of home-based US available for consumers is unknown.

Purpose: To determine survival and recovery of *Escherichia coli* TVS 353 and *Listeria innocua* on romaine lettuce treated with home-based US technology.

Methods: Romaine lettuce leaves were dip inoculated with rifampicin-resistant strains of *E. coli* TVS 353 and *L. innocua,* and air-dried for 2 hr. A two-way factorial design of treatment type (US, water bath) and time (5, 10, 15 min) was used in a randomized complete block design, yielding six treatment combinations per replicate, with a total of three replicates. Following treatment, 10g samples of lettuce leaves were diluted in buffered peptone water

and spread plated onto tryptic soy agar with 80 ppm and 100 ppm rifampicin for *E. coli* TVS 353 and *L. innocua,* respectively. Plates were incubated at 37°C and enumerated after 24 hr. Color measurements (L*, a*, b*) of uninoculated lettuce leaves were also taken. Data were analyzed using a mixed-model ANOVA, with pairwise comparisons made at alpha = 0.05.

Results: For *E. coli* TVS 353, no significant main effects or their interaction were observed, (p>0.05). Overall, the mean reduction of *E. coli* TVS 353 was 1.15 log ± 0.58 CFU/g. For *L. innocua*, a significant interaction between treatment type and time was observed (p<0.05). The smallest reduction of *L. innocua* was observed in samples treated with water bath for 5 min (0.35 log CFU/g), with all other treatment combinations yielding an average reduction of 0.58 log CFU/g. For L*, a*, and b* values, a significant main effect of treatment type was observed (p<0.05). US provided a mean of 47.7 ±1.57, -14.3 ±0.40, and 20.3 ±0.91 compared to water for L*, a* and b* values, respectively.

Significance: These findings will inform consumer food safety education on the effectiveness of home-based U.S. for safely cleaning produce.

P3-060: REDUCTION OF AFLATOXIN IN PEANUT KERNELS USING PULSED LIGHT

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Peanuts (*Arachis hypogaea)* are consumed globally but are susceptible to *Aspergillus* contamination and aflatoxin production. Aflatoxins are heat stable and resistant to thermal degradation, limiting the effectiveness of conventional thermal decontamination.

Purpose: This study evaluated the effectiveness of pulsed light (PL) in reducing aflatoxins in peanut kernels.

Methods: Peanut kernels were adjusted to 13% moisture content and inoculated with an *Aspergillus flavus* NRRL 3357 spore suspension prepared to 1.96 x 10^6 spores/mL. Peanut kernels (500 g) were inoculated with 10 mL of the suspension and incubated at 28°C for 14 days to induce aflatoxin production. Inoculated peanuts were treated in the sterilizing chamber of a Z-1000 PL generator at lamp distances of 5, 7, and 10 cm for 60 and 90 s using broad-spectrum (200–1100 nm) high-intensity pulses. Aflatoxin levels before and after treatment were measured in triplicate and analyzed by one-way ANOVA followed by Tukey’s

test ($p < 0.05$).

Results: The PL treatment significantly reduced aflatoxin concentrations in peanut kernels. The highest reduction (86.44%) was observed at 5 cm for 60 s, followed by 85.59% at 7 cm for 90 s and 84.81% at 5 cm for 90 s, respectively. Lower reductions were observed at 10 cm, with 76.78% and 41.19% at 90 s and 60 s, respectively. There was no statistically significant difference in reduction at 5 and 7 cm for both treatment times. However, at 10 cm, 90 s showed a significantly higher toxin reduction ($p < 0.05$), indicating strong effects of both distance and exposure time on treatment efficacy. The findings indicate that PL effectively degrades aflatoxins in peanut kernels.

Significance: This study establishes pulsed light as a promising nonthermal intervention for aflatoxin reduction in peanut kernels thereby improving postharvest mycotoxin control strategies.

P3-061: INACTIVATION OF SALMONELLA ON PECAN KERNELS USING LACTIC ACID

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: The high fat content and low-water activity of pecan kernels facilitate survival of foodborne pathogens, while conventional decontamination methods may negatively affect quality. Consequently, clean-label interventions such as lactic acid are of increasing interest.

Purpose: The objective of this study was to evaluate the effect of lactic acid concentration and exposure time on the inactivation of *Salmonella* on pecan kernels.

Methods: Five pecan kernels (~15 g) were individually spot-inoculated with 10 µL of a mixed culture of 80 µg/mL rifampicin-resistant *Salmonella* cocktail (Enteritidis PT9C, Montevideo, Saint Paul, and Newport) at ~6 $\log_{10}$ CFU/g and air-drying for 45 min. Kernels were spot treated with 12 µL of 0.5% or 1% (v/v) lactic acid for 0, 1, 5, 10, and 20 min, homogenized in 20 mL sterile peptone water, serially diluted, plated on 80 µg/mL rifampicin supplemented TSA, and incubated. Experiments were conducted using five kernels per replicate with six

replications (n=6). Results were analyzed using a GLM and means were generated using LSM and separated with the Tukey-Kramer Test (p□0.05).

Results: Reductions at 1% lactic acid, increased from 1.36 ± 0.15 $\log_{10}$ CFU/g at 1 min to 2.37 ± 0.19 $\log_{10}$ CFU/g at 5 min and to 2.88 $\log_{10}$ ± 0.19 CFU/g at 20 min, indicating rapid inactivation within the first 5 min. In contrast, 0.5% lactic acid showed the highest reduction at 10 min of 2.25 ± 0.09 $\log_{10}$ CFU/g, while reduction at 1, 5, and 20 min treatments were not significantly different. Overall, lactic acid concentration had a stronger effect on *Salmonella* reduction than exposure time. Pecan kernel showed no noticeable color changes visually.

Significant: Lactic acid effectively reduces *Salmonella* on pecan kernels, providing a simple, low-cost, food-grade intervention for enhancing microbial safety in commercial processing. Further research is needed to assess its effect on pecan kernel quality.

P3-062: PHASE-DEPENDENT INACTIVATION OF MICROBIAL SURROGATES BY HYDROXYL-RADICAL TREATMENTS GENERATED USING UV-C, HYDROGEN PEROXIDE, AND OZONE

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Gas-phase hydroxyl-radical treatments generated using UV-C, hydrogen peroxide, and ozone are emerging non-thermal decontamination technologies for foods. However, limited comparative data exists on microbial resistance to these treatments.

Purpose: To determine microbial sensitivities to hydroxyl-radical treatments and identify surrogate organisms for process validation.

Methods: *Escherichia coli* K-12, *Lactobacillus fructivorans*, *Enterococcus faecalis*, bacteriophages MS2 and Phi6, and *Bacillus subtilis* and *Geobacillus* endospores were evaluated in aqueous and gas-phase systems. Aqueous treatments used a UV-C collimated-beam reactor with ozone and hydrogen peroxide. Gas-phase treatments involved inoculated glass slides exposed to ozonated hydrogen-peroxide mist with UV-C. Survivors were enumerated to determine D-values.

Results: In the aqueous phase, MS2 and Phi6 were most sensitive (D 5.2 s), followed by *L. fructivorans* (28 s) and endospores (30–82 s), while *E. faecalis* showed minimal inactivation (D > 440 s). In the gas phase, a similar resistance pattern was observed, with MS2 and Phi6 again showing the fastest inactivation (D 4.9 s), followed by endospores (12–28 s) and vegetative bacteria (*E. coli* 35 s; *Lactobacillus* 51 s). Notably, *E. faecalis* showed a strong phase-dependent shift, with its D-value decreasing from >440 s to 4.98 s.

Significance: Gas-phase hydroxyl-radical treatments enhance microbial inactivation compared with aqueous systems while maintaining a similar resistance hierarchy. Identification of resistant microbial surrogates supports validation of hydroxyl-radical decontamination processes for food and surface sanitation.

Award Application: Developing Scientist Award

P3-063: PILOT-SCALE EVALUATION OF AN OZONATED WATER GENERATION SYSTEM USING A STACKED WIRE DIELECTRIC BARRIER DISCHARGE (SWDBD) REACTOR FOR MICROBIAL INACTIVATION AND ENERGY EFFICIENCY IN FRESH PRODUCE WASHING

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Current ozonated water (OW) generation technologies are primarily laboratory-scale and energy-intensive, limiting their practical application. This study addresses the need for pilot-scale, energy-efficient OW systems for fresh produce washing.

Purpose: To evaluate the system performance and antimicrobial efficacy of OW generated by a pilot-scale stacked-wire dielectric barrier discharge (SWDBD) system for washing baby lettuce and cherry tomatoes.

Methods: OW was generated using a pilot-scale SWDBD system, dissolving ~10 ppm ozone into a 2.5 gal/min water stream per batch. A commercial ozone generator producing comparable ozone concentration and flow rate was used for comparison. Deionized water (DI) served as a negative control, and 60 ppm chlorine (CL) served as a conventional sanitizer. Baby lettuce and cherry tomatoes were surface-inoculated with *E. coli* or *Listeria innocua* (>6 log CFU/produce), air-dried, and washed with 10 mL of each solution under orbital shaking (200 rpm, 10 min). Microbial populations on produce and in wash water were enumerated by

surface plating. All experiments were conducted in triplicate.

Results: Pilot-scale SWDBD OW achieved bacterial reductions comparable to CL and commercial OW ($P > 0.05$), while consuming significantly less energy than the commercial ozone system ($P < 0.05$). All treatments resulted in >3 log reductions on cherry tomatoes and ~2 log reductions on baby lettuce. No bacteria were detected in CL post-wash water, while OW and commercial OW post-wash water contained <3.5 log CFU/mL. The SWDBD system also offers improved scalability and a compact footprint compared to conventional ozone generators.

Significance: The pilot-scale SWDBD-based OW system demonstrated effective microbial inactivation with enhanced energy efficiency, supporting its potential for scalable and sustainable fresh produce washing applications.

P3-064: PLASMA-ACTIVATED MEDIA: A NOVEL APPROACH TO MICROBIAL INACTIVATION IN CULTIVATED MEAT USING COLD ATMOSPHERIC PLASMA

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Microbial contamination is a major challenge in cultivated meat production, reducing productivity and quality while increasing reliance on antibiotics. Cold atmospheric plasma (CAP) offers an antibiotic-free alternative, but its effectiveness in complex cell culture media has not been studied.

Purpose: To determine factors (gas composition and flow rate, media composition) that impact on the ability of CAP to inactivate *Escherichia coli* in cell culture media.

Methods: *E. coli* ATCC 25922 (~7 $\log_{10}$ CFU·mL^{-1}) was suspended in 200 mL of either DI water or DMEM/F-12 basal Culture Medium (CM) and treated with CAP generated using Leap100-liquid frit reactor. CAP was applied at 120 V, 1500 Hz for 10–300 s (water) and 30–3600 s (CM). The effect of feed gas composition (air, O_2, N_2, and 1:1 and 3:1 O_2: N_2 mixtures), gas flow rate (0.1–3 L·min^{-1}) and exposure time on bacterial survival was determined. Survivors were

quantified with $\log_{10}$ reductions compared to untreated controls. All experiments were performed in triplicate.

Results: CAP treatment achieved rapid bacterial inactivation in DI water: ~6 $\log_{10}$ CFU/mL reduction within 30 s; no (< 1.70 CFU/mL) survivors were detected after 60 s ($p < 0.001$). Inactivation was significantly reduced in CM ($p < 0.001$), with a maximum reduction of 3.12 $\log_{10}$ CFU/mL at 900 s. In CM, cell death was strongly influenced by feed gas composition and flow rate ($p < 0.001$). Oxygen and air achieved significantly higher reductions (3.81 and 3.12 $\log_{10}$ CFU/mL respectively) than nitrogen (0.08 $\log_{10}$ CFU/mL). A flow rate of 1.0 $L \cdot min^{-1}$ achieved maximum inactivation (3.70 $\log_{10}$ CFU/mL), beyond which efficacy declined, indicating plasma saturation.

Significance: The study identified oxygen-rich feed gas and gas flow rate as key parameters in maximizing bactericidal efficacy of CAP in complex culture media, providing a foundation for scalable, antibiotic-free application of CAP to ensure microbial safety in cultivated meat bioprocessing.

Award Application: Developing Scientist Award

P3-065: SYNERGISTIC INACTIVATION OF SALMONELLA TYPHIMURIUM BY PULSED LIGHT AND PERACETIC ACID

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: *Salmonella* contamination in raw foods remains a major food safety and public health concern, highlighting the need for effective nonthermal interventions that reduce microbial risks while preserving product quality.

Purpose: This study evaluated the effectiveness and potential synergy of pulsed light (PL) combined with peracetic acid (PAA) or hydrochloric acid (HCl) for inactivating *Salmonella* Typhimurium on agar surfaces as a model system for future application in food matrices.

Methods: Acidified agar surfaces (6 cm diameter) were prepared with PAA or HCl and adjusted to similar pH values (5.08–7.02). Samples were inoculated with *S.* Typhimurium (~7.0 log CFU/sample) and exposed to acids for 0, 30, or 60 min. PL was applied at 2000 V (1 pulse; 75 or 150 µs) either before or after acid exposure. Control treatments included acid-only and PL-only conditions. Surviving populations were enumerated by plate counting. Inactivation kinetics were modeled using the Weibull equation, and treatment interactions were assessed using a synergy index (*S*).

Results: PAA alone produced pH-dependent reductions of *S.* Typhimurium, with Weibull

models providing good fits ($R^2 > 0.86$). Combined PAA+PL treatments resulted in significantly greater inactivation than individual treatments ($p < 0.05$). Organic acid treatments achieved higher reductions than inorganic HCl both alone and combined with PL ($p < 0.05$). Synergistic interactions were consistently observed for PAA+PL combinations ($S > 1.0$).

Significance: The combination of PL and PAA represents a promising nonthermal hurdle strategy for controlling *Salmonella* on food contact surfaces, supporting the development of effective and energy-efficient decontamination approaches for food safety.

Award Application: Developing Scientist Award

P3-066: SYNERGISTIC EFFECTS OF ATMOSPHERIC COLD PLASMA AND CHLORINE ON PATHOGEN REDUCTION IN SPROUT SEEDS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Sprouts are rich in health-promoting bioactive compounds, making them highly popular among consumers. However, sprouts have also been associated with numerous foodborne illnesses outbreaks. Traceback investigations have identified contaminated seeds as the primary source of pathogens. Therefore, there is a growing need for effective decontamination strategies for sprout seeds.

Purpose: This study aims to evaluate the efficacy of atmospheric cold plasma (ACP), alone or in combination with chlorine, in inactivating Shiga toxin-producing *E. coli* on alfalfa and red clover sprout seeds, as well as to assess the impact of these treatments on seed vitality.

Methods: Alfalfa and red clover seeds inoculated with a cocktail (10^8 CFU/ml) of *E. coli* O157:H7 were treated with ACP, chlorine, or a combination of both, and the pathogen inactivation data were collected. For the seed vitality study, non-inoculated seeds were treated under the same conditions, and germination rates were collected. RO water as a

control. Inactivation and vitality data were processed using analysis of variance (ANOVA) in SAS to compare mean pathogen reductions and to evaluate treatment effects on seed vitality.

Results: The combined ACP and 200 ppm chlorine treatment exhibited a synergistic effect, resulting in significantly greater pathogen reduction for alfalfa (4.9 log CFU/ml) and red clover (4.84 log CFU/ml) compared with ACP alone (2.06 and 1.89 log CFU/ml) or 200 ppm chlorine alone (2.14 and 1.92 log CFU/ml) for alfalfa and red clover, respectively). All treatments had no significant effect on the vitality of either seed type.

Significance: This study demonstrates that ACP, particularly when combined with low-level chlorine, is a highly effective non-thermal decontamination strategy that does not compromise seed vitality. This approach represents a promising alternative to high-concentration chemical treatments, offering enhanced microbial safety while preserving germination potential.

Award Application: Developing Scientist Award

P3-067: ANTIMICROBIAL EFFICACY OF ULTRAVIOLET-C LIGHT (UV-C) ON LISTERIA INNOCUA AND ESCHERICHIA COLI IN COLD BREW COFFEE USING A NOVEL CONTINUOUS FLOW UV-C LIGHT REACTOR

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: UV-C light (254 nm) is a non-thermal germicidal processing technology that could be used to treat cold brew coffee, which minimizes the microbial risk without heat application.

Purpose: Develop a continuous flow UV-C light treatment device to attain sufficient microbial inactivation in cold brew coffee.

Methods: Cold brew coffee was prepared by steeping ground coffee in deionized water (75 g/L) for 18 h at room temperature. A UV-C light treatment prototype was developed for treating 30 ml of cold brew coffee at 500 ml/min. 1 mL of 20-hour bacterial culture (*Listeria innocua* or *Escherichia coli*) was inoculated in 99 mL of cold brew coffee. 30 mL of the inoculated cold brew coffee was continuously treated with UV-C light, with sampling at 1, 2, 3, 4, 5, and 10 minutes for bacterial enumeration by surface plating. A control was run through the UV-C light treatment prototype without the use of the UV-C lamp. The

experiments were performed in triplicate.

Results: The bacterial population levels of *Listeria innocua* at 0, 1, 2, 3, 4, 5 and 10 min were 6.3±0.3, 4.4±0.1, 1.7±0.3, 1.3±0.6, 1.7±0.4, 1.2±0.1 and 0.7±0.6, respectively, while the population levels of *Escherichia coli* were 7.1±0.0, 3.2±0.3, 2.2±0.2, 1.8±0.4, 1.8±0.1, 1.6±0.3 and 0.7±0.7 respectively. For *Listeria innocua*, there was 4.5±0.5 and 5.02±0.2 log reduction after 2 and 5 minutes of UV-C treatment ($p>0.05$), while for *Escherichia coli,* there was a significant decrease of 4.9±0.1 and 5.5±0.3 log reduction after 2 and 5 min of UV-C treatment ($p<0.05$). In both experiments, after 10 minutes of UV-C treatment, the microbial populations were below the limit of detection (1 log CFU/mL) with *Listeria innocua* and *Escherichia coli* decreasing by 5.5±0.6 and 6.4±0.7 log, respectively.

Significance: The use of UV-C light could provide a potential non-thermal kill step, which would eliminate the microbial risk in cold brew coffee at low temperatures (<30℃).

Award Application: Developing Scientist Award

P3-068: VALIDATING EFFICACY OF HIGH-PRESSURE PROCESSING FOR INACTIVATION OF LISTERIA MONOCYTOGENES AND NONTYPHOIDAL SALMONELLA IN PRESENCE OF CARVACROL

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: To ensure safety of pressure-treated commodities, validation studies are required for Food Safety and HACCP plans, typically to show 5-log reduction of the most relevant pathogen(s) of public health concern associated with a product.

Purpose: The objective of the current study was to validate the performance of this technology to ensure a 5-log reduction of *Listeria monocytogenes* and nontyphoidal *Salmonella* serovars in industrial settings in the context of multiple hurdle technology for liquid and solid products.

Methods: Both liquid and solid products were inoculated with a cocktail of the above-mentioned pathogens and were exposed to elevated hydrostatic pressure of 85K PSI (c. 586.1 MPa) in the presence and absence of 0.5% carvacrol. Studies were randomized complete block designs with two blocks (i.e., two biologically independent repetitions). Each block consisted of three replications and two microbiological repetitions. The log-transformed data were statistically analyzed using Proc GLM of SAS by conducting Tukey-adjusted ANOVA at type I error level of 5%.

Results: The treatment at 85K PSI for 3 minutes with come-up time of 240 seconds at 5°C was able to achieve 5 log CFU/mL and 5 log CFU/g reduction in the tested liquid and solid foods, respectively, for both pathogens. While the treatment with and without the carvacrol was efficacious for the 5-log reduction ($P < 0.05$) of both pathogens, application of carvacrol provided additional benefits for reducing the counts of background microbiota and protecting the products during shelf life due to the residual effect.

Significance: Our results illustrate that stakeholders of high-pressure processing could benefit from the synergism of pressure-based treatments with bioactive compounds in the context of multiple hurdle technology to ensure safety and quality of pressure-treated commodities.

Award Application: Developing Scientist Award

P3-069: ENHANCED INACTIVATION OF ESCHERICHIA COLI O157:H7 IN POST-HARVEST LETTUCE USING NONTHERMAL HURDLES OF COLD PLASMA AND PULSED LIGHT

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Contamination of produce like lettuce continue to be a major concern. Existing chlorine-based antimicrobial decontamination method has only limited efficacy. Safe and effective method is warranted.

Purpose: Objective of this work was to evaluate the effectiveness of cold plasma (CP), pulsed light (PL) and their combinations for decontamination of *E. coli* O157:H7 in Romaine lettuce.

Methods: A bacterial cocktail containing three outbreaks strains of *Escherichia coli* O157:H7 was prepared and was used to spot inoculate the lettuce to a level of about 6.2 log CFU/g. Experiments with PL and CP were conducted for 60 s to evaluate optimum inactivation condition which was utilized for hurdle combination. Homogenates of lettuce sample were prepared by stomaching in peptone water. Pathogen populations were quantified by plating 100 µL of homogenate dilutions on SMAC selective medium. For populations below detection limit, pathogen absence in sample was confirmed by enrichment methods. Experiments were conducted in triplicates. Data were analyzed with ANOVA to assess significance of treatment using statistical analysis system (SAS Institute, Cary, NC).

Results: Experimental results indicated 30 s of PL and 45 s of CP treatments as optimum for inactivation of *E. coli* in lettuce. PL (30 s) and CP (45 s) treatments individually, inactivated

2.7±0.65 and 2.1±0.43 logs of *E. coli,* respectively. Significant (p<0.05) synergistic inactivation was achieved by the combination treatments as no *E. coli* cells were detected (detection limit, 1 log) after combination treatments, indicating > 5 log reductions of the pathogen on Romaine lettuce.

Significance: These results indicate that PL and CP treatments combination is an excellent decontamination strategy which can ensure the microbial safety of Romaine lettuce.

P3-070: ENZYME INACTIVATION AS A QUANTITATIVE SURROGATE FOR PREDICTING MICROBIAL REDUCTION AT DIFFERENT DOSAGES OF PLASMA-ACTIVATED WATER

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Plasma-activated water (PAW) is a promising sanitizer generated by exposing water to plasma discharge. However, the variation in PAW reactive species across varying operating conditions and equipment highlights the need for reliable process-validation methods. Oxidative reactive species inactivate specific enzymes, consistent with mechanisms related with microbial reduction using PAW. Therefore, using an enzyme-based surrogate could enable rapid estimation of PAW efficacy.

Purpose: This study aimed to validate a catalase-based surrogate to estimate PAW antimicrobial efficacy using a rapid spectrophotometric method.

Methods: PAW was generated using an atmospheric plasma jet (22.5 kHz, 295 V) for 1–15 minutes at two discharge gaps (5 cm and 5 mm). Catalase inactivation was measured using a kinetic spectrophotometric assay, and microbial reduction (*E. coli* and *L. innocua*) by TSA spread plating (log CFU/mL). Physicochemical parameters (pH, EC, ORP, nitrate, nitrite) were

also measured. Finally, statistical models were used to correlate catalase inactivation with microbial reduction.

Results: Catalase inactivation increased linearly with PAW exposure at both 5 cm (R^2 = 0.864) and 5 mm (R^2 = 0.96) gaps. *E. coli* showed a non-linear correlation with catalase inactivation, following an exponential trend at 5 cm (R^2 = 0.951) and logarithmic curve at 5 mm (R^2 = 0.893). *L. innocua* showed a linear correlation with catalase inactivation at 5 cm (R^2 = 0.923) and at 5 mm (R^2 = 0.946), respectively. Plasma treatment produced significant physicochemical changes in PAW; at 15 min (5 cm vs. 5 mm gaps), pH dropped to 2.55 and 1.7, EC increased to 646 and 4052 µS/cm, and ORP rose to 893 and 789 mV, nitrate concentrations reached 3.22 and 15.51 mM, and nitrite measured 2.94 and 0.80 mM, respectively.

Significance: The findings show that catalase inactivation can predict microbial reduction, enabling fast and standardized validation of PAW treatment efficacy.

Award Application: Developing Scientist Award

P3-071: EVALUATING THE SAFETY AND THERMAL PROCESS OF HOME-CANNED SEAFOOD STOCK

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Seafood stock is a popular ingredient prepared by home food preservers, yet no validated canning recipe currently exists in validated resources such as the *USDA Complete Guide to Home Canning* or the National Center for Home Food Preservation.

Purpose: The objective of this study was to validate a seafood stock recipe for home canning.

Methods: Seafood stock was prepared with salmon and farmed catfish heads and frames,

and packed into quart-size jars for process validation. Cold spot testing was conducted to determine the slowest heating zone. Temperatures were monitored at six locations in the bottom half of six jars across five canner runs at 11 PSI. Heat penetration (HP) studies were conducted using closed jars processed in 17- and 22-quart canners, with temperatures recorded every 10 seconds during the come-up, processing, and cooling phases over nine total runs. Further analyses were done to determine viscosity and fill temperature. Analysis of variance was used to determine if significant differences existed between the treatments (viscosity and temperature at filling) using the Linear Mixed-Effects Model procedure in R v4.5.2.

Results: Analyses of f(h) values showed the slowest heating zone of the quart jar was located at 5 ⅞ inches below the rim of the jar. Subsequent HP runs with thermocouples at the cold spot showed that the viscosity and temperature at the filling main effects and their interaction did not significantly impact F_0=6 during processing. Mean viscosity (mPa·s) was 12.5 ± 0.45 (heads) and 12.0 ± 0.63 (frames) for salmon, and 13.0 ± 0.69 (heads) and 13.0 ± 0.30 (frames) for farmed catfish. During thermal processing, a mean F_0 value of 12.5 ± 0.67 min was achieved, with the total F_0, including cooling, reaching 23.31 ± 0.38 min.

Implications: These findings establish a validated pressure canner process for home-canned seafood stock, addressing a gap in current guidance for this type of product.

P3-072: EFFECT OF FAT CONTENT ON HIGH-PRESSURE PROCESSING INACTIVATION OF LISTERIA INNOCUA AND LISTERIA MONOCYTOGENES IN PORK MEAT EMULSIONS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: High-pressure processing (HPP) is widely used as a post-lethality intervention to control *Listeria* spp. in ready-to-eat meat products. However, its efficacy depends not only on processing parameters but also on food matrix characteristics, such as fat content.

Purpose: This study evaluated the effects of pressure and fat content on microbial inactivation of *Listeria innocua* Seeliger SLCC 3379 (ATCC® 33090™) and *Listeria monocytogenes* ATCC® 19111™ in pork meat emulsions.

Methods: A full factorial experiment was conducted using pork meat emulsions formulated with 5, 15, or 25% fat. Samples were surface-inoculated with either *L. innocua* or *L. monocytogenes* to approximately 10^8 CFU/g, vacuum-sealed, refrigerated for 48 h, and treated at 0, 450, and 600 MPa for 3 min at 4°C. Microbial populations were quantified by direct plate counts on modified Oxford agar topped with tryptic soy agar following

incubation at 32°C for 48 h.

Results: Pressure level and fat content significantly affected microbial inactivation, with a significant interaction between fat and pressure ($p < 0.05$). At 600 MPa, reductions exceeding 5 log CFU/g were achieved, with no significant differences among fat levels. In contrast, at 450 MPa, fat content significantly influenced inactivation. For both microorganisms, the lowest reductions occurred at 15% fat. Reductions for *L. monocytogenes* (log CFU/g) decreased from 2.63 ± 0.26 at 5% fat to 1.95 ± 0.22 at 15% fat, before increasing to 2.77 ± 0.52 at 25% fat. Similarly, *Listeria innocua* reductions were 2.55 ± 0.66, 1.98 ± 0.06, and 2.39 ± 0.44 log CFU/g at 5%, 15%, and 25% fat, respectively, with no significant differences between microorganisms ($p > 0.05$).

Significance: These results demonstrate that fat content modulates HPP efficacy in meat emulsions in a pressure-dependent manner. Comparable inactivation of both microorganisms suggests that *L. innocua* ATCC® 33090™ may be used as a potential surrogate for HPP validation studies.

Award Application: Developing Scientist Award

P3-073: EFFECT OF FREEZE DRYING ON ENTEROCOCCUS FAECIUM, LISTERIA INNOCUA, AND E. COLI IN RAW SALMON

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Home freeze drying is becoming increasingly popular as consumers show interest in creating long-lasting, shelf-stable foods, and as pilot- and kitchen-scale freeze dryers have become more accessible and affordable. Despite its convenience, home freeze-dried foods carry potential risks, as contamination can occur at multiple steps in the process. Additionally, many home users lack the knowledge needed to ensure the safety of freeze-dried foods.

Purpose: To quantify the reduction of *Enterococcus faecium* NRRL B-2354, *Listeria innocua* and generic *Escherichia coli* in raw salmon by freeze-drying.

Methods: Raw diced salmon was inoculated with 6.5 log CFU/g of *Enterococcus faecium* NRRL B-2354 (surrogate for *Salmonella* spp.), *Listeria innocua* (surrogate for *Listeria monocytogene*s), and generic *Escherichia coli* and kept frozen at -20°C prior to freeze drying in a pilot-scale unit (Medium Scientific, Harvest Right, Salt Lake City, UT). The samples were dried using a programmed mode. The programmed mode maintained the shelf temperatures at or below 50 and 74°C. The ambient temperature inside the dryer was maintained at or below 22°C throughout the process. Three replications were conducted, and the following response

variables were compared: microbial log reductions, final water activity and moisture content values.

Results: Less than 5-log reductions were observed in all surrogates. Average log reductions were 0.57 ± 0.33 for *E. faecium*, 0.39 ± 0.31 for *L. innocua*, and 1.08 ± 0.72 for generic *E. coli*. Final water activity values were <0.1 with moisture contents <10%.

Significance: Freeze drying of raw salmon resulted in minimal bacterial reduction despite achieving low water activity. Home freeze-dried foods remain potentially hazardous unless additional validated lethality steps are applied.

P3-074: EVALUATION OF OZONE NANOBUBBLES FOR REDUCING LISTERIA ON FOOD-CONTACT SURFACES

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Surfaces and packaging materials in food processing environments can contribute to cross-contamination of *Listeria monocytogenes* and ozone nanobubbles offer a residue-free alternative antimicrobial approach for multiple surface types.

Purpose: This study evaluated the efficacy of ozone nanobubbles for reducing *L. innocua*, a surrogate for *L. monocytogenes*, on food-grade PVC conveyor belts and plastic food packaging.

Methods: Two laboratory studies were conducted. First, food-grade PVC conveyor belt sections were inoculated with a five-strain cocktail of *L. innocua* at high (~8 log CFU/cm^2) or low (~5 log CFU/cm^2) levels and treated with a 5 ppm ozone nanobubble spray for 30–90 s. Microbial recovery was performed using validated surface swabbing methods, and treatments were performed in triplicate (n=3). Second, 3-oz condiment packets were surface-inoculated with a four-strain *L. innocua* cocktail (~6 log CFU/packet), stored at 4°C for 18 h, and treated with a 5 ppm ozone nanobubble solution for 60 s via overhead spray or immersion. Treatments and controls were performed using 8 packets per trial and repeated in triplicate (n=24).

Results: A 30-s ozone nanobubble spray reduced *L. innocua* on PVC conveyor belts with low and high inoculum levels by 0.66 and 1.30 log CFU/cm^2, respectively. *Listeria* reductions significantly (p <0.05) increased to a maximum of 3.93 log CFU/cm^2 for high inoculum level belts after 60 s, while low inoculum belts achieved a maximum reduction of 1.45 log CFU/cm^2 after 90 s. On condiment packets, spray and immersion treatments resulted in mean reductions of 4.66 and 4.28 log CFU/packet, respectively. Spray treatments also resulted in a higher percentage of non-detectable samples (71%) than immersion (42%).

Significance: Ozone nanobubbles effectively reduced *Listeria* on food-contact surfaces and packaging. These results support its potential as a practical intervention to mitigate *Listeria* risks in food processing environments.

Award Application: Developing Scientist Award

P3-075: EVALUATION OF SMART WASH® AND THE ADDITION OF A SILVER ION PRETREATMENT, SMARTWASH® BOOST, TO MITIGATE E. COLI O157:H7 IN FRESH-CUT ROMAINE LETTUCE

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Presentation type/Format: Technical

Category: Food Processing Technologies

Abstract:

Introduction: Fresh-cut lettuce processors are moving beyond the “do-no-harm” of cross-contamination control to controlling wash systems to reduce pathogens. Sanitizer performance is affected by organic load, product quality, and process variability. These factors can impact chlorine efficacy and complicate validation of preventive controls under processing conditions**.**

Purpose: *Escherichia coli* O157:H7 (ECH) on romaine lettuce during fresh-cut processing**.**

Methods: Four independent trials were conducted in a pilot scale facility. Romaine lettuce heads (n = 25 per tote) were inoculated with an ECH cocktail (□5 log CFU/sample) and processed including coring, outer-leaf removal, and shredding. Three treatments were evaluated per trial: SW, SWB, and a water-only control. For each treatment, four 1.5-kg bags of shredded lettuce were collected and subdivided into five 150-g analytical units. ECH was

recovered by centrifugation, resuspension, and plating, with parallel enrichment to confirm viability. Data were analyzed by analysis of variance with mean separation at α = 0.05.

Results: Treatment significantly affected ECH recovery ($p < 0.05$), the control was highest at 5.25 ± 0.02 log CFU/g (essentially no change), followed by SW at 4.70 ± 0.01 log CFU/g and lowest, SWB (4.18 ± 0.02 log CFU/g). SWB consistently achieved greater reductions than SW across all trials. Lettuce quality influenced sanitizer performance in two of four processing events.

Significance: These results indicate that SW and SWB provide mitigation compared to water alone. Chlorine efficacy was variable, with SWB with silver ions providing more robust and consistent reductions. This study supports the use of silver ion enhanced wash systems as part of the preventive controls for human food under FSMA and buyer-driven food safety programs.

P3-076: EVALUATION OF THE INJURY CHARACTERISTICS OF BACTERIAL SPORES WITH HIGH HYDROSTATIC PRESSURE PROCESSING BY NEAR INFRARED SPECTROSCOPY

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: High pressure processing (HPP) can reduce the heat resistance of bacterial spores, but it is difficult to evaluate the polymers that are produced inside and outside the spores.

Purpose: To evaluate the changes in the characteristics of bacterial spores caused by damage due to high-pressure treatment based on spectral characteristics obtained by near-infrared (NIR) spectroscopy.

Methods: *Bacillus spizizenii* NBRC3134 was used as the test strain. Spores were formed and then adjusted to an absorbance of 1.0 at 600 nm to prepare a spore suspension. The spore pellet obtained by centrifugation was subjected to repeated stirring and centrifugation in a 1% surfactant solution (Tween 20 and 80), followed by HPP (50 MPa, 1 hr, 30 or 50°C) in order to reduce their heat resistance. Near-infrared absorption spectra of the suspension were obtained using a FT-NIR spectrometer. Growth curves were also obtained by continuously monitoring the viable cell count by TPC and the absorbance at 600 nm upon recovery in TSB.

Results: The heat resistance of spores was significantly reduced by the combined use of HPP and Tween 20, but the combined use of Tween 80 had almost no effect. Growth curves also showed a significant prolongation of the lag phase. Tween 80 also showed an increase in the lag phase, suggesting that HPP affects growth activity by causing surfactants to adhere to the spore surface or penetrate into the spore. Principal component analysis of the NIR spectra of treated samples revealed differences among untreated, heated, and HPP treated samples.

Significance: Near-infrared spectroscopy could be suggested to characterize the reduction in heat resistance caused by HPP-induced injury.

P3-077: PROCESS PARAMETER OPTIMIZATION AND MODELING OF PLASMA-ACTIVATED WATER FOR MICROBIAL INACTIVATION

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Cold plasma inactivates pathogens via Reactive Oxygen and Nitrogen Species (RONS); however, efficacy varies with environmental factors and equipment parameters. To validate the process, it is essential to quantify inactivation levels independent of equipment variability and establish non-bacterial surrogates.

Purpose: To quantify and optimize the operational parameters of a plasma jet for Plasma Activated Water (PAW) and develop a predictive model for microbial inactivation.

Methods: A Definitive Screening Design (DSD) in JMP initially evaluated five parameters: voltage (280–300 V), frequency (19–22.5 kHz), cycle time (50–100%), generation time (2–10 min), and contact time (1–5 min). With the significant parameters, a full factorial design RSM model was developed.

Results: The DSD indicated that frequency, voltage, and cycle time had negligible effects (p>0.05) on PAW efficacy. Hence, Generation Time (G Time) and Contact Time (C Time) were chosen for further experiments. A full factorial design (n=75) was used to optimize significant

parameters. The optimized model using G Time and C Time demonstrated a good fit (Predicted RMSE=0.316 RSq=0.94 p=< 0.0001) with an insignificant lack-of-fit (p=0.1756). Reductions increased significantly (p<0.05) with G and C times, ranging from 0.23±0.07 log CFU/ml (2 min G, 1 min C) to 5.21±0.7 log CFU/ml (10 min G, 5 min C).

Significance: This study provides a statistically validated model for PAW generation for antimicrobial purposes, identifying that process efficacy is driven by time rather than voltage or frequency. This simplifies the critical process parameters required for food safety optimization.

Award Application: Developing Scientist Award

P3-078: WITHDRAWN

WITHDRAWN

P3-079: Q-SWITCHED UVA LASER IRRADIATION AS A CHEMICAL-FREE STRATEGY FOR SURFACE INACTIVATION OF SALMONELLA ENTERITIDIS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Microbial surface contamination poses a persistent threat to food safety; this necessitates the development of rapid, residue-free decontamination technologies. Q-switched UV lasers, which combine high peak pulse energy with germicidal UV effects, offer a sustainable and high-precision alternative for surface sanitation.

Purpose: This study evaluated the efficacy of a 5-watt Q-switched UVA (355 nm) laser for the inactivation of *Salmonella* Enteritidis PT30 on glass surfaces.

Methods: Laser parameters, including Spot diameter, defocus distance (0–22 cm), and scanning duration, were varied to optimize inactivation. After simulations ensured full coverage, *Salmonella* inactivation on 1 cm^2 was assessed using 65–142 s scans and SD ratios (0.71–0.044) at 4–10 cm defocus distances. Survival was measured via serial dilution and plate counting.

Results: The UV laser treatment achieved strong inactivation efficacy (>6 log reduction) following log-linear kinetics relative to treatment time or energy. The D-values (decimal reduction dose) were significantly governed by defocusing distance and SD ratio ($p < 0.05$). While beam defocusing increased D-values, this effect was mitigated by reducing the SD ratio to increase overlap. Calculated D-values ranged from 18.9 to 100.8 J/cm^2. While these values

are higher than those of typical UVC light, the laser demonstrated superior penetration through a thick culture layer. Results suggest that microbial inactivation results from the synergy of direct UV irradiation and accumulated thermal energy.

Significance: This work establishes a geometry-based method for point-to-point laser sanitation. For the first time, this study demonstrates the robust capability of Q-switched UVA lasers for disinfecting *Salmonella* on abiotic surfaces.

P3-080: FREEZING AND MICROWAVE HEATING AS CONTROL STRATEGIES FOR CAMPYLOBACTER SPP. IN READY-TO-EAT FROZEN CHICKEN FOODS

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: The increasing consumption of frozen, microwavable ready-to-eat (RTE) products, together with the high prevalence of *Campylobacter* spp. in chicken-derived foods, has driven interest in evaluating freezing and microwave heating as potential control strategies for this pathogen in frozen RTE chicken products.

Purpose: This study aimed to evaluate the effect of domestic freezing and microwave heating on the inactivation of *Campylobacter* spp. in selected frozen RTE chicken products.

Methods: Three commercially available frozen RTE products containing chicken (wontons and two nugget brands) were inoculated with a five-strain *Campylobacter* spp. cocktail to an initial level of approximately 5 log CFU/g. Samples were frozen overnight under household conditions and evaluated using a randomized block design with a 2×2 factorial arrangement, including two microwave power levels (high and medium) and two heating times (label-recommended time and double the recommended time), using a 1100 W microwave oven. Experiments were conducted in triplicate and data were statistically analyzed. *Campylobacter* spp. populations were enumerated on blood agar incubated at 42°C for 48 h.

Results: Freezing resulted in an average reduction of 3.0 ± 0.1 log CFU/g of *Campylobacter* spp. Microwave heating produced reductions ranging from 0.1 to 2.3 log CFU/g, which were significantly affected ($P < 0.05$) by treatment conditions. These results indicate that microwave heating under the evaluated domestic conditions is insufficient as a sole control measure for *Campylobacter* spp.

Significance: This study highlights the limitations of domestic freezing and microwave heating as standalone interventions for controlling *Campylobacter* spp. in frozen RTE chicken products and underscores the importance of upstream safety controls and cross-contamination prevention prior to consumer handling.

P3-081: SOME ENGINEERING AND PHYSICAL PROPERTIES OF PECANS FOR PULSED LIGHT STERILIZATION

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: Pulsed light (PL), a non-thermal processing method, offers an alternative to traditional food processing methods and has been used in reducing microbial loads in foods, including pecans. The development of PL system for effective microbial reduction in pecans is highly dependent on the determination of the engineering and physical properties of the nuts.

Purpose: This study determined the engineering and physical properties of pecans in designing PL systems for effective microbial reduction in the nuts.

Methods: The measurements of mass and dimensional properties (length, width, and

thickness) of pecans were taken. Other physical properties, such as arithmetic mean diameter, geometric mean diameter, sphericity, surface area, and volume, were calculated from the dimensional values. Pecans were treated with PL at distances of 7, 9.5, and 13.4 cm for 0– 60 s. Temperature measurement and cooling rate for all the distances and treatment times were evaluated after PL treatment with 20 pecan halves samples for each treatment. Significance was determined by ANOVA and Tukey's test (P□0.05).

Results: The results indicated the range values for mass, length, width, thickness, arithmetic mean diameter, geometric mean diameter, sphericity, surface area, and volume as 0.85– 4.19 g, 22.06–38.05 mm, 13.35–21.59 mm, 5.24–10.31 mm, 14.55–22.39 mm, 12.21–19.75 mm, 45.89–67.69%, 644.59–1525.84 mm^2, and 954.24–4033.87 mm^3, respectively. Temperature of closer distance to the light source and longer time indicated a higher temperature. Cooling rate revealed that longer treatment time and closer distance to the light source had a longer cooling time.

Significance: The result from the determination of engineering and physical properties contributes to the design of PL system specific to pecan processing, thereby effectively improving microbial reduction in the nuts. Pulsed light may be applied to pecans, but the treatment time and distance should be considered.

P3-082: DEVELOPMENT OF A CONTINUOUS COLD PLASMA SYSTEM AND ITS APPLICATION IN BLUEBERRY PRESERVATION

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: The fresh-food sector urgently requires an efficient, safe, non-thermal decontamination technology that can ensure both microbial safety and quality of packaged fresh products.

Purpose: To address this need, we developed a high-efficiency, continuous-operation cold plasma decontamination system and evaluated its antimicrobial performance and quality impacts.

Methods: Key reactor structural parameters, including dielectric thickness and electrode length, were systematically optimized using discharge power and discharge uniformity as primary performance criteria. The optimized design was integrated in a continuous-processing dielectric barrier discharge (DBD) cold plasma unit. Using blueberries as a representative high-value commodity, 120 g portions were surface-inoculated with *Escherichia coli* O157:H7 and *Listeria monocytogenes* to determine decontamination efficacy and to assess quality changes during refrigerated storage.

Results: The optimized system achieved stable, large-area discharges with enhanced uniformity. Treatment at 90 kV for 48 s resulted in a 3-log (99.9%) reduction of both pathogens on blueberry surfaces. After 7 d of refrigerated storage at 4°C, plasma-treated

samples exhibited significantly ($p < 0.05$) lower total aerobic counts and yeast/mold populations than the untreated control. Meanwhile, key quality attributes–including color, firmness, and antioxidant capacity–were better preserved than those of the control, demonstrating a favorable balance between microbial inactivation and quality retention.

Significance: This continuous, non-thermal plasma intervention offers a sustainable strategy to mitigate foodborne microbial risks and reduce spoilage-related losses in packaged fresh produce, thereby supporting both public health protection and waste reduction.

P3-083: SYNERGISM OF CARVACROL AND HIGH-PRESSURE PROCESSING FOR INACTIVATION OF SALMONELLA TYPHIMURIUM LT2

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Presentation type/Format: Poster

Category: Food Processing Technologies

Abstract:

Introduction: With recent improvements in the engineering of the commercially available high-pressure processing units, this technology is gaining increased adaptation across various sectors of the food industry. Nontyphoidal *Salmonella* serovars continue to be one of the leading causes of foodborne hospitalizations and deaths in the United States.

Purpose: The current study is a hurdle validation challenge study to investigate the synergism of elevated hydrostatic pressure and carvacrol against a novel strain of nontyphoidal *Salmonella.*

Methods: *Salmonella enterica* subsp. Typhimurium, strain designation LT2 (*Salmonella* LT2) was exposed to elevated hydrostatic pressure of 400 MPa with and without 3% carvacrol (v/v) in apple cider. The studies were complete randomized block design, with two biologically independent repetitions (i.e., two blocks), each consisting of three replications. After treatment time of up to 10 minutes, samples were neutralized using D/E neutralizing broth and were then spread-plated on to the surface of XLD and non-selective media. Treatments were conducted at 4°C in a chamber that was surrounded by a steel jacket, connected to a

circulating water bath.

Results: Pressure-based treatments alone at 400 MPa resulted in modest reductions (P<0.05) after 5 minutes of exposure of <1 log CFU/ml. The same treatment synergized with 3% carvacrol resulted in significant reductions of *Salmonella* Typhimurium LT2 after 1 minute, resulting in reductions (P<0.05) up to >5 log CFU/ml.

Significance: Our results, thus, indicate that stakeholders of high-pressure processing, after conducting proper validation studies, could benefit from the synergism of this bioactive compound and mild pressure to achieve reductions comparable to the use of pressure and intense levels. This could optimize the cost of the product and further protect the quality and organoleptic properties of pressure-treated commodities.

P3-084: CLEAN-LABEL PRESERVATION FOR MOLD CONTROL IN SEMI-MOIST PET TREATS UNDER ACCELERATED STORAGE CONDITIONS

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Mold contamination remains a persistent challenge in semi-moist pet treats due to their water activity and formulation limitations, with implications for product quality, safety, and consumer acceptance. Increasing humanization of pet food has driven interest in clean-label approaches to fungal control that maintain product integrity.

Purpose: This study aimed to assess mold control in semi-moist pet treats formulated with a clean-label preservation system under accelerated storage conditions.

Methods: Semi-moist pet treats were manufactured to a target moisture content of 23.0%, water activity (a_w) of 0.72, and pH 6.10. Six formulations were prepared, including an untreated control, potassium sorbate (0.15%), lactic acid (0.30%), and EverWild® D01 (natural flavor dried whey) applied at 0.50%, 1.00%, and 1.50%. Treats were inoculated with *Eurotium herbariorum*, *Penicillium roqueforti*, and *Aspergillus oryzae*, then stored under accelerated conditions (21°C, 84% relative humidity). Time to visible mold growth was recorded through

visual inspection. Treatment performance was compared using one-way ANOVA.

Results: Days to first visible mold growth in the negative control and lactic acid treatments occurred between day 25–29. The 1.50% EverWild® D01 treatment delayed the first sign of visible mold growth to 165, 155, and 171 days for *E. herbariorum, A. oryzae*, and *P. roqueforti*, respectively, significantly outperforming 0.15% potassium sorbate (126, 91, and 115 days; p 0.05). Relative to potassium sorbate, the 1.50% treatment extended mold-free duration by approximately 31%, 70%, and 49% for *E. herbariorum*, *A. oryzae*, and *P. roqueforti*, respectively.

Significance: The natural flavor dried whey significantly delayed mold growth in semi-moist pet treats under accelerated storage conditions, outperforming a conventional commercial preservative. These findings highlight the potential for clean-label antifungal strategies to improve product stability and shelf life.

P3-085: IMPROVING MICROBIAL SHELF LIFE OF HIGH-MOISTURE RAW PET FOODS USING MULTI-HURDLE PRESERVATION

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: High-moisture pet foods present food safety and shelf-life challenges due to elevated water activity and refrigerated storage conditions that favor microbial proliferation. Multi-hurdle preservation strategies integrating processing technologies with ingredient-based antimicrobial systems may enhance microbial stability in these products.

Purpose: This study aimed to determine the impact of natural antimicrobial ingredients combined with high pressure processing (HPP) on the refrigerated shelf life of high-moisture pet foods.

Methods: Raw pet food formulations included a no-antimicrobial control (T1) and a natural flavor-based antimicrobial treatment (T2: EverWild® 40L, 3.50%; EverWild® S100, 0.20%). Samples were vacuum-packaged and stored at 4°C throughout the study. Aerobic plate counts (APC), lactic acid bacteria (LAB), and psychrotrophs were enumerated over time by duplicate plating on tryptic soy agar (TSA), de Man, Rogosa and Sharpe (MRS) agar, and plate

count agar (PCA), respectively. A spoilage threshold of □6 log CFU/g was applied. Statistical analysis was performed using one-way ANOVA (JMP Pro v15.1.0; p □ 0.05).

Results: The no-antimicrobial control exceeded spoilage thresholds for APC, LAB, and psychrotrophs by approximately Day 45 (p □ 0.05), indicating limited refrigerated stability. In contrast, the natural flavor-based antimicrobial treatment in combination with HPP demonstrated significantly improved microbial control throughout storage. APC, LAB, and psychrotrophic populations remained well below spoilage thresholds for up to 120 days, averaging approximately 2.0, 4.25, and 2.48 log CFU/g, respectively (p □ 0.05).

Significance: These findings demonstrate that integrating natural flavor-based antimicrobial ingredients with HPP effectively enhances refrigerated microbial stability and extends shelf life in high-moisture pet foods.

P3-086: ANTILISTERIAL AND SHELF-LIFE EFFICACY OF VINEGAR-BASED INGREDIENTS IN COOKED REFRIGERATED PET FOOD

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Cooked refrigerated pet foods have high moisture and water activity, increasing susceptibility to microbial growth during refrigerated storage. *Listeria monocytogenes* remains a key food safety concern due to its ability to grow at refrigeration temperatures, highlighting the need for clean-label antimicrobial strategies that enhance safety and shelf life.

Purpose: To evaluate the impact of a vinegar-ferment solution on *Listeria monocytogenes* control and overall microbial stability in cooked pet food stored at 4°C.

Methods: An acidified commercial control and a cooked pet food formulation containing 3.0% vinegar-ferment ingredient (EverWild® [EW] 20L) were evaluated. Products were inoculated with *L. monocytogenes* at 2–3 log CFU/g, packaged under modified atmosphere conditions (70% N_2, 30% CO_2), and stored at 4°C. An uninoculated set was stored under identical conditions and monitored for aerobic plate counts (APC) and lactic acid bacteria

(LAB). Duplicate microbiological analyses were conducted throughout storage. *L. monocytogenes*, LAB, and APC were enumerated on Modified Oxford (MOX), de Man, Rogosa and Sharpe (MRS), and Tryptic Soy Agar (TSA), respectively. Antimicrobial failure was defined as □2 log CFU/g *Listeria* outgrowth, and spoilage as □6 log CFU/g. Data were analyzed using one-way ANOVA (JMP Pro v15.1.0; p □ 0.05).

Results: Acidified control exhibited a 2-log *Listeria* outgrowth by day 35 of refrigerated storage. In contrast, the vinegar-ferment treatment suppressed *Listeria* growth for up to 161 days at 4°C. The vinegar-ferment treatment significantly delayed LAB and APC growth (p □ 0.05), extending refrigerated shelf life to 161 days, whereas control reached spoilage thresholds by day 119, representing an approximate 35% shelf-life extension.

Significance: The findings show that vinegar-ferment ingredients offer effective clean-label protection against *Listeria* while prolonging shelf life.

P3-087: EXTENDING MOLD-FREE SHELF LIFE IN SEMI-MOIST PET TREATS AT ELEVATED WATER ACTIVITY

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Higher water activity enhances softness and palatability in semi-moist pet treats but increases susceptibility to mold growth, making effective antifungal strategies essential for maintaining shelf life at elevated moisture levels.

Purpose: To evaluate mold control strategies that allow increased a_w and moisture content in semi-moist pet treats without compromising microbial quality under accelerated storage conditions.

Methods: Semi-moist meat treats were manufactured with target a_w values of 0.70 (commercial control) and 0.75 (no-antimicrobial control and natural flavor-based treatments at 1.00% and 1.50%). Samples were inoculated with *Aspergillus oryzae, Penicillium roqueforti*, and *Aspergillus montevidensis* (100 spores/mL) and stored at 21°C and 84% RH for 180 days. Visible mold growth was monitored daily. Statistical comparison between treatments were performed using one-way ANOVA (p 0.05).

Results: The untreated control (a_w = 0.75) showed rapid mold onset: Day 13 for *A.*

montevidensis, Day 42 for *P. roqueforti*, and Day 55 for *A. oryzae*. The commercial control (a_w = 0.70) remained mold-free for 180 days for most strains, with *P. roqueforti* showing mold at Day 86. At higher a_w (0.75), the 1.00% treatment delayed mold onset to Day 158 for *A. montevidensis* and Day 93 for *P. roqueforti*, while 1.50% treatment prevented visible mold growth across all strains for the full 180-day study. These findings confirm that high-a_w formulations, when paired with effective antifungal systems, can deliver mold-free shelf life equivalent to lower-a_w commercial control (p>0.05).

Significance: Higher moisture and palatability can be achieved without compromising microbial quality, as optimized mold control strategies enable shelf life at par with low-a_w products, supporting clean-label innovation and consumer-preferred textures.

P3-088: REVEAL Q+ MAX ZEARALENONE ENHANCEMENT

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: In this study, we evaluated the performance of the enhanced Reveal Q+ MAX Zearalenone kit and its impacts to the current list of validated commodities, analyst comparison, temperature, reagent volume and lot to lot comparison.

Purpose: To determine the enhanced performance of the Reveal Q+ MAX Zearalenone kit performs accurately.

Methods: 27 samples were tested following the 10-gram sample Extraction. For each matrix and method each sample was tested unspiked, low and high when unspiked results were below the Limit of detection of the kit. Each spiked result needed to fall within 30% recovery. Samples that tested above the limit of detection needed to fall within 30% difference of HPLC.

Results: Eight commodities did not need to be adjusted on the validated list of commodities. Sixteen commodities needed an adjustment to their extraction ratios to work with the new antibody. It is also shown that the results are comparable between Analysts, the kit performed correctly at the low end and high end of the temperature listed on the kit insert, the kit worked appropriately from 360–440ul reagent volume into the Raptor and with three new lots the results were comparable and within 30% difference of HPLC.

Significance: Zearalenone "*Fusarium graminearum*" is a mycotoxin that impacts the quality of many ingredients in animal and human diets. Zearalenone causes reproductive and estrogenic disorders and has an Advisory Level of <500ppb. Having a rapid enhanced kit with hygiene and be more productively directed toward individual restaurants. Findings also provide evidence-based insights for regulators and policymakers to enhance training/ certification policies and enforcement strategies at the national and international levels.

P3-089: SHELF-LIFE EXTENSION OF REFRIGERATED PET FOOD USING CLEAN-LABEL ANTIMICROBIALS

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Refrigerated pet food remains highly susceptible to microbial spoilage due to post-processing contamination that can occur during shredding, mixing, and packaging. Because these products are often sold with extended refrigerated shelf lives (up to 160 days), effective control of spoilage organisms is essential to maintain product quality and consumer confidence.

Purpose: To evaluate the efficacy of clean-label antimicrobial solutions in controlling spoilage microflora in refrigerated pet foods.

Methods: A pet food model, consisting of chicken and brown rice with vegetables, was prepared, shredded, portioned into 30-g samples, vacuum-packed, and stored at 4.4°C. Four variables of pet food were evaluated: i) Negative control [no antimicrobial] ii) 2% Verdad® Opti Powder N510 [Natural flavor, Vinegar] + 0.45% Verdad® Powder N6 [Vinegar] iii) 1.5% Certeza® Powder 716 [Natural flavor, Vinegar] + 0.8% Verdad® Powder N20 [Natural flavor] iv) 1.26% Verdad® Powder N6 [Vinegar] + 1.08% Verdad® Powder N20 [Natural flavor]. Samples (n=3) were enumerated for lactic acid bacteria (LAB; 30°C for 48 h) and aerobic plate counts

(APC; 35°C for 48 h) throughout the shelf life of the samples.

Results: All variables had a similar starting population of ca. 1 log CFU/g LAB and 2.7 log CFU/g APC on day 0. The APC population in the negative control reached 6 log CFU/g by day 14 and 7 log CFU/g by day 42; the LAB population increased more slowly than the APC population. The APC population in the negative control sample reached 3 log CFU/g by day 14 and 5 log CFU/g by day 42. In the treated samples, both LAB and APC populations remained bacteriostatic throughout the study.

Significance: Refrigerated pet food is a rapidly growing segment of the pet food industry, posing a distinct challenge in controlling spoilage organisms due to their extended shelf life. Clean-label antimicrobials are an effective way to maintain freshness by preventing the outgrowth of spoilage microorganisms.

P3-090: CONTROL OF SPOILAGE MICROORGANISMS IN TOMATO SALSA USING CLEAN-LABEL ANTIMICROBIALS

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Although tomato salsa is acidic, it remains vulnerable to spoilage by yeasts, lactic acid bacteria (LAB), and molds under refrigeration. Understanding how clean-label antimicrobials affect microbial growth is crucial for enhancing product quality, extending shelf life, and providing safer options that meet consumer needs.

Objective: To assess the efficacy of clean-label antimicrobials for extending the shelf life of tomato salsa stored under refrigeration.

Methods: Tomato salsa samples were prepared without antimicrobial (control), with 0.5% Verdad® Powder N30 [Cultured sugar], or 2% Verdad® F340 [Vinegar]. Samples were inoculated with a yeast cocktail (*D. hansenii*, *P. membranifaciens*, *C. zeylanoides*), a LAB cocktail (*L. curvatus*, *L. sakei*, *L. carnosum*, *L. mesenteroides*) at 2.5 log CFU/g, or a mold spore mix (*P. brevicompactum*, *P. roqueforti*, *P. palitans*) at 10,000 spores/sample. Inoculated samples were packaged aerobically and stored at 4.4°C. Yeasts and LAB were enumerated (n=3) using standard plating methods, while mold was visually assessed. Microbial growth was modeled using the Baranyi–Roberts equation, and treatment effects were analyzed by one-factor

ANOVA.

Results: The yeast population reached 6-log on days 18, 36, and 26 for the control, Verdad® Powder N30, and Verdad® F340, respectively. Antimicrobials significantly (p<0.05) increased the lag time and decreased the growth rate of yeast compared to the control. Similarly, the antimicrobials inhibited the outgrowth of LAB to below the detection limit (0.30 log CFU/g) during storage. The visual mold in the control sample was first observed on day 18, whereas the inclusion of antimicrobials extended the time to visual mold by 10–12 days.

Significance: Clean-label antimicrobials can significantly slow the growth of spoilage microorganisms in tomato salsa. These findings support practical, consumer-friendly strategies for reducing waste and improving safety in refrigerated, acidified foods.

P3-091: SCREENING FOR SALMONELLA IN PET FOOD SAMPLES IS CRITICAL TO PROTECT BOTH ANIMAL AND HUMAN HEALTH

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Infection with *Salmonella* can cause gastrointestinal diseases in pets but may also lead to zoonotic transmission to humans. Handling contaminated pet food or an insufficient hand hygiene after feeding can also lead to an infection. Vulnerable populations, particularly young children and elderly individuals are at increased risk of infection.

Purpose: This study demonstrates the high performance of BACGene GO *Salmonella* PCR Assay for the detection of *Salmonella* in pet food, including large sample sizes of up to 375 g in a reduced sample-to-medium ratio.

Methods: Probability of Detection (POD) studies were conducted according to AOAC Appendix J on 375 g dry dog food and 375 of canned pet food. After an enrichment in BPW in a 1:6 ratio, DNA was extracted via rapid mechanical extraction and the PCR was performed on the Analytik Jena qTOWERiris/GSD version, the Bio-Rad CFX OPUS (Deepwell and Standard) and ThermoFisher QuantStudio™ 5 (Fast). The BACGene GO *Salmonella* PCR Assay was compared to ISO 6579-1:2017 reference method using an unpaired study design for all matrices. All samples were analyzed with and without the optional treatment with the free-DNA removal solution PREraser.

Results: There were no statistically significant differences in the detection of *Salmonella* in both sample types. Positive detected samples with the BACGene GO *Salmonella* PCR were

culturally confirmed by ISO 6579-1:2017. Additionally, there was no negative influence observed from the free-DNA treatment.

Significance: BACGene GO *Salmonella* PCR provides reliable detection of *Salmonella* in large sample sizes offering possibility of sample pooling using low volume of enrichment media, to improve enrichment handling and reduce cost testing.

P3-092: ISOTHERMAL INACTIVATION OF SALMONELLA ENTERICA IN BLACK SOLDIER FLY FRASS AND LARVAE

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Temperature and water activity (a_w) strongly influence the thermal inactivation efficiency of microorganisms in dry products. However, data describing the impact of elevated temperatures on the thermal resistance of *Salmonella* in low-a_w black soldier fly (BSF) frass and larvae used as animal feedstocks are not yet available.

Purpose: To determine the inactivation kinetics of *Salmonella enterica* in low-moisture larvae/frass at elevated temperatures.

Methods: Combined frass:larvae (3:1, w/w) was dry inoculated to ~7 log CFU/g with one of three *Salmonella enterica* strains (Senftenberg 775W, Enteritidis PT30, and Typhimurium ATCC 14028) and equilibrated to a_w 0.25 ± 0.05. A 0.5 g portion of the inoculated sample was

loaded into a sanitized thermal death time (TDT) disk and heated isothermally at 80, 87.5, or 95°C using a circulating water bath. Disks were removed at five predetermined timepoints for each temperature (n = 6 disks per timepoint), immediately cooled in ice for 45 s, placed in room-temperature water (60 s), and surviving cells were enumerated by most probable number (MPN).

Results: Survival curves were well described by first-order kinetics (R^2 ≥ 0.84), with D-values decreasing as temperature increased from 80 to 95°C. At 80°C and a_w 0.25 ± 0.05, Senftenberg 775W and ATCC 14028 showed the highest thermal resistance (D_{775W} = 7.99 min, and $D_{ATCC\ 14028}$ = 7.91 min), followed by PT30 (D_{PT30} = 6.67 min). Thermal treatment at 80°C resulted in > 4 log CFU/g reductions across all the tested strains.

Significance: These data provide a quantitative basis for validating rotary dryer lethality conditions and support the safe use of low-moisture BSF material in animal feed applications.

P3-093: KILL-STEP VALIDATION OF BACILLUS AND CLOSTRIDIUM VEGETATIVE CELLS IN THERMALLY PROCESSED PET FOOD USING CANNING

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Spores of *Bacillus* and *Clostridium* species can survive inadequate thermal processing and pose food safety risk in shelf-stable pet food. Inefficient time-temperature-pressure parameters might result in insufficient microbial inactivation and compromise the validated thermal lethality.

Purpose: This study aims to validate the lethality of commercial canning conditions against spores of *Bacillus* and *Clostridium* species by evaluating critical processing parameters, including time, temperature, and pressure.

Methods: Meat pâté was formulated at pH levels (5.0, 5.5, 6.0, 6.5, 7.0) and inoculated with *C. sporogenes* (ATCC 7955) and *G. stearothermophilus* (ATCC 12980) spores (~ 10^8CFU/g). Samples were sealed in cans and processed in a pilot-scale retort at varying temperatures. Internal temperatures were monitored via needle-type thermocouples at the geometric cold point. Surviving spores were enumerated using the pour-plate method on RCA (37°C, anaerobic) and TSA (55°C, aerobic). Inactivation kinetics (D and z-values) and F_0 were determined using a first-order log-linear model to evaluate the impact of processing parameters on thermal

resistance.

Results: Thermal processing significantly reduced the *Bacillus* and *Clostridium* spore populations compared to the controls. A canning process of 121°C for 15 min at 15 PSI successfully achieved > 5.0 log reductions from an initial 8.0 log CFU/g with surviving populations reduced to below the detection limit (1.0 log CFU/g). Reductions were significantly greater than those observed under lower lethality conditions ($p < 0.05$).

Significance: This validation supports compliance with Food Safety Modernization Act (FSMA) preventive controls and industry standards, reducing risks associated with vegetative pathogens for canned pet food, reducing the risk of vegetative pathogens and strengthening food protection efforts.

Award Application: Developing Scientist Award

P3-094: PHAGE BIOCONTROL OF SALMONELLA ON RAW PET FOOD

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Presentation type/Format: Technical

Category: Animal and Pet Food Safety

Abstract:

Introduction: Consumer demand for minimally processed raw pet food continues to increase. The U.S. Food and Drug Administration maintains a zero-tolerance policy for the foodborne pathogen *Salmonella enterica* in raw pet food. Bacteriophage-mediated biocontrol has emerged as a viable non-antibiotic, non-chemical intervention for controlling foodborne pathogens across diverse food matrices, while preserving their organoleptic properties. Previous studies have focused on phage efficacy on raw pet food ingredients but not in the multi-component products themselves.

Purpose: We tested the efficacy of a commercial bacteriophage preparation, SalmoFresh™, containing lytic bacteriophages specific for *Salmonella,* against *S. enterica* contamination in four raw pet food formulations: beef, pork, chicken, and a beef/chicken combination.

Methods: *Salmonella* Enteritidis strain SE949 was spray-inoculated (5 log CFU/g) onto 10-lb batches of raw pet food, allowed to attach, then mixed into the matrix. Phage ($1x10^8$ PFU/g) was sprayed at a rate of 9mL/lb onto product while constantly mixing in a lab-scale ribbon blender. Treated product was transferred to 4°C for storage. Phage efficacy was determined by comparing viable *Salmonella* in treated and untreated control samples using direct plate

count 1h and 24h after application.

Results: Phage application resulted in a significant (P<0.01) reduction of *Salmonella* on beef (1.5 and 1.8 log CFU/g), pork (1.3 and 1.7 log CFU/g), chicken (1.0 and 2.0 log CFU/g) and chicken/beef combo (1.0 and 1.3 log CFU/g) raw pet food at 1h and 24h post-treatment, respectively, compared to untreated control.

Significance: Collectively, these findings demonstrate that bacteriophage-based biocontrol provides a statistically significant, matrix-independent reduction of *Salmonella* in raw pet food. These results support its applicability as a targeted antimicrobial intervention for mitigating pathogen load in raw pet food formulations with diverse meat matrices.

P3-095: MICROBIAL ECOLOGY AND BIOFILM FORMATION IN COMMERCIAL WHEAT MILLING FACILITIES: IMPLICATIONS FOR LOW-MOISTURE GRAIN PRODUCT SAFETY

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Emerging foodborne illness outbreaks linked to wheat products highlight critical gaps in understanding microbial communities and dry-biofilm formation in milling facilities, where inadequate sanitation poses persistent contamination risks.

Purpose: Characterize microbial ecology and identify dry biofilm-associated taxa across a commercial wheat-milling facility.

Methods: Environmental samples (n=160) were collected from a Kansas wheat-milling facility before milling (BM, n=70), after milling (AM, n=65), and from milling fractions (MF, n=25). DNA was analyzed using 16S rRNA amplicon sequencing with the DADA2-SILVA pipeline to generate sequence variants and taxonomic profiles. Alpha and beta diversity were calculated using Shannon indices and community differentiation analysis. Differential abundance analysis identified enriched taxa ($\log_2$ FC > 1.8).

Results: Distinct microbial assemblages dominated each stage: BM harbored spore-forming *Bacillus* spp. (*B. subtilis*, 4.5% relative abundance; 92% prevalence); AM transitioned to biofilm-forming *Pseudomonas* spp. (*P. putida*, 6.7% relative abundance; 99% prevalence). MF exhibited grain-fermentation-associated *Lactobacillus/Weissella* communities (*L. plantarum* 5.6%; 88% prevalence; *Leuconostoc mesenteroides* 4.8%; 92% prevalence). Alpha diversity was preserved across stages (Shannon: 2.96–4.01), while beta diversity revealed significant community differentiation. Post-milling enrichment of biofilm-formers, including *P. putida*, *Acinetobacter johnsonii*, and *Staphylococcus epidermidis* ($\log_2$ FC >1.8), identified the facility as a biofilm-contamination reservoir. *Bacillus cereus* enrichment (2.4% post-milling) identified a potential contamination reservoir, with dried biofilm deposits persisting during grain processing.

Significance: Comprehensive microbial characterization and biofilm-targeted monitoring are essential for designing food safety interventions and contamination-control strategies in grain-milling facilities.

Award Application: Developing Scientist Award

P3-096: VALIDATION OF DOG BISCUIT BAKING PROCESS TO CONTROL SALMONELLA CONTAMINATION

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Dog biscuits are widely consumed pet treats, and their safety is critical due to the risk of *Salmonella* contamination. Cereal-based ingredients such as whole-wheat flour may act as potential sources of *Salmonella*. Validating the baking process is essential to ensure the microbiological safety of finished dog biscuit products. This study evaluated the effectiveness of baking as a control step and determined the thermal inactivation kinetics of *Salmonella*, including *D*- and *z*-values.

Purpose: To validate dog biscuit baking as an effective kill step and determine *Salmonella* thermal inactivation kinetics.

Methods: Whole-wheat flour was mist-inoculated with 5-serovar *Salmonella* cocktail to achieve an initial concentration of 10.1 ± 0.33 log CFU/g and incorporated to make dog biscuit dough. The dough was shaped and baked at 350°F (175°C) for 25 minutes. Samples were collected at selected time points for *Salmonella* enumeration, water activity (a_w), and pH

analysis. *Salmonella* population in pre- and post-baked samples were enumerated using injury recovery media consisting of Brain Heart Infusion agar overlaid with Xylose Lysine Deoxycholate (XLD) agar.

Results: Baking resulted in 6.6 ± 0.1 log CFU/g reduction of *Salmonella* population. Water activity decreased significantly during baking for the crust samples, and it remained constant for the crumb, while pH exhibited minor variation throughout the process. *D*-values of *Salmonella* at 57°C, 61°C, and 65°C were 17.7 ± 1.8, 7.09 ± 0.5, and 1.4 ± 0.1 minutes, respectively, and the mean *z*-value was 7.3 ± 0.6°C. The *D*- and *z*-values indicate progressive thermal inactivation of *Salmonella* in the dog biscuit matrix.

Significance: The results demonstrate that baking dog biscuits at 350°F (175°C) for 25 minutes serves as an effective kill step to control *Salmonella* ensuring safety of dog biscuits.

Award Application: Developing Scientist Award

P3-097: ENHANCING MICROBIAL SAFETY OF MECHANICALLY SEPARATED CHICKEN IN PET FOOD USING NATURAL PRESERVATION APPROACHES

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Microbial contamination by spoilage bacteria presents a persistent challenge in the pet food industry, often resulting in reduced shelf life and increased risk of product recalls. Mechanically separated chicken (MSC), used as a protein source in pet food, is susceptible to microbial proliferation due to its high moisture and nutrient content.

Purpose: This study assessed the effectiveness of a natural antimicrobial solution composed of a blend of cultured sugar and vinegar in optimized proportions, for controlling microbial growth in MSC.

Methods: Fresh MSC samples were treated with the antimicrobial at 1.0% inclusion and compared to untreated control. Both groups were stored at 4°C and periodically analyzed over 14 days for total viable counts and *Enterobacteriaceae* using standard microbial plate count methods.

Results: Results show significant antimicrobial activity against bacterial contamination, providing substantial protection against Enterobacteriaceae proliferation. The monitoring data shows that the untreated control sample allows an increase of 3 log whereas the treated sample does not allow any growth during the 14 days of follow-up. Additionally, monitoring of the total viable count indicates that the control sample without the preservative shows a 2

log increase beginning on day 3. The sample treated with the natural antimicrobial allows for delaying microbial growth to 7 days. After 14 days, the treated sample shows a lower contamination compared to the control (4.5 log vs 6 log). Importantly, the application of antimicrobial did not affect the sensory attributes of the MSC or the palatability of the final pet food product.

Significance: These findings highlight the potential of this new natural antimicrobial solution to enhance the microbial safety and shelf life of high-risk raw materials such as MSC, supporting the industry's transition toward cleaner label and more natural preservation methods without compromising product quality.

P3-098: NATURAL PRESERVATION STRATEGIES USING CULTURED INGREDIENTS FOR FUNGAL INHIBITION IN PET FOOD MATRICES

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: One of the most critical challenges for the pet food industry is fungal contamination, as it drives product spoilage, reduces shelf life, and increases the risk of costly recalls and consumer dissatisfaction. At the same time, the growing demand for clean-label products is accelerating the need for natural preservative alternatives.

Purpose: In this context, we developed a natural antifungal solution based on an optimized blend of cultured ingredients, designed to provide strong protection across pet food matrices while preserving palatability.

Methods: Two matrices prone to fungal spoilage were evaluated. A semi-moist pet food model and a liquid palatability enhancer typically protected with potassium sorbate. Challenge tests were conducted using 6-strain mold and yeast cocktails inoculated at 5.5 log CFU/g and stored at 25°C and monitored over time.

Results: In the semi-moist model, 1.25% dosage maintained yeast counts at the inoculum level (□5 log CFU/g) after 60 days, while the untreated control exceeded 8 log CFU/g. Mold counts decreased by more than 3 log CFU/g over the same period, demonstrating fungicidal activity. Even stronger effects were observed in the palatability enhancer: yeasts dropped from ~4 log CFU/g to below count limit (<1 log CFU/g) within 2 weeks (vs. 2 days with

sorbate), whereas the control exceeded 6 log CFU/g. Mold counts fell below 1 log CFU/g after 9 days, while remaining at 2.7 log CFU/g in the control after 28 days. Due to their intrinsic characteristics (low pH, high salt content, etc.) palatability enhancers create multiple hurdles that weaken or sensitize microorganisms increasing the efficacy of preservatives. Palatability tests in dogs (n=40) and cats (n=40) showed no impact on acceptance ($p > 0.5$).

Significance: This natural solution provides robust antifungal performance in multiple pet food applications without affecting palatability, positioning it as a credible alternative to potassium sorbate.

P3-099: A NATURAL MULTIPURPOSE ANTIMICROBIAL SOLUTION FOR PET FOOD: CONTROLLING FUNGAL SPOILAGE AND FOODBORNE PATHOGEN

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: Microbial contamination—from spoilage to pathogenic—remains a major challenge in the pet food industry, driving shelf-life reduction, recalls, and loss of consumer confidence. In parallel, demand for clean-label products is accelerating the shift from synthetic preservatives to natural alternatives.

Purpose: We developed a natural multipurpose solution based on an optimized blend of citric acid and vinegar, designed to control microbial risks without compromising palatability.

Methods: Two pet food models with distinct water activities were used: a low-a_w model (<0.65) representing dry kibble supporting pathogen survival, and a high- a_w model (0.85) representative of semi-moist pet food supporting fungal growth. Our solution was incorporated into the pet food through an extrusion line. Challenge tests were performed using *Listeria monocytogenes* and *Salmonella enterica* (3 strains at 5 log CFU/g), and a 6-strain mold cocktail (3 log CFU/g). Samples were stored at 25°C and monitored over time.

Results: In the high- a_w model, 1% dose achieved complete mold inhibition with no contaminated samples after 5 months (n=10), while 100% of untreated samples were spoiled after 1 month. A 0.3% dose was insufficient, and intermediate contamination was observed with another recipe, emphasizing the need for dose adjustment. In the low- a_w model, 1%

dose showed activity against *Listeria*, reaching the count limit within 14 days (4-log reduction), compared with 28 days in untreated control. For *Salmonella*, inactivation occurred within 28 days (4-log reduction), while controls remained more than 1.5 log CFU/g higher. Palatability testing on a 40-dog panel showed no significant impact on pet food acceptance *($p > 0.5$)*.

Significance: Our solution demonstrates multipurpose antimicrobial effectiveness across pet food model while maintaining palatability. However, it is crucial to validate the effective dosage, which can vary depending on the pet food and microorganisms involved.

P3-100: OCCURRENCE OF ANTIMICROBIAL RESISTANCE IN INDICATOR BACTERIA AND CAMPYLOBACTER SPP. ISOLATED FROM COMMERCIAL RAW MEAT-BASED PET FOOD IN BELGIUM

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Purpose: Raw meat-based diets (RMBDs) for companion animals may serve as reservoirs for antimicrobial-resistant bacteria, posing risks to animal and public health. This study investigated the occurrence and risk factors of antimicrobial resistance (AMR) in indicator bacteria and *Campylobacter* spp. in commercial RMBDs sold in Belgium.

Methods: Between May and August 2023, 50 RMBD samples representing 21 brands from five European countries were collected from retail sources in Belgium. Samples were analyzed for *Escherichia coli*, *Enterococcus faecalis*, *E. faecium*, and *Campylobacter* spp. using standard isolation procedures. Selective media supplemented with cefotaxime (1 mg/L) and

linezolid (4 mg/L) were used to screen for resistant strains. Antimicrobial susceptibility testing was performed using broth microdilution following EUCAST guidelines. Risk factors were analyzed using logistic regression.

Results: High bacterial contamination rates were observed: *E. coli* (90.0%), *E. faecalis* (62.0%), *E. faecium* (46.0%), and *Campylobacter* spp. (6.0%). ESBL/AmpC-producing *E. coli* was detected in 34.0% of samples. Notably, one carbapenem-resistant *E. coli* strain was isolated—the first reported in commercial pet food. Multidrug resistance was identified in 17 isolates from 15 samples (30.0%), including 14 MDR *E. coli*, 1 MDR *E. faecalis*, and 2 MDR *E. faecium*. Belgian-origin samples showed significantly higher *E. faecium* prevalence (76.5%) compared to Dutch samples (21.4%) (OR=11.11, $p<0.001$). Minor livestock sources were associated with increased MDR risk (OR=5.56, $p=0.017$).

Significance: Commercial RMBDs exhibit widespread bacterial contamination with concerning AMR rates, including critically important resistance phenotypes. These findings highlight the need for enhanced regulatory oversight and consumer education regarding safe handling practices for raw pet foods within the One Health framework.

P3-101: PRESSURE RESISTANCE VARIATION AMONG SELECTED SALMONELLA SEROVARS IN A MODEL POULTRY-BASED PET FOOD DIET

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Presentation type/Format: Poster

Category: Animal and Pet Food Safety

Abstract:

Introduction: High-pressure processing (HPP) is a promising nonthermal pasteurization technology that effectively reduces microbial populations while preserving the sensory and nutritional quality of raw meats. However, pressure resistance varies among *Salmonella* serovars, necessitating identification of the most resistant strains for surrogate validation studies.

Purpose: This study evaluated the HPP susceptibility of five *Salmonella* serovars in a poultry-based pet food matrix to identify appropriate target organisms for surrogate validation.

Methods: A model poultry-based raw pet food diet was inoculated with each *Salmonella enterica* serovars (Agona, Enteritidis, Typhimurium, Heidelberg, and Senftenberg) at ~ 10^7 CFU/g. Samples were treated at 300 or 400 MPa for 2 min at 4°C using a laboratory-scale HPP unit. Surviving populations were enumerated on XLD with TSA overlay, and log reductions were calculated relative to non-pressurized controls.

Results: Five *Salmonella* serovars reveal distinct pressure resistance patterns in poultry-based pet food. *S.* Enteritidis exhibited the greatest resistance at both pressure levels ($P < 0.05$), showing the lowest reduction of 2.01 log at 300 MPa and 3.18 log at 400 MPa. At 300 MPa, *S.* Agona, Heidelberg, and Senftenberg showed similar intermediate resistance, with reductions ranging from 2.55–2.60 log ($P > 0.05$). Increasing pressure to 400 MPa resulted in greater inactivation across all serovars, with *S.* Agona achieving the highest reduction (4.53 log), followed by Senftenberg (4.23) and Heidelberg (4.02). *S.* Typhimurium exhibited intermediate resistance at both pressure levels, with reductions of 2.44 and 3.75 log at 300 and 400 MPa, respectively.

Significance: These findings show distinct serovar-dependent HPP resistance patterns in a complex pet food matrix. Based on their contrasting barotolerant profiles, *S.* Enteritidis and *S*. Typhimurium will be selected for surrogate validation studies with generic *E. coli* and *E. faecium.*

Award Application: Developing Scientist Award

P3-102: QUANTIFYING RESIDUE OF ADDITIVES AND CROSS-CONTAMINATION IN FEED MILL BUCKET ELEVATORS USING MAGNETIC IRON TRACERS AS A SCREENING METHOD

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Category: Animal and Pet Food Safety

Abstract:

Introduction: The concern over carryover of drug and other additives residue in animal foods is primarily associated with the production of medicated feed when non-medicated feed is also manufactured using the same equipment. It also applies to other additives when customers of the same feed mill require a non-detectable presence of animal source proteins, and fermentation process residuals in their respective feed.

Purpose: This research focused on validating feed sequencing and equipment flushing in two “self-cleaning” bucket elevators using Tracer F-blue, a food dye-coated magnetic iron particle, and developing an equation for ease of quantification.

Methods: Flushing was studied between two similar bucket elevators: one for pre-heat-treated and the other for post-heat-treated animal feed. For a bucket elevator, three replicates with each consisting of a tracer-carried feed, flush material, and carryover feed, resulting in a total of 162 samples. The tracer was extracted from the feed mash using a magnetic rotary detector and blue spots developed on the filter paper, counted and recorded. The percentage of cross-contamination in the carryover feed was determined.

Results: There existed a significant difference in the residual amount of material in the boots of the two bucket elevators, which was not affected (p = 0.89) by the tonnage of feed produced or run days before equipment cleanout. Also, the recovery of Tracer F-blue varied

significantly (p =0.017) among replicates but consistently predicted the CV of mixer uniformity. Also, Tracer F-blue indicated that cross-contamination was less than 1% across replicates and the two bucket elevators.

Significance: The consistent amount of residue recovered in the boots of the two 'self-cleaning' bucket elevators suggests that running a base mix to fill the boots and other spaces immediately after equipment cleanout can effectively help control cross-contamination.

Award Application: Developing Scientist Award

P3-103: IMPACT OF CLEANING AND SANITATION ON SALMONELLA ADHESION ON STAINLESS STEEL SURFACES FOR MULTIPLE FOOD POWDERS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* is the primary pathogen of concern in low-moisture foods and processing environments. *Salmonella*'s ability on powder particles to adhere to processing equipment surfaces is poorly understood, with the impact of food composition and appropriate cleaning/sanitation practices remaining a major gap.

Purpose: This study is designed to evaluate 6 food powders to determine the impact of dry brush cleaning and UV sanitation on *Salmonella* adhesion to stainless steel surfaces.

Methods: Animal-based (egg white, whey protein, whole milk) and plant-based powders (almond, cocoa, and coconut) were tested. Powders (100 g) were inoculated with an agar-grown 6-serovar *Salmonella* cocktail (1 mL), then hand massaged to distribute inoculum throughout the sample. A custom-built, triboelectric powder coating device was used to fully coat stainless steel coupons. Coupons were brushed (10 strokes/cardinal direction) until

visibly clean, then UV-treated for 20 min. All coupon samples were combined with peptone water and hand massaged. Samples were serially diluted, plated on a differential/non-selective agar, incubated (37°C, 48 h) and survivors enumerated. ANOVA (α = 0.05) was used to evaluate the impact of powder type on *Salmonella* adhesion and subsequent removal via dry cleaning and sanitation.

Results: For coupons fully coated with powders, significantly ($p < 0.05$) more *Salmonella* was present for animal-based powders than for plant-based powders. Once visibly cleaned, *Salmonella* adhering to the coupons ranged from 0.87–3.00 log CFU/cm^2. No significant differences in the efficacy of UV treatment were observed between animal and plant-based powders; however, additional data are needed to quantify the impact of food composition on cleaning and sanitation efficacy.

Significance: These results demonstrate a need to consider the food powder when determining dry cleaning and sanitation efficacy for reducing *Salmonella* populations on food processing equipment.

Award Application: Undergraduate Student Award

P3-104: FROM SOIL TO SHELL: A STRUCTURAL EQUATION MODELING FRAMEWORK TO UNDERSTAND MICROBIAL CONTAMINATION OF IN-SHELL PECANS DURING HARVEST

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Presentation type/Format: Technical

Category: Low-Water Activity Foods

Abstract:

Introduction: The manner pecans are harvested lends to the potential for foodborne pathogen contamination. Understanding the factors that contribute to contamination during harvest is important to determine best practices.

Purpose: Evaluate environmental dynamics that influence the potential transfer of *E. coli* from the soil to in-shell pecans during harvest.

Methods: Each week over a 5-week period, plots (1x2 m) in a pecan orchard were inoculated with a rifampin-resistant strain of *Escherichia coli*. Following inoculation pecan trees near the plots were harvested. Pecan and soil samples were collected in the plots immediately, 1 day, and 2 days after harvest to determine *E. coli* levels in the soil and on the pecans were determined by plating on TSA supplemented with rifampin. Pecan and soil moisture, temperature, relative humidity, soil composition, shell moisture, and rainfall was measured. A structural equation model (SEM) was used to characterize the direct, indirect and total effect of these factors on *E. coli* transfer to pecans during harvest.

Results: The factor most strongly associated with *E. coli* concentration on pecans was *E. coli* concentration in the soil (0.51; 95% CI=0.40, 0.63), but did it not significantly associated with transfer rate (0.06; 95% CI=-0.14, 0.25). Other factors strongly associated with *E. coli* concentrations on pecans were week (0.47; 95% CI=0.43, 0.51), soil moisture (0.44; 95% CI=0.33, 0.55) and soil temperature (-0.27; 95% CI=-0.36, -0.17). Soil-to-shell transfer was also strongly associated with rain (0.22; 95% CI=0.016, 0.30), week (0.21; 95% CI=0.15, 0.27), shell moisture (-0.14; 95% CI=-0.26, -0.04), and days post-inoculation (0.14; 95% CI=0.04, 0.24).

Significance: The results of this study show how SEM can be utilized to understand microbial transfer in food production settings, and how environmental factors impact microbial transfer to pecans during harvest which can be used to determine optimal conditions to harvest.

P3-105: QUANTIFYING TRANSFER OF SALMONELLA AND SHIGA TOXIN-PRODUCING ESCHERICHIA COLI ON WHEAT GRAIN DURING TEMPERING

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) are two major bacterial pathogens responsible for outbreaks linked to wheat flour. The survival of these pathogens on wheat grain is well documented. However, the subsequent addition of moisture during grain tempering may facilitate movement of the pathogens, contaminating previously uncontaminated kernels during processing.

Purpose: This study aims to quantify the potential for cross-contamination during wheat tempering.

Methods: For both pathogens, 5 strains of each were streaked onto Luria Bertani agar supplemented with rifampicin (LBr) and incubated at 37°C. Lawn cultures were harvested and transferred to Butterfield's buffer to create an inoculum. Soft white wheat was inoculated and equilibrated for 48 hrs before tempering. After equilibration, 55 g quantities each of the inoculated white wheat and uninoculated hard red wheat – confirmed negative for pathogens – were combined with 5.2 mL of water to achieve a 4% increase in moisture content and tempered. After 16.5 hrs tempering, kernels were separated by color, diluted

with Butterfield's buffer, homogenized, and plated onto LBr to enumerate cross-contamination. For each pathogen, three biological replicates were assessed.

Results: Log reduction was calculated from pre- and post-tempering pathogen counts. Reductions from the initial inoculation level of 4.1 ± 0.3 log CFU/g for *Salmonella* and STEC ranged from 1.73 ± 0.13 and 1.89 ± 0.25 CFU/g, respectively. Pathogen transfer was quantified by comparison of pre- and post-tempering pathogen levels on each color of wheat, expressed as the percentage of total recovered cells present on previously uninoculated grain. Transfer of *Salmonella* and STEC ranged from 4–34% and 22–46%, respectively.

Significance: These results, demonstrating the potential for pathogen transfer during wheat tempering, are important for the development of microbial risk assessments of S*almonella* and STEC on wheat grain.

Award Application: Developing Scientist Award

P3-106: SWEET PROTECTION: THE IMPACT OF MATRIX PROFILES ON SALMONELLA PERSISTENCE IN CHOCOLATE

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* is a leading cause of foodborne hospitalizations, with increasing outbreaks linked to low-water activity (low a_w) foods. In high-fat matrices like chocolate, low moisture protects the pathogen from environmental desiccation and gastric acid, resulting in high virulence even at low contamination levels.

Purpose: This study aimed to correlate the chemical composition of raw ingredients with *Salmonella* survival mechanisms during storage and thermal processing of chocolate.

Methods: Two cocoa powders (Dutch and Dark; with Dark having the higher pH) and two milk powders (MP) were inoculated with *Salmonella* Typhimurium ATCC SL1344 (10^6 CFU/g; n=9, 1-g samples) and stored at 22°C for 14 days. Inoculated milk powders were then used at a 20% inclusion rate in laboratory chocolate production, with survival evaluated during heating at 50°C and 80°C for 60 minutes. Statistical analysis performed using one-way ANOVA followed by Tukey-Kramer HSD.

Results: Initial populations were 10^6 CFU/g. During storage, *Salmonella* survival was dependent on the type of cocoa. Highly alkalized Dark cocoa showed a 4-log CFU/g reduction over 8 days, while the Dutch cocoa powder exhibited higher persistence, with only a 2-log reduction. For MP, populations remained stable after a 1-log decrease after 2 days of storage.

In chocolate processing at 50°C, Israeli MP maintained $8x10^5$ CFU/g, whereas samples using MP originating from the USA dropped to $4x10^5$ CFU/g. Increasing temperature to 80°C reduced survival to $2x10^4$ CFU/g and $5x10^4$ CFU/g, respectively. Metabolic analysis confirmed distinct fatty acid and sugar profiles between varieties.

Significance: These findings demonstrate that higher alkalization in cocoa and specific lipid/sugar profiles in MP significantly enhance *Salmonella* resistance to desiccation and thermal stress, impacting safety protocols in chocolate manufacturing, and informing better risk assessment for chocolate manufacturing.

P3-107: INACTIVATION OF SALMONELLA MONTEVIDEO AND ENTERITIDIS IN WHEAT FLOUR THROUGH A COMBINATION OF L-CYSTEINE, ACETIC ACID, AND ULTRAVIOLET (254 NM) TREATMENT

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Flour has been implicated in several *Salmonella*-associated outbreaks and recalls. Although flour is commonly used in baked goods, *Salmonella* can be transmitted through handling and consumption of raw flour products such as cookie dough.

Purpose: This study aimed to develop a non-thermal, moisture-less treatment to reduce *Salmonella* in flour while maintaining flour functionality. The approach targets the susceptibility of *Salmonella* to oxidative and acid stress.

Methods: Wheat flour (100 g) was spray-inoculated with *Salmonella* serotypes Enteritidis and Montevideo to ~5 log CFU/g. The flour was supplemented with L-cysteine and sprayed with 0.1% (w/v) acetic acid, then passed through a UV-C tunnel. Treated flour was packaged in Ziplock bags and stored at 23°C for 15 days. Samples (10 g) were collected periodically to enumerate *Salmonella* survivors after resuscitation in TSB-glycerol. Two replicates of each sample were analyzed. *Salmonella* counts (log CFU/g) were analyzed using two-way ANOVA to

evaluate treatment, storage time, and their interaction. Significant differences were determined using Tukey's test ($p < 0.05$). Log reductions were analyzed using one-way ANOVA with Tukey's test.

Results: *S.* Montevideo declined by 0.47 log CFU in control flour during 15 days of storage. Flour treated with L-cysteine or acetic acid before UV-C did not produce significantly greater reductions than the control. However, the combined treatment of L-cysteine, acetic acid, and UV-C resulted in a 1.21 log CFU reduction, significantly greater than untreated flour. Treatment with L-cysteine and UV-C achieved approximately a 2-log CFU reduction of *S.* Enteritidis. Bread prepared with treated flour showed no significant differences in loaf volume or crumb hardness compared with untreated flour. L-cysteine also enhanced gluten aggregation, reducing dough mixing time.

Significance: The combination of L-cysteine, acetic acid, and UV-C reduces the risk of *Salmonella* in milled flour.

Award Application: Developing Scientist Award

P3-108: SURVIVAL DYNAMICS OF ESCHERICHIA COLI IN FOOD POWDERS DURING STORAGE AT DIFFERENT TEMPERATURES

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Outbreaks of human gastrointestinal infections have been linked to low-water activity foods.

Purpose: The study aimed to assess the effects of storage temperature, storage time, and bacterial strain type on the survival trends of inoculated *E. coli* in different food powders.

Methods: Three types of low-moisture foods, sprouted chia/flax seed powder, whole wheat flour, and moringa powder, were inoculated with ~5 log CFU/g of four individual strains of nalidixic acid (NA) resistant *E. coli*, 177, 178, 1212, and 1238. Inoculated samples were stored at refrigeration or room temperature for 6 months. NA-resistant bacterial populations and the populations of total aerobes (TA), total yeasts and molds (YM), and total coliforms (TC) were determined monthly throughout the storage. Uninoculated food powers served as controls. Data was analyzed using the Statistical Analysis Software ($\alpha = 0.05$).

Results: All evaluated groups of microorganisms exhibited higher population in samples stored at refrigeration than at room temperature. Most of the microbial groups demonstrated a declining trend from the initial to subsequent months of storage, except for the populations of YM. Chia/flax seed powder and wheat flour had similar TC and NA-resistant bacterial counts, and these counts were higher than those in the moringa

powder. Chia/flax seed powder had the highest YM counts, followed by wheat flour and moringa powder, and wheat flour has the highest TA counts, followed by chia/flax seed powder and moringa powder. Samples inoculated with isolate 1238 had the highest surviving populations of TC, TA, and NA resistant bacterial counts, and those inoculated with isolate 177 had the lowest TC- and NA-resistant bacterial counts.

Significance: These findings suggest that *E. coli* can persist for extended periods in low-water activity food powders under certain storage conditions, underscoring the importance of incorporating microbial persistence into food safety risk assessments.

Award Application: Developing Scientist Award

P3-109: SIGNIFICANCE OF UV-C AND ACIDIC WATER TEMPERING TREATMENTS ON INACTIVATING PATHOGENIC E. COLI ON HARD RED WINTER (HRW) WHEAT GRAINS AND WHOLE WHEAT FLOUR QUALITY

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Applying multiple non-thermal treatments product could improve their decontamination efficacy in food products without compromising quality.

Purpose: This study evaluated the effects of UV-C and acidic water tempering treatments in reducing the Shiga toxin-producing *E. coli* (STEC) contamination on wheat grains and its potential effects on the wheat flour produced.

Methods: Hard red winter (HRW) wheat grains were inoculated (high: 6.4 ± 0.1 log CFU/g and low: 3.3 ± 0.1 log CFU/g) with STEC O121/O26 and treated with UV-C (255 to 280 nm; 0 to 80 mins exposure time) alone or in combination with acidic water (5% lactic acid - LA) tempering (18 h). The experiment was done with three independent replications.

Results: UV-C exposure (280 nm) from 10–80 mins resulted in STEC load reductions ($P < 0.0001$) ranging from 1.4–2.3 logs and 0.4–1.3 logs at high and low inoculation levels, respectively. Combining both acidic water tempering and UV-C (280 nm, 40 mins) resulted in an STEC load reduction of 2.3–3.3 logs within 12 h of tempering. The combined treatments

(UV-C and LA) showed a synergistic effect (O/E > 1; 1.1–1.3) on reducing the pathogenic *E. coli* load of wheat; this combined treatment shortened the time required (6 h) for a 3-log reduction relative to the other treatments. This was supported by the lower ($P < 0.05$) predicted time required for a 3-log reduction (9.4 to 13.0 h) relative to the individual treatments (28.9 h). The LA/UV tempering treatment produced whole wheat flour with comparable quality as whole wheat flours produced from the other treatments (control, LA, and UV).

Significance: This study demonstrated the potential of UV-C and acidic water tempering as an effective hurdle approach combination for mitigating *E. coli* contamination on wheat grains without affecting quality. These findings can be used as basis for addressing enteric pathogen contamination of whole wheat flour products.

Award Application: Developing Scientist Award

P3-110: EVALUATING ENTEROCOCCUS FAECIUM AS A SURROGATE FOR PATHOGENIC BACTERIA FOR CHLORINE DIOXIDE TEMPERING OF WHEAT

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Wheat milling lacks a validated pathogen ‘kill step’ allowing contamination to persist through flour production and recalls. Aqueous chlorine dioxide (ClO_2)-assisted tempering improves microbial safety while preserving grain quality. However, commercial validation requires a safe surrogate representing pathogen behavior under low-moisture conditions.

Purpose: This study evaluated ClO_2 efficacy against pathogens and surrogates while assessing effects on enzymatic activity and bake quality. ClO_2 concentrations and exposure times were optimized using kinetic modeling.

Methods: Pathogenic strains included *Salmonella enterica* (Newport ATCC 6962, Typhimurium ATCC 14028, Kentucky ATCC 9263) and *Escherichia coli* O121 & O26 (ATCC 2219 & 2196), with *Enterococcus faecium* as surrogate. Inoculated wheat kernels were exposed to ClO_2 (300–1500 ppm) for 0–24 h. Microbial enumeration, enzymatic assays (lipase, lipoxygenase, peroxidase, polyphenol oxidase), germination, and baking tests were conducted. Inactivation mechanisms were explored using molecular and spectroscopic techniques. Kinetic modeling and ANOVA with Tukey HSD were applied.

Results: ClO_2 treatment reduced pathogenic populations in a concentration-dependent manner, achieving 4.1 and 4.5 log CFU/g reductions at 1500 ppm after 24 h for *Salmonella* and *E.coli,* respectively. Kinetic modelling showed biphasic inactivation with t3D values from 80 h to 4 h. Surrogate organisms showed comparable or greater resistance (3.9 log CFU/g reduction at 1500 ppm/24 h) confirming treatment robustness. Molecular analysis indicated microbial inactivation resulted from oxidative disruption of cellular components. Enzymatic activities linked to oxidative damage decreased >90% at 1500 ppm. Conventionally and ClO_2-tempered wheat showed similar bake quality (loaf volume 564 ± 2.08; cells 4209 ± 110; $p > 0.05$).

Significance: ClO_2 tempering is a promising intervention that targets pathogens while enabling surrogate validation

Award Application: Developing Scientist Award

P3-111: A COMPREHENSIVE MEASUREMENT OF THERMAL DEATH KINETICS OF SALMONELLA AND LISTERIA MONOCYTOGENES AND THEIR SURROGATES UNDER ISOTHERMAL AND CONSTANT-RH CONDITIONS RELEVANT TO DRYING AND ROASTING

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Salmonella* and *Listeria* are critical pathogens of concern in low-moisture foods, and their surrogates are widely used by industry for process validation. However, limited data are available on their thermal resistance under complex drying and roasting conditions.

Purpose: The objective is to measure the thermal death kinetics of *Salmonella* (Sa) and *Listeria monocytogenes* (Lm) and their surrogates, *E. faecium* NRRL B-2354 (Ef) and *Listeria innocua* (Li) at different temperature and RH relevant to drying/roasting processes.

Methods: Silica gel (SG) was conditioned at 100°C and RH (0–40%). The moisture content (MC) and high-temp (80–140°C) a_w of the conditioned SG were measured. SG with varying MC was then placed in thermal a_w cells to control RH during isothermal treatments of bacterial cultures. They are 1) Sa cocktail *(S.* Enteritidis PT30 ATCC BAA-1045, *S.* Tennessee K4643, and *S.* Mbandaka 698538), 2) Ef NRRL B-2354; 3) Lm cocktail (Lm NRRL B-33053, B-33466 and

B-57618); and 4) Li NRRL B-33197. Cultures were harvested from TSAYE plates, mixed (for cocktail), inoculated into sand, dried at room temp (<16% RH), and subjected to thermal-resistance testing (80–140°C) under controlled RH (0–40%).

Results: All four cultures exhibited log-linear inactivation kinetics to survival and thermal treatment time and demonstrated similar responses to temp and RH. The Dref (at 110°C & 0% RH) of Lm & Li were 10.9 & 12.6 min, comparable to each other. Whereas Sa and Ef showed higher Dref values (40.3 and 80.8 min), indicating greater thermal resistance. ZRH value, indicating sensitivity to RH change, were similar for Lm and Li (14.4 & 16.0% RH), while Sa showed the highest ZRH (19.2% RH), followed by Ef (17.5% RH). Likewise, ZT values were comparable for Lm and Li (20.7 & 22.5°C), higher than that of Ef and Sa cocktail (17.1°C & 18.6°C).

Significance: Data presented herein will contribute to enhancing process validation of food drying, thereby improving food safety.

Award Application: Developing Scientist Award

P3-112: OPTIMIZATION OF CITRIC ACID MARINADE – HEAT PRETREATMENTS FOR PATHOGEN INACTIVATION IN FREEZE-DRIED BEEF JERKY

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Beef jerky is a popular ready-to-eat, shelf-stable product increasingly made by home preservers using freeze-drying (FD); however, FD does not eliminate foodborne pathogens, requiring validated pretreatment strategies for microbial inactivation.

Purpose: Identify a citric acid-marinade-heat pretreatment combination that provides a 5-log CFU/cm^2 bacterial reduction in jerky prior to FD.

Methods: Beef samples (1 in × ¼ in × 2.5 in) were irradiated at 12 kGy before pretreatment. Eight pretreatment combinations of citric acid (2.5 g or 7.5 g; marinade pH 3.3 and 2.65, respectively), marination time (2 or 24 h), and oven heating (5 or 10 min) at 275°F were evaluated. Samples were spot-inoculated with surrogate strains of *Escherichia coli* and *Salmonella enterica* and air-dried to allow bacterial attachment prior to marination. Microbial populations were enumerated at four stages: untreated control, post-inoculation, post-marination, and post-heat treatment. The experiment was conducted as a randomized

complete block design with three biological replicates per treatment combination.

Results: Reductions of both *S. enterica* and *E. coli* were significantly influenced by the interaction of citric acid concentration, marination duration, and heat treatment ($p<0.001$). The pretreatment combination of 24 h marination with 7.5 g citric acid followed by 10 min heating produced the greatest lethality, achieving 7.85 ± 0.16 log CFU/cm² reduction of *S. enterica* and 7.58 ± 0.19 log CFU/cm² reduction of *E. coli*. The limit of detection for pathogen enumeration was 0.9 log CFU/cm².

Significance: Establishing evidence-based interventions that achieve 5-log CFU reductions prior to FD can reduce foodborne illness risk as home use of freeze-dryers continues to expand.

Award Application: Developing Scientist Award

P3-113: EFFICACY OF SALMONELLA REMOVAL USING WHEAT FLOUR AND CORN MEAL PUSH-THROUGH IN AUGER CONVEYOR SYSTEMS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Dry-cleaning interventions in low-moisture food (LMF) production environments remain poorly understood, particularly regarding the efficacy of pushing-through material for removing *Salmonella* from food equipment contact surfaces.

Purpose: To evaluate the removal of *Salmonella* in two auger systems contaminated with *Salmonella*-inoculated wheat flour (WF) using dry push-through with WF and corn meal (CM).

Methods: The auger system was primed by pushing through 250g of non-inoculated WF, then contaminated with 100g of *Salmonella*-inoculated WF to mimic an adverse event caused by contaminated ingredients. Non-inoculated WF or CM (1000g) was then used to purge the auger. Triplicate samples (1–2g) were collected after various amounts (n□8) of product push-through, and the internal surfaces of the auger assembly were swabbed. *Salmonella* was enumerated on differential media, and population was compared using analysis of variance to evaluate the cleaning performance of WF and CM in seamless and multi-section sanitary tube auger setups. Three trials were performed.

Results: After 110g of push-through, >5log CFU/g of *Salmonella* removal was seen,

with remaining *Salmonella* populations ranging from 1.05 (WF in seamless) to 2.53 (CM in multi-section) log CFU/g. In the seamless setup, CM exhibited significantly higher (p<0.05) *Salmonella* decay rate than WF, but no difference was found in the multi-section tube. Auger design did not significantly affect the *Salmonella* decay rate for either WF or CM (p>0.05). However, irregularly high *Salmonella* populations were intermittently detected with multi-section tube, whereas no *Salmonella* above the limit of detection (~0.7 log CFU/g) was enumerated after 110g of push-through in seamless tube.

Significance: Although dry push-through effectively reduced *Salmonella*, even minor changes in the hygienic design of equipment can limit the effectiveness of product push-through to remove pathogens from processing equipment in LMF processing.

Award Application: Developing Scientist Award

P3-114: HARMONIZED AFNOR AND AOAC CERTIFICATION EXTENSIONS OF THE BAX® REAL-TIME PCR ASSAY FOR SALMONELLA DETECTION IN 375 G PET FOOD

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Over the past seven years, 21 of 22 pet food outbreaks were linked to *Salmonella*, demonstrating the public health risk of contaminated pet food through human illness, recalls, and economic losses. Detecting *Salmonella* in low-moisture pet food is challenging, requiring rapid, highly sensitive PCR methods to identify low-level contamination before distribution.

Purpose: This study evaluated the performance of a real-time PCR assay for the detection and molecular colony confirmation of *Salmonella* in 375 g dried pet food samples.

Methods: Method performance was assessed using relative level of detection (RLOD), inclusivity, and sensitivity studies, with results compared to FDA and ISO reference methods. RLOD and sensitivity testing employed multiple enrichment protocols followed by real-time

PCR analysis incorporating an optional DNA removal step and PCR-based colony confirmation. Additional inclusivity testing was conducted using *Salmonella* strains isolated from low-moisture matrices (n = 12), prepared at 10^6 CFU/mL. All studies were performed at ADRIA (Quimper) or Q Laboratories (Cincinnati).

Results: The assay demonstrated 100% inclusivity for target organisms, RLOD and sensitivity performance comparable to reference methods, and full agreement between PCR-based and reference colony confirmations. Difference of POD (Probability of Detection) analysis showed no statistically significant differences between alternate and references methods, with all 95% confidence intervals containing the zero point.

Significance: These results indicate that the BAX® System Real-Time PCR Assay for *Salmonella* provides a reliable approach for detecting low-level contamination in pet food, supported by flexible, globally certified enrichment protocols, a DNA removal step, and an alternative molecular colony confirmation strategy that may help reduce product release times.

P3-115: HARMONIZED AFNOR AND AOAC EXTENSION OF THE BAX® REAL-TIME PCR ASSAY FOR SALMONELLA DETECTION IN 375 G CHOCOLATE SAMPLES

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Category: Low-Water Activity Foods

Abstract:

Introduction: Although infrequent, *Salmonella* contamination in chocolate poses a significant public health risk because the low-moisture, high-fat matrix supports long-term survival, illness may result from very low-contamination levels, and routine hygiene monitoring may fail to detect the organism.

Purpose: This study evaluated the performance of a real-time PCR assay for screening and molecular colony confirmation of *Salmonella* in 375 g cacao and chocolate matrices.

Methods: Method performance was assessed using relative level of detection (RLOD), inclusivity, and sensitivity studies, with results compared to FDA and ISO reference methods. RLOD and sensitivity testing employed multiple enrichment protocols followed by real-time PCR analysis incorporating optional DNA removal and a PCR-based colony confirmation procedure. Additional inclusivity testing was performed using *Salmonella* strains isolated

from low-moisture matrices (n = 12), prepared at 10^6 CFU/mL. All studies were conducted at ADRIA (Quimper) or Q Laboratories (Cincinnati).

Results: The assay demonstrated 100% inclusivity for target organisms, RLOD and sensitivity performance consistent with reference methods, and full agreement between PCR-based and reference colony confirmations. Difference of POD (Probability of Detection) analysis showed no statistically significant differences between alternate and reference methods, with all 95% confidence intervals containing the zero point.

Significance: Collectively, results show the BAX® System Real-Time PCR Assay for *Salmonella* enables sensitive detection in chocolate matrices with flexible enrichment options, optional dead DNA removal, and molecular colony confirmation providing further ease of use.

P3-116: CO-INOCULATION METHODS FOR PATHOGENS SALMONELLA, ESCHERICHIA COLI, LISTERIA MONOCYTOGENES AND THEIR SURROGATES ENTEROCOCCUS FAECIUM AND PEDIOCOCCUS ACIDILACTICI ON DRIED LOW WATER ACTIVITY FOODS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Dried foods may be contaminated with human pathogens through transfer from dry surfaces including soil, other products or facilities surfaces during processing. Ready-to-eat dry products are often pasteurized and require validated processes.

Purpose: Develop a repeatable dry transfer inoculation method to mimic dry contamination on dried products that vary in size, shape, and proximate composition.

Methods: Pathogens (*Salmonella*, *E. coli,* and *Listeria monocytogenes*) and non-pathogenic surrogates (*Enterococcus faecium* and *Pediococcus acidilactic*i) were grown to ~10 $\log_{10}$ CFU/mL and applied to sterile silicon beads or sand, producing a target of 8.5 $\log_{10}$ CFU/mL. Carriers

were dried overnight in a biosafety cabinet. Products were combined with carriers at varying weight ratios, mixed in 50 mL tubes using a rotator (n=3) for different times and then separated by sieving. Bacteria were enumerated from products following serial dilution and plating on selective media, with incubation at 37°C for 24–48 h.

Results: Product-specific dry inoculation methods consistently achieved target levels of 6–7 $\log_{10}$ CFU/g for individual pathogens and surrogates. Product size was the most important factor for carrier selection, as void spaces influenced carrier transfer ($p < 0.05$); proximate properties also affected the carrier selection. No significant differences in bacterial load were observed among different product-to-carrier ratios ($p > 0.05$).

Significance: Dry inoculation simulates real-world contamination of dried foods, and co-inoculation with surrogates enables improved thermal inactivation studies and more reliable pathogen comparisons to enhance food safety.

P3-117: SEASONED FOR SAFETY: HERB AND SPICE VALIDATIONS USING HYGIENA'S BAX® SYSTEM METHOD TO RAPIDLY DETECT SALMONELLA

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Category: Low-Water Activity Foods

Abstract:

Introduction: Spices are often perceived as an unlikely source of foodborne illness due to their natural antimicrobial compounds; however, recent recalls and outbreaks have raised concerns about their microbiological safety. The detection of pathogens in spices remains a challenge as bioactive compounds may adversely affect microbial survival, recovery, and growth.

Purpose: This study evaluated the performance of a rapid real-time PCR method compared to the FDA BAM reference method for the detection of *Salmonella* species in a variety of herbs and spices, following AOAC INTERNATIONAL Official Methods of Analysis Appendix J.

Methods: Separate validation studies were conducted for ground basil (125 g), black pepper (375 g), cumin (375 g) and cinnamon (37.5 g). Each matrix was inoculated with a different *Salmonella* species at a low level expected to yield fractional positives and a high level expected to yield all positives. Additional samples were included as controls. Following a 2-week equilibration at room temperature, 30 test portions were prepared for each method (test and reference). Samples were enriched and analyzed according to the respective method protocols.

Results: At the low-inoculation level, the real-time PCR assay detected *Salmonella* in 5/20 ground basil samples, 12/20 black pepper samples, 18/20 cumin samples, and 15/20 cinnamon samples. At the high inoculation level, all matrices produced 5/5 positive samples, with the exception of cinnamon (4/5). All real-time PCR results were identical to culture. The probability of detection (POD) between the test and reference methods showed no significant differences at a 95% confidence level for any matrices tested.

Significance: These results demonstrate that the BAX® System Real-Time PCR assay is accurate and reliable for the detection of *Salmonella* in herb and spice matrices, with results equivalent to the reference method but with a substantially faster time to results, providing timely food safety decisions.

P3-118: MATRIX VALIDATION OF 375 G OF CREAMY PEANUT BUTTER FOR THE DETECTION OF SHIGA TOXIN-PRODUCING E. COLI USING HYGIENA'S BAX® SYSTEM METHOD

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Pathogenic *E. coli* is recognized as a "known or reasonably foreseeable food-related biological hazards" in nuts and seeds. Studies have demonstrated that this organism can survive for extended periods of time in low-moisture foods and withstand thermal processing that would typically inactivate them. Together these factors present an ongoing challenge to product safety and pose adverse implications for consumer health.

Purpose: In this study, the performance of three real-time PCR assays was compared to the FDA BAM reference method for the detection of Shiga toxin-producing *Escherichia coli* in peanut butter.

Methods: Bulk portions of peanut butter were inoculated with a heat-stressed *E. coli* O157:H7 culture to create low and high levels of contamination. Samples were blended for uniform distribution, equilibrated for 2 weeks and enumerated right before use. Thirty unpaired samples were then created for the test method (375 g) and the FDA BAM method (25 g), consisting of 5 uninoculated controls, 20 low fractional and 5 high. Test method samples were enriched in BPW and analyzed by real-time PCR. Both test method and reference method samples were confirmed by culture.

Results: For the test method, all three real-time PCR assays returned consistent positive

results for 8/20 low inoculated samples and 4/5 high inoculated samples at 20 and 24 hours. All 5 uninoculated controls returned negative results. All PCR results were identical to culture with 100% sensitivity and 100% specificity. The difference in the probability of detection (dPOD) between the test and reference method showed no significant difference.

Significance: Overall, the BAX® System Real-Time PCR assay for *E. coli* O157:H7 Exact, Real-Time *E. coli* O157:H7 and STEC Screening are validated fit-for-purpose with statistical equivalence to the reference method for the detection of STEC in 375 g samples of peanut butter.

P3-119: DETECTION OF SALMONELLA FROM 375 G OF POWDERED INFANT FORMULA USING THE HYGIENA® BAX® SYSTEM REAL-TIME PCR ASSAY

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Powdered infant formula is not a sterile product, and despite stringent manufacturing controls, it can become contaminated with foodborne pathogens. *Salmonella* has been implicated in both outbreaks and sporadic cases of illness in infants. Given the vulnerability of infants, safeguarding the microbiological safety of powdered infant formula is critical.

Purpose: This study was designed to evaluate the performance of a commercial real-time PCR assay compared to the FDA BAM reference standard for the detection of *Salmonella* from 375 g test portions of powdered infant formula using an optional secondary 3-hour regrowth.

Methods: Paired test portions were inoculated with a heat-stressed *Salmonella* Agona culture at a low level expected to yield fractional positive results (n=20) and a high level expected to be all positive (n=5). Five additional samples were left uninoculated for controls.

Samples were equilibrated at room temperature for 2 weeks and then enriched following the FDA BAM Chapter 5 reference method. Aliquots were removed and tested directly from the primary enrichment and with a secondary 3-hour BHI regrowth. All samples were confirmed by culture.

Results: For the test method, 14/20 low-inoculated and 5/5 high-inoculated samples were detected by real-time PCR, and all were confirmed to be correct by culture. There was no difference in results when processed with or without the BHI regrowth. Statistical significance was determined using the probability of detection (POD), which indicated no differences between the test and reference method results.

Significance: The study results demonstrate that the BAX System Real-Time PCR assay is sensitive, specific, and accurate for the detection of *Salmonella* species in 375 g samples of powdered infant formula in 22–26 hours.

P3-120: SAMPLE-HANDLING PARAMETERS TO SUPPORT ALMOND DRY ROASTING VALIDATION STUDIES USING ENTEROCOCCUS FAECIUM NRRL B-2354

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Almond Board of California (ABC) guidance requires removing hot inoculated sample packs from the roaster before the cooler, chilling them in an ice or chilled water bath, and then processing and microbial enumeration within 24 h. Introducing water into low-moisture facilities is problematic, and processing samples in 1 day can be logistically challenging.

Purpose: To evaluate cooling method (crushed ice and ice packs) and extended hold times on the recovery of *Enterococcus faecium* NRRL B-2354 from thermally treated almonds.

Methods: Almonds were inoculated with a lawn-harvested cell suspension of *E. faecium* and then dried to a moisture level of 4.0–5.5% at ambient temperatures. Dried almonds (100 g) were spread on a metal mesh tray, heated in a convection oven at 168°C for 12 min, held at room temperature for 30 s, transferred to sterile bags, cooled on either crushed ice or ice packs for 1 h, and then stored at 4 ± 2°C. *E. faecium* was enumerated on tryptic soy agar (TSA) and Slanetz and Bartley (S&B) agar at 0, 24, 48, and 72 h. Data from two biological replicates with triplicate samples (n = 6) were analyzed using ANOVA in JMP Student Edition 18.

Results: There was no significant difference in counts on TSA or S&B agar at any time point ($P < 0.05$). Initial counts of *E. faecium* on almonds were 8.0 ± 0.3 log CFU/g. No significant difference ($P < 0.05$) in *E. faecium* reductions was observed between the two cooling methods or across holding times up to 72 h. Mean and range reductions were 5.8 ± 0.5 (5.2–6.6), 5.2 ± 0.8 (3.9–6.5), 6.1 ± 0.8 (4.8–6.9), and 5.6 ± 0.9 (4.0–6.8) log CFU/g for samples cooled with crushed ice, and 5.5 ± 1.0 (4.3–6.7), 5.6 ± 1.0 (4.2–7.0), 5.6 ± 1.0 (4.6–7.1), and 5.2 ± 0.6 (4.0–6.3) log CFU/g for samples cooled with ice packs, at 0, 24, 48, and 72 h, respectively.

Significance: These findings support updating ABC guidance with the use of ice packs for sample cooling and by extending sample holding times to 72 h.

P3-121: AN INVESTIGATION OF SALMONELLA RICHMOND INFECTIONS LINKED TO IMPORTED MORINGA LEAF POWDER, 2025

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: In October 2025, USFDA, CDC, and state/local partners investigated a *Salmonella* Richmond outbreak linked to moringa leaf powder imported from India, resulting in 11 illnesses in seven states and three hospitalizations.

Purpose: Provide investigational findings and outcomes for an outbreak involving a rare *Salmonella* serotype in the U.S. linked to an uncommon vehicle.

Methods: An illness was defined as infection with *S*. Richmond with an isolate related by whole genome sequencing (WGS) to the outbreak strain and illness onset from May 12 to September 4, 2025. Traceback analysis and facility inspections were conducted per standard FDA practices.

Results: Of ill people with information available, 90% (9/10) specifically reported consumption of moringa leaf powder, or dietary supplements containing moringa leaf powder, prior to illness. FDA's traceback investigation included nine ill people from six states who purchased product from eight points of service (POS). Products containing a common lot of moringa leaf powder, sourced from a single manufacturer in India, supplied to the eight POS could explain all nine illnesses included in the traceback investigation. Two dietary supplement retain samples containing moringa leaf powder collected by FDA from a domestic manufacturer were positive for the outbreak strain. Open samples of three unique brands of product, either moringa leaf powder or a dietary supplement containing moringa leaf powder, collected by state partners from the household of ill people, were also positive for the outbreak strain. Four brands of moringa leaf powder, or dietary supplements, were recalled as a result of this outbreak investigation.

Significance: Epidemiologic, traceback, and laboratory evidence confirmed moringa leaf powder sourced from a single manufacturer in India was the source of this outbreak. This is the second outbreak of *Salmonella* in the U.S. linked to moringa leaf powder.

P3-122: PERSISTENCE OF CRONOBACTER SAKAZAKII BIOFILMS ON STAINLESS STEEL IN THE PRESENCE OF POWDERED INFANT FORMULA RESIDUE

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Cronobacter sakazakii* is a foodborne pathogen associated with low-moisture processing environments. The pathogen is desiccation-tolerant, especially in its biofilm state, and can persist on food contact surfaces for extended periods. However, survival outcomes can vary based on relative humidity (RH) and the presence of food residues.

Purpose: To determine the survival of *C. sakazakii* biofilms on stainless steel (SS) during storage at 33 or 53% RH with or without powdered infant formula (PIF) matrix.

Methods: Biofilms of *C. sakazakii* were prepared on SS at 7 log CFU/coupon with or without water-reconstituted PIF (RPIF). Coupons were stored at 25°C and 33 or 53% for up to 60 d. At selected intervals, triplicate coupons for each condition were rinsed with Bufferfield's phosphate buffer (BPB) to remove loosely attached cells and then coupons were massaged with BPB to dislodge biofilm cells. Both cell types were serially diluted and plated onto brain heart infusion agar. Three trials were conducted and statistical differences in populations were assessed via ANOVA with significance set at $p<0.05$.

Results: Initial *C. sakazakii* biofilm populations on SS with and without RPIF were 7.48±0.08

and 6.66±0.05 log CFU/coupon, respectively. After 7 d of storage at 33 or 53% RH, no significant differences in biofilm populations were observed on SS. After 60 d of storage at 33 and 53% RH, biofilm populations on SS without RPIF significantly reduced to 5.87±0.26 and 5.89±0.38 log CFU/coupon, respectively. With RPIF, the biofilm population on SS after 60 d of storage at 33% RH was significantly reduced to 6.84±0.23 log CFU/coupon, however the population did not significantly decrease at 53% RH (7.17±0.17 log CFU/coupon).

Significance: Results of this study indicate that *C. sakazakii* biofilms can persist on SS and that RH and food residue may contribute to survival.

P3-123: PERSISTENCE OF CRONOBACTER SAKAZAKII BIOFILMS ON STAINLESS STEEL TREATED WITH CHEMICAL SANITIZERS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: *Cronobacter sakazakii* is a desiccation-resistant pathogen that forms persistent biofilms in low-moisture processing environments. Despite routine sanitation, these biofilms may survive chemical interventions. This study aimed to evaluate the survival of biofilms on stainless steel (SS) treated with chemical sanitizers.

Purpose: To evaluate the persistence of *C. sakazakii* biofilms on SS treated with benzalkonium chloride (BZK), peroxyacetic acid (PAA), and gaseous chlorine dioxide (ClO_2).

Methods: *C. sakazakii* biofilms were established on SS at 7 log CFU/coupon and stored at 25°C for 7 d. For liquid interventions, duplicate coupons were submerged in 100 ppm of BZK or PAA for 30 or 60 s, followed by neutralization. For the gaseous intervention, duplicate coupons were subjected to ClO_2 at 2 mg/L to achieve a cumulative exposure of 1,600 ppm-h. Following treatment, cells were recovered by manual agitation of coupons for 1 min. Surviving populations were serially diluted and enumerated. Three independent trials were conducted, and data were analyzed using ANOVA ($p<0.05$) to identify significant differences in sanitizer efficacy.

Results: The *C. sakazakii* biofilm population on untreated SS was 6.47±0.40 log CFU/coupon. For the liquid interventions, BZK demonstrated a significantly higher efficacy than PAA, reducing the population to 4.19±0.45 log CFU/coupon after only 30 s (a reduction of 2.28 log CFU/coupon, compared to 0.60 log CFU/coupon for PAA). No further significant population reductions were observed when the BZK or PAA treatment times were extended to 60 s. For the gaseous intervention, limited efficacy was observed for ClO_2. The *C. sakazakii* biofilm population was reduced by 1.00 log CFU/coupon relative to the control.

Significance: Results from this study provide information on the varying persistence of *C. sakazakii* biofilms on stainless steel exposed to different sanitizer interventions.

P3-124: BEHAVIOR OF LISTERIA MONOCYTOGENES IN COCOA PRODUCTS AND CHOCOLATES UNDER DIFFERENT STORAGE CONDITIONS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: While *Salmonella* in low-moisture foods is well-studied, data on *Listeria monocytogenes* in cocoa-based products remain scarce. This knowledge gap challenges risk assessments for chocolate and cocoa ingredients.

Purpose: To evaluate *L. monocytogenes* survival in cocoa powders, chocolates, liquor, and butter under industry-relevant storage conditions.

Methods: A four-strain outbreak cocktail (10^6 CFU/g) was used for inoculation. Liquids were inoculated at storage temperature; solids at 40°C then solidified. Powders were mist inoculated. Samples were dried to original water activity and stored: liquid chocolates at 40–55°C; cocoa liquor at 50–70°C; cocoa butter at 45–70°C; solids at 15°C, RH 60%. Sampling extended up to 6 months. Enumeration used MOX/TSA overlay; ISO 11290-1 was used when no colonies were detected.

Results: Liquid milk and dark chocolates achieved >6-log reduction within 7 days, undetectable by day 11; white compound required 14–18 days. Solid chocolates needed 5–6

months for similar reductions, with occasional residual counts. Cocoa liquor showed rapid decline: natural liquor >6 log in 7 days at 50°C, 1 day at □60°C; alkalized liquor required 14 days at 50°C. All liquid liquor tested negative by 21 days; solids required 4–5 months. Cocoa butter liquids reached >6-log reduction within 1 day at □50°C; solids required 21 days (natural) or 2 months (deodorized). Cocoa powders reduced >6 log after 3 months.

Significance: These findings provide critical survival data for *L. monocytogenes* in cocoa-based products, enabling manufacturers and risk assessors to refine hazard analyses and implement targeted controls.

P3-125: ANTICIPATION OF FOOD SAFETY RISKS WITH AN EXPERT DRIVEN HORIZON SCANNING SYSTEM

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Food safety authorities and control services have a need to anticipate food safety risks in order to prepare, adapt and manage risks before they develop into a food safety crisis.

Purpose: Presentation of a method that enables systematic identification of issues and risks with a high crisis potential including measures for foodborne outbreak prevention.

Methods: A team of experts with interdisciplinary backgrounds monitors a range of information sources to identify topics with high crisis potential. Monitoring covers outbreaks and foodborne diseases, risks and issues with crisis potential that already occur, emerging risks and disease outbreaks in other countries. Observations are merged into a database that allows for further research and analysis. A root cause analyses is performed for issues and risks with high crisis potential. Results are published as reports on a website with restricted access for a network of members from food safety authorities and control services. Moreover, a quarterly newsletter informs the network.

Results: Reports cover a wide range of topics, such as foodborne infections and outbreaks caused by *Yersinia* spp., *Edwardsiella tarda, Cryptosporidium*, or agricultural biocontrol agents. Further examples are the use of toys and occupying materials in animal husbandry and the correlation between the contamination of sesame seeds with salmonella and the use of ethylene oxide.

Significance: The continuous anticipation and analysis of food safety risks, including the collection and transformation of information to intelligence, is a proactive approach. It enables authorities and food control services to take action timely and to prevent foodborne outbreaks.

P3-126: ADVANCING HACCP IMPLEMENTATION IN NIGERIA: INSIGHTS FROM A REGIONAL SURVEY

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Category: Food Safety Systems

Abstract:

Introduction: The Hazard Analysis and Critical Control Point (HACCP) system is a globally recognized preventive food safety framework designed to identify, evaluate, and control hazards throughout food production. While HACCP principles are incorporated into food safety legislation worldwide, adoption in Nigeria has been largely voluntary, driven by companies aiming to meet internal food safety objectives and enhance brand credibility.

Purpose: This study evaluates HACCP adoption and implementation among Nigerian food processors, examining their understanding of the system, operational integration, and perceived barriers. The goal is to identify challenges and opportunities for strengthening food safety practices in Nigerian food facilities.

Methods: A quantitative survey was conducted with 135 professionals across diverse sectors of the Nigerian food industry. Participants represented various food handling and processing operations and were familiar with HACCP principles and supporting food safety programs. Data were analyzed to assess certification, knowledge, and practical application of HACCP.

Results: Among respondents, 98% had formal education in quality or food safety, but only 55% had completed formal HACCP certification based on the seven principles and twelve steps. While 95% recognized HACCP’s role in identifying and controlling hazards, and 85%

understood the importance of critical control points, only 80% could identify distinct food safety hazards, and 45% demonstrated competence in developing or documenting food safety plans. Additionally, 90% cited inadequate training, education, and technical knowledge as key barriers to adoption.

Significance: The findings highlight critical gaps in training and technical capacity, underscoring the need for structured education and operational support to enhance adoption, strengthen food safety culture, and improve compliance across Nigerian food facilities.

P3-127: FRAMEWORK FOR ANONYMIZATION OF FOOD SAFETY DATA FOR PRIVACY PRESERVED DATA SHARING DEMONSTRATED ON PUBLICLY AVAILABLE DATA

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Data sharing across food safety systems can improve environmental monitoring, risk modeling, and early warning capabilities, yet concerns surrounding confidentiality and re-identification risk continue to restrict data availability.

Purpose: This study evaluates whether data privacy techniques can enable food safety data sharing while maintaining predictive model performance.

Methods: A methodological pipeline was developed to apply 3 data privacy techniques (k-anonymity, l-diversity, and t-closeness) to food safety datasets and evaluate downstream model performance. To balance the level of protection and data usability, privacy techniques were implemented using percentile-based binning rather than fixed-width intervals, allowing adaptive granularity while limiting the influence of outliers and data omission. The impact of the protection level on data usability was evaluated using 6 machine learning models, with hyperparameters tuned to optimize accuracy, and the training/testing data split of 85:15. The framework was demonstrated using 3 publicly available datasets with binary outcomes representing the presence of *Listeria* in soil (n=612), fecal contamination in watersheds (n=197), and *Salmonella* and *Campylobacter* in chicken carcasses (n=4888).

Results: On the original datasets, Logistic Regression, Neural Networks, SVM, Decision Trees,

KNN, and Gradient Boosted Classifiers achieved relatively high classification accuracy. Low (k=2–4), moderate (k=5–7), and high (k=8–10) privacy protection levels produced comparable but reduced performance across models. Similar trends were observed for precision and recall, indicating improved model stability under stronger protection.

Significance: These findings demonstrate that privacy-preserving anonymization can effectively enable safer data sharing, supporting broader collaboration in food safety surveillance and public health risk assessment.

P3-128: UTILIZING FLUORESCENCE IMAGING SYSTEMS TO DETECT FECAL CONTAMINATION ON SHELL EGGS

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Fecal contamination on eggshells is a primary route for *Salmonella* transmission, allowing the pathogen to penetrate the shell and survive inside the egg. Some of this contamination cannot be seen by the naked eye, fluorescence imaging technologies

are needed to help identify contaminated eggs before entering into the food supply.

Purpose: Visible and invisible fecal contamination detection on shell eggs with fluorescence imaging systems can improve food safety. Through data analysis of the video dataset, detection of unidentified fecal residue of shell surface can be analyzed to reduce pathogen risk found in egg products.

Methods: Fresh, shell eggs (n=360) and fecal matter were procured from a local layer operation. Fecal slurry was inoculated on the shell's surface for detection purposes. A florescence imaging system (CSI-D+) was used to collect video of the eggs divided in three categories; unwashed, fecal contaminated, and washed. Two supervised multi-class classification models were curated. A convolutional neural network CNN model architecture include spatial and temporal cues across video frames, pooling, and linear classifier-learning motion and feature vectors. The second model contained long short-term memory (LSTM) model combined with 2D-CNN to aggregate per-frame features producing a representation sequence for classification.

Results: The CSI-D+ system successfully detected contamination invisible to the naked eye. To validate this data, CNN and LSTM algorithms were assessed. Video validation analysis concluded to have a 59.2% accuracy for CNN while LSTM shown a 40.5% accuracy.

Significance: These results allows a promising stride forward in contamination detection on shell eggs which can be transversed into detection systems on conveyor belts in egg processing plants.

Award Application: Developing Scientist Award

P3-129: TECHNOLOGY-ENABLED HAND-HYGIENE COMPLIANCE: A CROSS-SECTOR REVIEW AND PRIVACY-PRESERVING MACHINE VISION EVALUATION

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Consistent and effective hand-hygiene (HH) practices are essential in food and healthcare to prevent contamination-events and healthcare-associated infections. Traditional monitoring systems face challenges in ensuring consistent HH compliance highlighting the potential for intelligent technologies to enhance safety protocols.

Purpose: This study aimed to review existing/emerging technologies supporting HH compliance and develop a generic system for real-time HH recognition.

Methods: A scoping review of peer-reviewed literature (2019–2025) in four academic databases examined technologies supporting HH compliance in food-manufacturing, food-service and healthcare. AI- and sensor-based monitoring approaches were examined, focusing on event detection, behavioral design and challenges related to accuracy, cost, scalability, deployment and privacy. In parallel, a privacy-preserving, machine vision-based

HH recognition system was implemented/evaluated using experimental video data processed in real-time.

Results: Overall, 44 studies met inclusion criteria; most frequent sources were from USA (28%), India (18%), and China (15%). Reported technologies included proximity sensors, RFID-enabled dispensers, smart wearables and AI-enabled tools. Machine vision approaches supported real-time recognition but raised privacy/practicality concerns. Sensor-based approaches often captured limited aspects of HH behavior. Implemented privacy-preserving, machine vision-based system demonstrated high recognition performance under experimental conditions, exceeding 90% across evaluation measures, operating in real-time without storing identifiable personal data.

Significance: This study demonstrates that privacy-preserving machine vision can support reliable real-time HH actions recognition while addressing key barriers including privacy/cost. The work highlights a feasible pathway for integrating intelligent monitoring technologies into food safety and healthcare environments.

P3-130: DEEP LEARNING-BASED RAPID DETECTION AND CLASSIFICATION OF BIO-AEROSOLIZED BACTERIA

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Aerosolized microbes pose significant food safety risks through cross-contamination. Current culture-based methods are labor-intensive and time-consuming, creating a need for rapid, low-cost method for detection of bio-aerosolized bacteria from environmental samples.

Purpose: To develop an AI-based approach for rapid detection and classification of aerosolized bacteria based on their microcolony morphologies.

Methods: Overnight cultures of *Salmonella enterica, Escherichia coli*, and *Pseudomonas fluorescens* were aerosolized in a closed chamber using an ultrasonic humidifier and captured on a tryptic soy agar plates. Plates were incubated at 37°C for 3 h, and microcolonies were imaged at 60X magnification. A dataset of 600 images (200/class) was split into training, validation, and testing sets (70:15:15). A Faster R-CNN object detection model was used to localize microcolonies and classify species. Performance was evaluated using mAP, precision, recall and confusion matrices across confidence thresholds.

Results: A method was developed to rapidly capture an aerosolized bacteria directly on a culture plate and form microcolonies of individual bacteria within three hours of initial capture. The model based on the white light microscopy images of the various bacterial

micro-colonies demonstrated robust identification capabilities, achieving peak performance at a 0.7 confidence threshold, with mAP 89.9% and recall 90.1%. *S. enterica* showed the highest sensitivity (recall 94.4%), while *E. coli* had the highest precision (96.4%). While lower thresholds (0.5) revealed minor phenotypic overlap between *S. enterica* and *E. coli*, raising the confidence threshold to 0.7 effectively resolved these misclassifications.

Significance: This study demonstrates an AI-based approach for rapid (3 h) detection and species-level classification bioaerosol bacteria, supporting faster contamination response and improved microbial risk management in food-processing environments.

P3-131: IMPACT OF VISUAL LAYOUT ON FOOD RECALL EFFECTIVENESS: AN EYE-TRACKING AND BEHAVIORAL OBSERVATION STUDY

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Despite having no empirical testing, effective recall communication is often assumed that the easier a message is to understand, the more likely consumers will take protective action.

Purpose: This study examined whether perceived message clarity predicts protective behavior (avoidance of recalled product), and explored the gap between recall awareness and actionable memory.

Methods: Fifty-nine adults (n=59) were randomly assigned to view one of three recall message templates embedded in a simulated food news website: an FDA-style Control, Template 1 (two-column layout), or Template 2 (stacked layout). A screen-based, eye-tracking device recorded eye movements. Participants were then asked to prepare a quick meal in the test kitchen, which was stocked with the ‘recalled’ product as well as alternatives. Post

observation, participants completed a semi-structured interview focusing on perceived clarity of the “recall” messages and their decisions while in the kitchen.

Results: Template 2 received the highest clarity ratings (84%) yet achieved only 56% product avoidance—comparable to Control (47%). Template 1, rated less clear (76%), achieved 85% avoidance (p=.019 vs. Control). Eye-tracking revealed T2 participants spent approximately 10 seconds less viewing the message than T1 participants. Interview findings highlighted this disconnect: T1 participants demonstrated higher spontaneous recall of the message (38% vs. 16% for T2) and described the template prompting active verification, while T2 participants more frequently reported understanding the message, but not connecting it to their kitchen decisions.

Significance: When trying to communicate to the public about safety information, it is important that the readers remember and comprehend the messages. The findings from this study indicate that perceived clarity and behavioral effectiveness may operate independently, suggesting the need for behavioral outcome measures when evaluating recall message design.

P3-132: EVALUATING THE RELATIONSHIP BETWEEN VISUAL FIXATION PATTERNS AND SPONTANEOUS RECALL IN FOOD SAFETY COMMUNICATIONS

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Category: Food Safety Systems

Abstract:

Introduction: While food recall notifications are a primary health tool, empirical data are limited regarding how specific message design elements influence consumer product avoidance behavior.

Purpose: This exploratory study examined how recall message format relates to visual attention, memory encoding, and subsequent product avoidance behavior to inform the development of evidence-based recall templates.

Methods: Fifty-nine adults (n = 59) were randomly assigned to view one of three recall message templates embedded in a simulated news website: an FDA-style Control, Template 1 with a (T1; two-column product-focused), or Template 2 (T2; stacked product-first). Using a deceptive design, eye tracking devices recorded fixations while participants viewed a simulated food news website containing the assigned recall message. Following exposure, participants were then observed in a test kitchen selecting ingredients for a recipe from a

pantry stocked with the "recalled" product and alternative brands. Post-observation semi-structured interviews were conducted to assess spontaneous recall and the decision-making processes used during the behavior task.

Results: Among participants who made a product selection (n = 57), T1 achieved significantly higher avoidance of the recalled product (85%, n = 17/20) compared to Control (47%, n = 9/19; p = .019), while T2 (56%, n = 10/18) did not differ significantly from Control. Eye tracking data revealed that T1 participants fixated on health risk content approximately 40% longer than Control. Qualitative interview analysis showed T1 generated the highest spontaneous recall integration (38%) compared to T2 (16%).

Significance: These findings indicate that recall message format, independent of content, significantly influences protective action. These results provide a framework for refining recall templates to improve consumer compliance and enhance public health outcomes.

P3-133: THERMAL INACTIVATION PARAMETERS OF SALMONELLA, SHIGA TOXIN-PRODUCING ESCHERICHIA COLI, AND CRONOBACTER SAKAZAKII IN WHEY PROTEIN CONCENTRATE (WPC80) DURING 120 DAYS OF STORAGE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Foodborne pathogens such as *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and *Cronobacter sakazakii* can survive in low-water activity foods such as whey protein concentrate (WPC80) powder. The ability of these pathogens to survive in desiccated, low-moisture foods poses significant public health risks when products are rehydrated or improperly handled.

Purpose: To investigate the effect of extended storage on the survivability and thermal resistance of *Salmonella*, STEC, and *C. sakazakii* in WPC80 over 120 days.

Methods: The WPC80 powder was mist-inoculated independently with an 8-serovar *Salmonella* or 6-strain STEC or 4-strain *C. sakazakii* cocktail, dried at 37°C for approximately 5 h, and stored in airtight containers. At 30-day intervals, bacterial survivability, pH, and water activity (a_w) were evaluated. Thermal inactivation studies were conducted at 80, 85, and 90°C

using thermal death time disks (5-g each) to determine *D*- and *z*-values, with temperature monitored via T-type thermocouples.

Results: After 120 days of storage, populations of *Salmonella*, STEC, and *C. sakazakii* declined by > 3 log CFU/g. the water activity of WPC80 powder changed during storage from 0.216±0.002 to 0.289±0.008 (*Salmonella*), 0.209±0.002 to 0.307±0.004 (STEC), and 0.210±0.001 to 0.318±0.004 (*C. sakazakii*). The pH of WPC80 remained comparable throughout storage. The *D*-values for *Salmonella* and *C. sakazakii* changed significantly at 80, 85, and 90°C, while STEC *D*-values remained statistically unchanged. The *z*-value of *Salmonella* decreased from 18.32±0.71°C to 11.29±0.47°C, STEC z-values remained unchanged, and *C. sakazakii* z-values increased from 10.77±0.46°C to 22.26±3.11°C.

Significance: The results strengthen predictive modeling and risk assessment for managing *Salmonella*, STEC, and *C. sakazakii* in WPC80 powders during extended storage and offer quantitative benchmarks for validating heat treatments in low-moisture protein ingredients.

P3-134: VALIDATION OF BUTTERMILK TEA BISCUIT-BAKING PROCESS TO CONTROL SALMONELLA CONTAMINATION

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* can survive for significant periods of time in low water activity (a_w) raw ingredients such as wheat flour. Furthermore, they have the ability to grow and multiply when hydrated to produce dough or batter. Therefore, effective inactivation of *Salmonella* during the baking step is critical for the safety of finished food products.

Purpose: Validate the effectiveness of a simulated commercial buttermilk tea biscuit baking process as an effective pathogen kill step for *Salmonella.*

Methods: The buttermilk tea biscuit mix was initially mist inoculated with a five serovar-cocktail of *Salmonella* and dried back to its pre-inoculation a_w level. In a stand mixer at low speed, 300 g of *Salmonella* inoculated mix was mixed with 150 ml of water to make dough. The buttermilk tea biscuit portions were baked at 400°F for 8 minutes, followed by ambient cooling for 15 minutes. Biscuit samples were collected during baking at 0, 2, 4, 6, 8, and 23 minutes for microbial enumeration, a_w, and pH analysis. The *Salmonella* population in pre-

and post-baked buttermilk tea biscuit samples were enumerated on injury recovery media and reported as colony forming units (CFU/g).

Results: The *Salmonella* population decreased from 7.99±0.02 log CFU/g to 2.87±0.08 log CFU/g after 8 min of baking at 400°F, followed by a 15 min ambient air cooling. The a_w decreased slightly during the baking process, while the pH remained statistically unchanged.

Significance: This study validates that baking buttermilk tea biscuits at 400°F for at least 8 minutes, followed by a 15-min ambient air cooling will ensure a >5 log (CFU/g) reduction in *Salmonella* population.

P3-135: VALIDATION OF PANCAKE MANUFACTURING PROCESS AND THERMAL DEATH INACTIVATION PARAMETERS OF AN EIGHT-SEROVAR SALMONELLA COCKTAIL

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* can persist for prolonged periods in low-moisture foods such as wheat flour, maintaining infectivity despite unfavorable conditions. Inadequate thermal processing of flour-based products may allow *Salmonella* to survive, increasing the risk of foodborne illness outbreaks.

Purpose: To validate pancake cooking as an effective kill step and determine thermal inactivation parameters of *Salmonella*.

Method: Wheat flour was inoculated with a freshly prepared eight-serovar *Salmonella* cocktail and dried to its original water activity (a_w) level. The inoculated flour was mixed with other ingredients to prepare the pancake batter. Batter portions were cooked on a preheated electric griddle at 350°F for 4 minutes (2 minutes per side) followed by 15-minute cooling at ambient temperature. Mean product internal temperature was recorded and samples

collected at 0, 2, 4 minutes were analyzed for pH, a_w, and *Salmonella* population. The *Salmonella* population in pre- and post-cooked samples were enumerated using injury recovery (BHI agar over-layered with XLD agar). Thermal inactivation kinetics of *Salmonella* were evaluated by heating inoculated batter in thermal death time disks at 57, 60, and 63°C.

Results: Cooking pancakes at 350°F resulted in >5 log CFU/g reduction in *Salmonella* population. The *D*-value of *Salmonella* at 57, 60, 63°C were 22.37±2.86, 8.48±.15, and 1.64±0.14 min respectively. The *z-value* of *Salmonella* was 5.36±0.35°C.

Significance: The results demonstrated that cooking pancakes at 350°F for 4 minutes served as an effective kill step, achieving >5 log CFU/g reduction in *Salmonella* population.

Award Application: Developing Scientist Award

P3-136: ENSURING MICROGREEN SAFETY: A COMPREHENSIVE REVIEW OF DECONTAMINATION STRATEGIES WITH EMPHASIS ON LED-BASED ANTIMICROBIAL TREATMENTS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Microgreens are immature and nutritious plants rich in micronutrients, and bioactive compounds. Significant knowledge gaps regarding microbial risks associated with microgreens highlight the need for risk mitigation practices, with Light Emitting Diode (LED) treatments presenting a promising opportunity.

Purpose: This review synthesizes current literature on the effects of various treatments/ conditions emphasizing LED light spectra, intensity, and photo period on microgreen growth, nutrient composition, and microbial safety.

Methods: A comprehensive review of eighty two published studies up to 2025 was carried out, focusing on microgreens grown under LED lighting using databases such as Google Scholar, PubMed, and Web of Science. The selected studies included investigations on the impact of various light spectra (e.g., red, blue, white) and intensity levels on growth, nutritional content, and microbial contamination (e.g., pathogenic *E. coli*). Photoperiod and photon flux density (PPFD), or light intensity, are the primary parameters used in previous studies to evaluate differences among treatments.

Results: Studies consistently report that light quality and intensity influence microgreen growth and nutritional value which varies with microgreen type and treatment. The limited research on food safety reveals that pathogens such as pathogenic *E. coli* can persist in microgreens, especially under suboptimal production and handling conditions. Only two research were found with focus on LED-based antimicrobial efficacy. For instance, blue LED-irradiated kale microgreens showed stronger antibacterial activity than those grown under white/red LEDs.

Significance: No microgreen-specific safety guidelines currently exist, and existing regulatory frameworks are taken from fresh produce and sprouts. This highlights a need for systematic research to develop food safety protocols for microgreen production, with LED light treatment holding significant potential.

Award Application: Developing Scientist Award

P3-137: MILLET-BASED PROBIOTIC AND ANALYSIS OF ITS INHIBITORY EFFECT ON COLON CANCER CELLS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Microbiologically safe non-dairy probiotic foods are increasingly important for food protection and public health, particularly for functional products targeting gut health and colorectal cancer (CRC) risk.

Purpose: This study developed a finger millet-based probiotic beverage and evaluated the food safety attributes and functional efficacy of *Lactobacillus* strains isolated from the product.

Methods: Finger millet malt (250 g) was heat-treated at 100°C and fermented with *Lactobacillus* spp. (10^8 CFU/mL) under aseptic conditions. Isolates were screened for Gram reaction, catalase activity, hemolysis, acid (pH 2.5) and bile (0.3%) tolerance, antimicrobial activity against foodborne pathogens, and antioxidant capacity (DPPH assay). Anticancer activity was evaluated on CT-26 colon adenocarcinoma cells (1.5×10^4 cells/well) treated with cell-free supernatant (30–80% v/v) using MTT assay. Data were analyzed by one-way ANOVA ($n = 3$, $p < 0.05$).

Results: Selected isolates were Gram-positive, catalase-negative, non-hemolytic, and showed >85% survival under simulated gastrointestinal conditions. Inhibition zones against *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* ranged from 14.2 ± 0.8 to 21.6 ± 1.1 mm. DPPH scavenging activity reached 71.8 ± 1.9%. CT-26 cell viability decreased by 68.7 ± 3.4% at 80% supernatant after 72 h ($p < 0.001$).

Significance: Millet-based probiotics showed acceptable microbiological safety and bioprotective potential, supporting their relevance to food safety-oriented functional foods and public health.

P3-138: IMPACT OF EXTRACTION METHODS ON THE ANTIMICROBIAL ACTIVITY OF EXOPOLYSACCHARIDES FROM PROBIOTIC CELLS AND SPENT CULTURE MEDIUM

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Optimizing extraction methods is essential to enhance the antimicrobial activity of bioactive exopolysaccharide (EPS) secreted by probiotics.

Purpose: Therefore, this study was conducted to evaluate the antimicrobial activity of EPS extracted from both probiotic cells and spent culture medium (SPM) using different extraction methods to determine their potential as novel antimicrobial agents.

Methods: Crude EPS were extracted from 9 probiotic strains and 3 types of SPM. Extraction was performed with or without trichloroacetic acid (TCA, 10%) treatment. Subsequently, EPS was precipitated by adding two volumes of chilled ethanol followed by incubation at 4℃ overnight. The agar disc diffusion test was performed against 7 foodborne pathogens to compare the antimicrobial activity of SPM with that of probiotic cells and to evaluate the effect of TCA treatment. The minimum inhibitory concentration (MIC) tests were determined with and without dialysis (MWCO 12 kDa) step to evaluate the necessity of purification.

Results: The results revealed that TCA treatment enhanced the antimicrobial activity of EPS.

Furthermore, the antimicrobial activity of EPS derived from SPM was generally comparable to t hat of EPS from probiotic cells. In the MIC tests for BGN4 SPM against *Escherichia coli*, the cru de EPS (without dialysis) exhibited a lower MIC (2.5%) compared to the dialyzed EPS (> 5%). Nota bly, purification via the dialysis step did not enhance the antimicrobial activity.

Significance: These findings suggest that upcycling probiotic SPM using simple extraction (TCA treatment without dialysis) is a cost-effective strategy for developing novel natural antimicrobial agents.

Award Application: Developing Scientist Award

P3-139: FERMENTATION IN NOVEL VEGETABLES FOR SAFE AND SUSTAINABLE PRESERVATION PRACTICE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Fermentation that meets food-safety guidelines is a cost-effective preservation process that can ensure food safety and can be readily practiced by both small- and large-scale producers.

Purpose: The purpose of this study was to evaluate the effects of raw material characteristics and fermentation practices on acidification dynamics and microbial safety of fermented tomatoes, onions, and carrots, and to assess food safety practices promoted in popular social media fermentation content.

Methods: The top 100 most-viewed niche vegetable and fruit fermentation videos on YouTube, TikTok, Instagram, and Facebook were systematically evaluated for the inclusion of food safety parameters, including hygiene, pH monitoring, fermentation time, and final pH targets using R Studio. Based on the results of which, fresh tomatoes, onions, and carrots from local grocery stores of Jefferson City were fermented in triplicate under controlled laboratory conditions (72°F) using 32 oz fermentation jars with 2.5% (w/w) NaCl brine, with or without 4.5% sucrose supplementation, following standard university extension guidelines. Lab-controlled fermentations were conducted for 21 days, with brine overflow monitored, and pH was measured on days 0, 7, 14, and 21 using a calibrated pH meter.

Results: The most-viewed videos on social media (n = 400) omitted pH monitoring

significantly (p = 0.009). Furthermore, the use of sensory terms varies significantly (p < 0.001) in assessing acidification levels across social media platforms. All produce that underwent controlled laboratory fermentation (n = 150) achieved a pH < 4.6 within 21 days.

Significance: Traditional fermentation practices that meet food safety guidelines ensure food safety when pH is monitored throughout the fermentation period. Popular recipes that gain attention on social media have failed to meet food safety standards.

Award Application: Developing Scientist Award

P3-140: RISK MITIGATION OF FOODBORNE PATHOGENS BY APPLYING OMICS APPROACHES WITHIN THE FOOD SYSTEM: A FOCUS ON LISTERIA MONOCYTOGENES

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: When combining culture-independent methods, such as Next Generation Sequencing (NGS), with standard food safety practices, gaps related to detection limits, quantitative estimates, and intraspecies dynamics of foodborne pathogens must be addressed to effectively refine the integrated approaches.

Purpose: This study aimed to improve detection limit, increase resolution, and investigate pathogen intraspecies dynamics by comparing NGS-based approaches—metataxonomics and shotgun metagenomics—with the culture-based ISO 11290-1:2017 method. The test pathogen investigated was *Listeria monocytogenes.*

Methods: Raw minced meat samples were inoculated with a cocktail of five serotype- and source-diverse *L. monocytogenes* strains (1–6 log CFU/mL) and selectively enriched according to ISO 11290-1:2017 in two independent replicates (R1, R2). Growth curves were obtained using ALOA agar. Colonies were randomly selected and fingerprinted by REP-PCR (R1, n=558; R2, n=258). Microbial community composition and *Listeria* spp. relative abundance were assessed by 16S rRNA (Illumina MiSeq; R1, n=69; R2, n=63). Shotgun metagenomics was performed at selected time points (R1, n=20; R2, n=3) using the NovaSeqX system.

Results: Growth curves reproduced initial inoculum levels across dilutions, with time-dependent shifts in strain dominance during enrichment. Background microbiota strongly influenced *Listeria* spp. relative abundance(%) and detection thresholds, with distinct trends between replicates. Shotgun metagenomics enabled species-level resolution, identifying two *subclades of L. monocytogenes*. Early detection was achieved at the highest inoculum level (6 log CFU/mL).

Significance: Integrating NGS-based approaches to the standard detection methods enhances sensitivity, timeliness, and resolution of *L. monocytogenes* detection. This study provides proof of concept for extending the approach to *Salmonella enterica* and other foodborne pathogens.

Award Application: Developing Scientist Award

P3-141: QUANTITATIVE RISK ASSESSMENT OF LISTERIA MONOCYTOGENES ON SNACK SANDWICHES THROUGH MICROBIOLOGICAL RISK IDENTIFICATION OF MEALS PROVIDED IN SMALL-SCALE PUBLIC FOODSERVICE SETTINGS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Small-scale child and elderly care foodservice facilities serve vulnerable populations, yet information on microbiological meal quality and risk of *Listeria monocytogene*s in snack foods remains limited.

Purpose: This study evaluated the microbiological quality of meals in small-scale child and elderly care foodservice facilities and assessed the quantitative microbial risk of *L. monocytogenes* on snack sandwiches.

Methods: Meat and vegetable side dishes served in thirteen foodservice facilities in Seoul, Gyeonggi, and Cheonan at Autumn in 2024 and Spring in 2025, respectively, were investigated. Total plate count, *Escherichia coli*, *Escherichia coli* O157:H7, and *L. monocytogenes* were enumerated and their confirmation tests were conducted with quantitative PCR (qPCR). Growth kinetics of *L. monocytogenes* on inoculated sandwiches were evaluated at 5, 12, 35, and 57℃ for 7 days. Quantitative microbial risk assessment (QMRA) was conducted with @Risk.

Results: Compliance rate was higher in meat side dishes (89%) than vegetable side dishes (80%), and in child care facilities (91%) than elderly care facilities (78%), with significantly lower *L. monocytogenes* compliance in elderly care facilities ($p < 0.05$). *L. monocytogenes* exhibited slow growth at 5°C, while growth rates increased to 0.039, 0.072, and 0.148 log CFU/g/h at 12, 25, and 37°C, respectively, reaching 8.5 log CFU/g within 72 h at 25–37°C. QMRA estimated a mean illness probability of 0.0046% per serving, increasing to 0.0104% at the 99th percentile. In the sensitivity analysis, the main influence factors were identified as initial contamination level (0.67), household storage time (0.64), and storage temperature (0.29).

Significance: These findings indicate that inadequate temperature abuse during storage and distribution can substantially increase population-level risk of *L. monocytogenes.*

Award Application: Developing Scientist Award

P3-142: ANALYSIS OF IMPORTED FOOD RECALLS IN SOUTH KOREA FROM 2015 TO 2024

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: In a globalized food supply chain, the investigation of imported foods plays a critical role in ensuring food safety. Food recalls provide valuable information for identifying recurring safety issues associated with imported foods. Despite the steady growth in the volume of imported foods in South Korea, systematic analyses of recalls involving imported foods remain limited.

Purpose: In this study, we analyzed recall data for foods imported into South Korea over a nine-year period (2015–2024).

Methods: The recall data investigated in this study (n = 1035) were collected by the Ministry of Food and Drug Safety and pre-processed to remove duplicates and standardize food types and hazard classifications. These data (n = 1027) were analyzed to identify characteristic hazards associated with different food types, recall trends caused by foodborne pathogens, and food categories associated with higher risks.

Results: Processed foods (n = 563; 54.8%) and dietary supplements (n = 170; 16.6%) were the primary categories of recalled products, while chemical hazards (n = 416; 40.5%) represented the most frequent cause of recall. Recalls for processed foods involved a diverse array of risks; specifically, chemical, biological, and physical hazards accounted for 35.7%, 32.3%, and 28.4% of cases, respectively. Conversely, dietary supplements were recalled mainly for other

factors (91.8%), with mislabeled ingredients being the primary cause. Recalls attributed to foodborne pathogens (n = 71) occurred predominantly in processed foods (76.1%) and animal products (19.7%). Major food types associated with high-risk recalls assigned to Classes 1 and 2 (n = 333) included processed foods (42.3%), produce (32.4%), and animal products (10.5%).

Significance: Our analysis identified distinct hazards and risks associated with various food categories imported into South Korea. These findings will be instrumental in devising targeted food safety management strategies.

P3-143: EVALUATING FOOD SAFETY RISKS AND MITIGATION STEPS IN LOW-MOISTURE FOOD SUPPLY CHAINS: A FOCUS ON DEVELOPING ECONOMIES

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: U.S. food import alerts permit detention when products violate safety requirements.Although low-water activity foods are generally considered microbiologically stable, they are frequently subject to import alerts due to contamination risks, chemical hazards, and preventive controls failures. Ghana, Nigeria, and Ivory Coast are critical suppliers of low-water activity foods to the United States, particularly cocoa and cocoa-based products, as well as spices, grains, oils, and dried produce. Disruptions affecting these countries therefore have outsized implications for supply chain reliability, trade continuity, and food system resilience.

Purpose: This study aims to characterize the scope, product categories, and country-level patterns of U.S. food import alerts affecting low-water activity foods exported from West Africa, to identify persistent regulatory pressure points within U.S.-bound supply chains.

Methods: U.S. food import alert records from 2002–2024 were analyzed for products originating from Ghana, Nigeria, and Ivory Coast and classified by product code, description, and country of origin. Temporal trends and product-category distributions were examined to assess long-term patterns in regulatory actions.

Results: Of 2,358 U.S. food import alerts linked to West African food imports, 1,031 (43.7%) involved low-water activity foods. Alerts were most frequent for spices and herbs (n = 326), cereals/grains (n = 224), dried fruits/veg. (n = 120), oils/fats (n = 116), seeds for consumption (n = 88), and confections/snacks (n = 74), including cocoa-based products. Ghana and Nigeria ranked among the top countries in most alerts, while Ivory Coast appeared frequently in cocoa-related alerts.

Significance: This study shows how U.S. food import alerts act as system-wide stressors, helping guide capacity building, risk communication, and targeted food safety culture improvement in key West African low-moisture food export sectors.

P3-144: RNA-SEQ-BASED TRANSCRIPTOMICS SHOWS THAT SALMONELLA TYPHIMURIUM IS EXPOSED TO OXIDATIVE STRESS DURING SURVIVAL IN CHICKEN MEAT MATRIX

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Even though chicken meat is an important source of salmonellosis, a molecular understanding of the *Salmonella* proliferation in chicken meat is lacking.

Purpose: Our aim was to obtain a molecular-level understanding of *Salmonella* survival in chicken meat using transcriptomics.

Methods: *S.* Typhimurium was inoculated on chicken tenderloins, and the growth pattern was identified using serial dilution and plating. Total RNA was isolated from *Salmonella*-spiked chicken meat at time points representing the mid-lag, mid-log, and mid-stationary phases using a commercial RNA isolation kit, sequenced on a HiSeq4000 platform, and analyzed using the DESeq2 package. Genes with an FDR-adjusted *p*-value 0.05 were considered significantly differentially expressed. The physiological relevance of the differential expression was analyzed by characterizing the growth of gene deletion mutants constructed using the λ-Red Recombination assay in chicken tenderloins. Mutants having a statistically significant growth aberration compared to the wild type, analyzed using Student's *t*-test, were considered physiologically relevant.

Results: The upregulation of genes *katE*, *katN, sodA,* and *sodC* indicates that oxidative stress is the primary stress faced by *Salmonella* during meat survival. The subsequent oxidation of the iron-responsive transcriptional repressor Fur resulted in the upregulation of the Fur regulon genes. The gene *sitA* was critical, confirming the importance of manganese transport in surviving meat-associated oxidative stress. The high upregulation of STM0347, combined with the hyper-replicative phenotype of the STM0347 mutant, suggests that STM0347 may be a food-specific DNA-binding transcriptional factor that enables *Salmonella* adaptation to foodborne stresses.

Significance: Besides gaining a molecular understanding of *Salmonella* meat survival, we also developed a database of molecular targets that can be utilized for pathogen mitigation in chicken meat.

Award Application: Developing Scientist Award

P3-145: COMPARISON OF PERSISTENCE AND VIABILITY OF LISTERIA MONOCYTOGENES ON DIFFERENT FOOD-CONTACT SURFACES

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: *Listeria monocytogenes* can persist on food-contact surfaces, with surface porosity influencing bacterial attachment and survival. Direct plating methods may fail to detect low-level or viable but non-culturable (VBNC) cells, making enrichment essential for accurate detection.

Purpose: The objective of this study was to evaluate the persistence and viability of *L. monocytogenes* on stainless steel (SS), high-density polyethylene (HDPE), rubber, and wood surfaces over 2 h to 3 weeks using direct and enrichment-based detection methods.

Methods: Sterile food-contact surface coupons were inoculated with *L. monocytogenes* Scott A and maintained under controlled conditions. Samples were collected from 2 h to 3 weeks of post-inoculation to assess persistence and viability. Direct (non-enrichment) detection was performed on samples recovered between 2 and 72 h, and the samples were processed immediately using selective media to enumerate culturable cells. Enrichment-based detection was conducted on samples collected after 1 week following ISO 11290-1 guidelines to recover stressed or low-level viable cells. PMA-qPCR was additionally used to assess VBNC cells. Experiments were performed in duplicates with three

independent replicates, and data were analyzed using ANOVA with post hoc Tukey tests (α=0.05).

Results: Following 72 h of incubation, plate count data showed statistically significant differences in *L. monocytogenes* recovery across surface materials ($p<0.05$). Pairwise comparisons indicated no significant differences between SS and rubber or between SS and HDPE ($p>0.05$). However, significant differences were observed between SS and wood, rubber and wood, rubber and HDPE, and wood and HDPE ($p<0.05$).

Significance: This work highlights the limitations of enrichment-free, culture-only detection methods and underscores the importance of integrating molecular viability assays and enrichment strategies for improved environmental monitoring of *L. monocytogenes*.

Award Application: Developing Scientist Award

P3-146: TRANSFER DYNAMICS OF LISTERIA MONOCYTOGENES BETWEEN FOOD CONTACT SURFACES AND AVOCADOS DURING PACKING PROCESS

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Cross-contamination between food contact surfaces and fresh produce remains a persistent food safety concern in avocado handling and processing.

Purpose: This study evaluated the effects of six food contact material types, three contact times, two avocado varieties, and transfer direction on *Listeria monocytogenes* transfer under simulated avocado packing conditions.

Methods: *L. monocytogenes* transfer experiments were conducted under controlled laboratory conditions using six food contact materials commonly found in produce packing environments (stainless steel, conveyor belt, plastic box, black roller, blue foam-backed curtain, and transparent strip curtain). Two avocado varieties (Hass and Fuerte) were evaluated at contact times of 36, 180, and 900 s in both material-to-avocado and avocado-to-material transfer directions. Surfaces were inoculated with *L. monocytogenes*, contacted for the specified duration, and sampled immediately after separation. Recovered populations were enumerated using Petrifilm™ plates and expressed as $\log_{10}$ transfer ratios. Data were analyzed using multi-factor ANOVA to assess the effects of material type, contact time, variety, and transfer direction.

Results: *L. monocytogenes* transfer differed significantly among food contact materials ($p <$

0.001) and increased with contact time. Material type was the dominant factor, with transfer lowest from conveyor belt material and highest from stainless steel (20.9-fold difference). Increasing contact time from 36 to 900 s increased transfer 6.1-fold. Hass avocados transferred more *L. monocytogenes* than Fuerte (p = 0.032). Overall transfer was symmetric between directions, although material- and variety-specific directional asymmetries were observed.

Significance: Surface material selection and contact time are key drivers of *L. monocytogenes* cross-contamination during avocado packing, highlighting the value of transfer-based metrics for risk-based contamination control.

Award Application: Developing Scientist Award

P3-147: DYNAMICS OF ATTACHMENT OF LISTERIA MONOCYTOGENES TO DIFFERENT TYPES OF FOOD-CONTACT SURFACES USED IN PRODUCE PACKING EQUIPMENT

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Listeria monocytogenes* can become strongly attached to food contact surfaces and resist routine sanitation, creating persistent contamination reservoirs.

Purpose: Compare *L. monocytogenes* attachment strength on six materials (stainless steel, black roller, conveyor belt, transparent strip curtain, blue foam-backed curtain, plastic box) and determine how surface properties and time influence attachment.

Methods: Six food-contact materials used in produce packing facilities were inoculated with *L. monocytogenes* (10^7 CFU/mL) and incubated at 25°C for 0, 1, 3, or 6 h. Loosely attached cells were removed by rinsing and enumerated, while strongly attached cells were recovered by sonication to calculate attachment strength (S_r = SA/(LA + SA) × 100). Materials were characterized by surface roughness (Rq), wettability (cos θ), and polarity, and data from two independent replicates with duplicate measurements (up to 96 observations) were analyzed using mixed-effects models and one-way ANOVA with Tukey's post hoc tests (α = 0.05).

Results: Attachment strength (S_r) differed significantly among materials at all time points (*p* □ 0.002), ranging from 7.5% to 91.2%, with black rollers consistently highest and transparent

strip curtains lowest. Significant material × time interactions ($p < 0.001$) demonstrated material-specific temporal changes, including a pronounced late increase on blue foam surfaces. S_r was moderately correlated with surface roughness (r = 0.41) but not with wettability or polarity, and discriminated material-associated contamination risk far better than total *L. monocytogenes* recovery (4.88–5.75 log CFU/cm^2).

Significance: Attachment strength reveals substantial differences in *L. monocytogenes* adherence among food-contact materials, offering a more practical indicator of cross-contamination risk than total CFU for guiding sanitation and material selection decisions.

Award Application: Developing Scientist Award

P3-148: BIOMAPPING SALMONELLA CONTAMINATION TRENDS THROUGHOUT A COMMERCIAL BEEF PROCESSING FACILITY

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: *Salmonella* remains a persistent safety concern in beef production, and understanding contamination changes along the processing chain is essential for control strategies.

Purpose: This study characterized *Salmonella* prevalence and contamination trends through biomapping and enviromapping within a commercial beef processing facility.

Methods: Biomapping was conducted at a commercial beef processing facility in the Western United States across three repetitions with two consecutive sampling days each, generating 219 samples prior to compositing. Environmental, carcass, trim, and lymph node samples were collected from lairage, harvest, chilling, and fabrication areas using boot swabs and sponge swabs following standardized protocols. Samples were composited in sets of three before analysis. *Salmonella* prevalence and quantification were determined using the

Hygiena BAX® System, while microbial indicators including aerobic counts, Enterobacteriaceae, and generic *Escherichia coli* were enumerated using the TEMPO® automated system (bioMérieux). Prevalence among processing areas was evaluated using Fisher's exact test ($\alpha = 0.05$).

Results: Contamination trends were observed across the processing chain. Aerobic counts, Enterobacteriaceae, generic *E. coli*, and *Salmonella* were highest in lairage areas, drains, and carcass-contact surfaces and decreased after antimicrobial interventions and throughout fabrication ($P < 0.05$). *Salmonella* prevalence was greatest at early process points, with 86% of lairage composites positive and levels up to 1.96 log CFU/sample. *Salmonella* was not detected in post-intervention areas including chilled carcasses, subprimals, trim, and fabrication environments. Limited detection occurred in mesenteric lymph nodes with 24% prevalence.

Significance: These findings show *Salmonella* contamination is concentrated at early processing points and highlight the value of biomapping to support monitoring strategies that enhance safety and health.

P3-149: DEVELOPMENT OF A BIO-BASED ANTIVIRAL COATING USING ZEIN AND CHLORINATED YEAST CELLS FOR STAINLESS STEEL FOOD-CONTACT SURFACE

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Category: Food Safety Systems

Abstract:

Introduction: Contamination of food-contact surfaces with foodborne viruses, such as norovirus, poses a serious food safety risk and economic concerns.

Purpose: This study was conducted to develop a bio-based antiviral coating that can be readily applied to stainless steel (SS) food-contact surfaces.

Methods: SS surfaces were coated with chlorinated yeast cells (YC@Cl) via a two-step brush-and-spray coating method, using zein as a polymeric binder. The antiviral activity of the coated SS surface was evaluated against bacteriophages T4 and T7 using a plaque-count assay. The mechanical resistance of the coating was assessed using the EPA-guided sponge-based abrasion test. The antiviral potential of the coated surface against norovirus was further validated using Tulane virus as a surrogate for norovirus, in the presence and absence of simulated fecal matter (SFM).

Results: A uniform zein and YC@Cl-based coating was deposited on the SS surface, with a thickness of ca. 38 ± 5.6 μm. The coated surface exhibited strong antiviral activities and

achieved more than 5-log reductions of bacteriophages T4 and T7 within 10 min. The coating formulation showed strong resistance against mechanical abrasion, and no significant loss of antiviral activities was observed even after 30 cycles of dry (480 single passes) and wet (240 single passes) abrasion. In addition, the coated SS surface exhibited strong antiviral activities against Tulane virus and achieved 2.1- and 3.0-log reductions in the presence and absence of the SFM, respectively, within 30 min.

Significance: These findings provide practical insights into developing bio-based antiviral coating formulations that can reduce the risk of viral contamination of food-contact surfaces.

P3-150: LONG-TERM SURVIVAL CHARACTERISTICS OF FOODBORNE PATHOGENS UNDER STRESS CONDITIONS DURING FROZEN STORAGE

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Presentation type/Format: Poster

Category: Food Safety Systems

Abstract:

Introduction: Frozen storage is widely used to preserve foods; however, the survival of foodborne pathogens during freezing remains a food safety concern.

Purpose: This study aimed to investigate the long-term survival characteristics of major foodborne pathogens under various stress conditions during frozen storage and to analyze survival kinetics using the Weibull model.

Methods: Five foodborne pathogens (*Salmonella* Typhimurium*, Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus,* and *Bacillus cereus*) were stored at −20°C for up to 420 days under four stress conditions (0.85% NaCl, pH 4.0 lactic acid, 3% NaCl, and 3% sucrose). Selective and non-selective media were used to evaluate bacterial survival and sublethal injury.

Results: *S*. Typhimurium exhibited the highest sensitivity to frozen storage, showing a reduction of 4.29 log CFU/mL and 2.83 log CFU/mL of sublethally injured cells after 420 days. In contrast, *S. aureus* and *L. monocytogenes* demonstrated high resistance to freezing,

maintaining viable counts of 7.90 and 6.21 log CFU/mL, respectively, after 420 days regardless of stress conditions. Notably, under 3% sucrose conditions, all five pathogens showed an increase of approximately 1.29 log CFU/mL at 420 days compared with 28 days of storage, indicating a cryoprotective effect during long-term frozen storage. The Weibull model adequately described these survival patterns ($R^2 > 0.90$), revealing non-linear inactivation trends ($n < 1$) associated with the persistence of resistant subpopulations.

Significance: These results demonstrate that frozen storage alone may be insufficient to control foodborne pathogens, particularly in sugar-rich environments, highlighting the need for improved food safety strategies.

Award Application: Developing Scientist Award

P3-151: AI-ASSISTED RISK NEGOTIATION TO SUPPORT ENVIRONMENTAL SAMPLING DECISIONS IN DAIRY PROCESSING

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Presentation type/Format: Technical

Category: Food Safety Systems

Abstract:

Introduction: Environmental sampling is central to food safety systems, yet developing appropriate plans, interpreting findings and choosing proportionate actions under time pressure is difficult, especially for microbiological hazards.

Purpose: To design and prototype a concept for an AI-assisted, negotiation-centered decision-support system to optimize environmental sampling-driven risk management in

dairy processing.

Methods: An agentic AI solution was conceptually developed, extending a previously described AI-assisted risk negotiation framework. The solution makes use of large language models, retrieval-augmented generation (RAG) over customizable knowledge bases (HACCP plans, SOPs, regulatory texts, process schemata, historical environmental sampling data) and external tools accessible via APIs. These tools include: (i) guided information capture, (ii) time-series and hotspot analysis for environmental data, (iii) simulation of predictive microbial models, (iv) multi-stakeholder negotiation agents capable of optimizing consensus finding between food safety, food production, food quality, financial and legal dimensions, and (v) dashboards that visualize generated analysis results and the rationale for recommended compromises. The system is designed to run with human oversight or, where appropriate, as a stand-alone customizable workflow.

Results: Illustrative example scenarios demonstrate how heterogeneous data, unstructured information and diverging objective function can be translated into explicit recommendations (e.g., number of samples to be collected, intensified sanitation, targeted resampling, process adjustments, root-cause investigations, hold/release decisions) without prespecifying outcomes.

Significance: A domain-specific and customizable AI-based solution exploiting the risk negotiation paradigm can improve environmental sampling and risk management decisions in dairy food safety systems while preserving accountability.

P3-152: BEST PRACTICES FOR IN VITRO BIOASSAYS TO ASSESS FOOD-RELATED COMPLEX MIXTURES: AN AOAC EUROPE INITIATIVE

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Presentation type/Format: Poster

Category: Food Law and Regulation

Abstract:

Introduction: Effect-based in vitro bioassays can support mixture risk assessment for food-related complex matrices, yet variability in sample preparation, assay qualification and data interpretation limits reliability and regulatory confidence.

Purpose: To develop harmonized best-practice guidance for applying in vitro bioassays to food and other complex mixtures across an end-to-end workflow.

Methods: AOAC Europe convened a cross-sector panel spanning environment, food/beverages and packaging, including academia, regulators, contract research organizations and suppliers. Four working groups addressed (i) sample preparation/extraction, (ii) fit-for-purpose qualification requirements for bioassays across sectors, (iii) criteria and reporting elements for mixture data interpretation, and (iv) the regulatory landscape for advancing acceptance of new approach methodologies. The groups reviewed current practices, identified critical control points that can bias outcomes, and iteratively drafted recommendations.

Results: A draft, end-to-end guideline was produced linking key decisions and minimum

reporting elements from sample handling through effect-based screening to follow-up characterization of bioactive contributors. The guideline highlights common sources of variability and misinterpretation and provides harmonized recommendations for controls, acceptance criteria and interpretation of mixture responses. Priorities for further refinement are defined, including elements needed to support validation and regulatory dialogue.

Significance: The guideline is expected to improve reproducibility and transparency of bioassay-based mixture assessment and facilitate broader uptake of NAMs in food protection and public health.

P3-153: DO YOU RECALL? BRINGING RECALL DATA UP TO DATE AND MAKING IT ACCESSIBLE

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Presentation type/Format: Poster

Category: Food Law and Regulation

Abstract:

Introduction: Analyzing the recall landscape is a perennial topic, and trends in the food safety landscape are continually reassessed. Creating a single view using multiple nations' food recall data and making it easily accessible and ready for analysis, however, has not been accomplished. The current state of data availability on a country-by-country basis is also not widely known.

Purpose: This review of the available research and data aimed to create a single, updatable repository of data, unified to allow for analysis as an aggregate, with geospatial attributes available, and with metadata describing the current state of data availability by country.

Methods: Recall histories were obtained from the U.S., UK, European Union, Australia, New Zealand, and Canada, where recall data has been consistently available. Comparable information was collected from other countries.

Results: We present a tool through which publicly available datasets on recalls are combined and mapped, and with select results presented. The detailed map shows individual data points, while the high-level map shows how data availability ranges from country to country. Natural language processing allowed the analysis of recalls with high accuracy, as well as the ability to incorporate multilingual data into the dataset. As of this submission, we are working on making mapping and data tools available through a web portal.

Significance: The variability in data influences the kinds of preventive actions taken and the

value attributed to food safety programs in the respective countries. Understanding this is of critical importance in a global and interconnected system such as the food industry. Transparency of high-level trends can help explain patterns, help with assessing risks and with making informed decisions when it comes to preventive actions, purchasing decisions, and in providing additional layers of data to support risk assessments.

P3-154: WITHDRAWN

WITHDRAWN

P3-155: DETECTION OF BACTERIAL AND FUNGAL CONTAMINATION IN FUEL SAMPLES USING THE AQUASNAP® TOTAL WATER ATP TEST

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Microorganisms, such as bacteria, yeasts, and molds, grow in fuel when they have access to both water and fuel as a nutrient source. Microorganisms that contaminate fuel cause various issues, i.e., blocked injectors. To prevent such issues, a method is required that can detect even low levels of contamination in the aqueous phase of the fuel. The AquaSnap® Total Water ATP Test, in combination with the EnSURE® Touch, is an easy-to-use test system that detects adenosine triphosphate (ATP) produced by living microorganisms.

Purpose: This study summarizes results generated using the Snap Test in combination with the fluorometer compared to an alternative microbial testing system that screens for bacterial and fungal contamination in fuel samples.

Methods: Diesel and gasoline samples were mixed 1:2 with TSB. After phase formation, the fuel phase was removed, and the aqueous phases were incubated at 30°C. After 16, 24, 48 and 72 hours, samples were analyzed using Swabs and the luminometer. In parallel, an alternative microbial method with culture plating on TSA and SDA was performed for results confirmation.

Results: After 48 to 72 hours of enrichment time, all results obtained with the ATP-based Swab analysis were in 100% agreement with the culture-based method, whereas other tested

kits showed deviations. After 16 hours of enrichment, the detection limit for bacteria in diesel samples was 10 to 100 CFU/mL. These results demonstrate the high sensitivity of this easy-to-handle ATP Test.

Significance: This Aqua-Snap ATP test from Hygiena is a highly sensitive test system for detecting microbial contamination in diesel samples. It provides positive results for microbial contamination within a maximum enrichment time of 48 hours.

P3-156: CLEANING AND SANITIZING EFFICACY AGAINST SALMONELLA ON NON-CONVENTIONAL FOOD-CONTACT SURFACES IN PRODUCE PACKINGHOUSES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Limited data exist on the efficacy of cleaning and sanitizing (C&S) for non-conventional food-contact surfaces (FCS) used in produce packinghouses.

Purpose: To evaluate the effectiveness of various C&S methods on non-conventional FCSs in the produce industry.

Methods: A *Salmonella enterica* cocktail (~9 log CFU/mL) of five serovars (Poona, Javiana, Typhimurium, Newport, Litchfield) was prepared from single colonies cultured overnight (16–18 h) in tryptic soy broth (TSB) at 37°C. Inocula were prepared in TSB or minimal medium (M9) and grown to early stationary phase at 21°C. Inoculum (0.1 mL) was applied to three FCS coupons: foam, rubber, and wood. Coupons were stored at 21°C and 50% relative humidity for 0, 12, or 168 h prior to treatment. Seven C&S treatments were evaluated: Clean; Sanitize (SaniDate, Alpet, or Oxine); and Clean+Sanitize (Clean+SaniDate, Clean+Alpet, or

Clean+Oxine). Percentage reduction was calculated relative to sham-treated controls processed in parallel. Data were analyzed using a factorial ANOVA model (7 treatments × 3 FCS × 2 growth media × 3 time points) with two-way interactions. Mean difference was further separated using Tukey's HSD (α=0.05).

Results: For the main effect of treatments, Alpet (86.44%) and Oxine (78.03%) groups had lower reductions (*P*<0.001) than other treatments (97.45%). This difference was mainly due to the significant treatment×FCS interaction (*P*<0.001). Combined C&S consistently achieved 94.98% reduction overall. Among sanitize-only treatments, SaniDate showed similar reductions (96.07%) on all FCS regardless of cleaning. Alpet was most effective on wood (96.89%), followed by rubber (90.03%), and foam (71.23%; *P*<0.001). Oxine was least effective on all FCS (79.66%) but improved to 94.98% when combined with cleaning (*P*<0.001).

Significance: Findings indicate that cleaning prior to sanitizing greatly enhances efficacy, particularly for Oxine, and FCS type affects sanitizer efficacy when used alone.

P3-157: COMPARATIVE ADHESION OF ENTEROCOCCUS FAECIUM AND NOVEL FLUORESCENT BIOMIMETIC PARTICLES TO SUPPORT SANITATION PRACTICES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Inconsistent sanitation practices in food processing environments contribute to persistent microbial adhesion on food-contact surfaces, increasing contamination risk, therefore new approaches should be explored to manage these issues to improve the sanitation and training gaps in the food industry.

Purpose: This study evaluated the adhesion-related physicochemical properties of a novel fluorescent biomimetic particle (fBMP) nanoemulsion compared with *E. faecium* to assess its suitability as a sanitation training surrogate.

Methods: The fBMP nanoemulsion was prepared by emulsifying curcumin in corn oil with potato protein and a non-ionic surfactant in phosphate buffer using sonication. Formulations were characterized for particle size, zeta potential, polydispersity index (PDI), and fluorescence stability over 30 days. Adhesion of optimized fBMP to stainless steel was compared with *E. faecium* and further evaluated using confocal microscopy. Each formulation

was measured in triplicates and analysed using two-way anova.

Results: The different fBMP formulations exhibited particle sizes of 0.2–1.0 µm and negative zeta potentials of -20 to 30 mV. Two-way ANOVA showed that surfactant ratio and curcumin concentration significantly affected particle size and zeta potential over time ($p < 0.05$). Formulations with smaller particle sizes and more negative zeta potentials were associated with greater stability and lower PDI indicating the primary determinant of stability. *E. faecium* displayed a comparable size range (0.5–1.0 µm) and surface charge (32.44 mV), with no significant difference compared to fBMP ($p > 0.05$). Confocal microscopy confirmed spherical, well-stabilized droplets with surface-associated curcumin, suggesting enhanced adhesion potential.

Significance: The fBMP nanoemulsion exhibits physicochemical and adhesion properties comparable to *E. faecium*, supporting its use as a stable, visual surrogate for sanitation training.

Award Application: Developing Scientist Award

P3-158: IMMUNOFLUORESCENCE-ASSISTED INVESTIGATION OF HUMAN ASTROVIRUS-1 IN OYSTER PROCESSING AND PRODUCTION

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Human astrovirus type 1 (HAstV-1) is an emerging foodborne virus frequently associated with shellfish consumption, yet validated inactivation strategies and infectivity-based detection methods remain limited. Oysters can bioaccumulate enteric viruses and facilitate viral persistence during processing, highlighting the need for effective control measures.

Purpose: This study evaluated the antiviral efficacy of chlorine dioxide (ClO_2) and peracetic acid (PAA) as chemical disinfectants, and UV-C irradiation as a physical treatment, against HAstV-1 applied individually or sequentially on three relevant matrices: oysters, stainless steel (SS; food-contact surface), and low-density polyethylene (LDPE; packaging surface).

Methods: Infectious viral titers were quantified using a modified immunofluorescence (IF) assay optimized for oyster matrices by adjusting virus adsorption time, antibody conditions, and buffer composition to improve reproducibility and signal clarity.

Results: On oyster tissues, ClO_2 (200 ppm, 5 min) and PAA (200 ppm, 1 min) achieved >2 $\log_{10}$ FFU/mL reductions, whereas UV-C alone showed limited efficacy (□1.8 $\log_{10}$ at 1,800 mJ/cm^2). On SS and LDPE, HAstV-1 was reduced below detection limits at □700 ppm ClO_2 and □1,500 ppm PAA on SS and □300 ppm ClO_2 and □900 ppm PAA on LDPE. Sequential treatments combining low-dose ClO_2 (50 ppm) or PAA (150 ppm) with UV-C (600 mJ/cm^2) significantly enhanced viral inactivation on oysters without affecting product quality.

Significance: These findings demonstrate that combined chemical–UV interventions, together with an optimized IF assay, provide practical strategies for controlling infectious HAstV-1 in oyster processing environments.

Award Application: Developing Scientist Award

P3-159: PATHOGEN PERSISTENCE ON PRODUCE PACKINGHOUSE FOOD-CONTACT SURFACES BASED ON TEMPERATURE AND INOCULATION METHOD

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Bacterial persistence on food-contact surfaces (FCS) is influenced by various factors, including inoculation method, temperature, and surface type.

Purpose: This study explores the effect of wet and dry inoculation on the persistence of *Listeria monocytogenes* (LM), *Salmonella*, and Shiga toxin-producing *Escherichia coli* (STEC) on FCS (stainless steel [SS], rubber, wood, high-density foam) used in produce packinghouses.

Methods: *Salmonella* (5 serovars), LM (5 serotypes), and STEC (4 strains) cocktails were prepared separately. Wet inoculum (4 log CFU/mL) was applied directly to FCS coupons, while dry inoculum (4 log CFU/g) was prepared by mixing bacterial cocktails with sterile sand, drying for 24 h at 40°C, and exposing the FCS for 2 min to the prepared sand. Coupons were stored at 21 or 27°C for 168 h (7 days). Pathogens were recovered by repeated pipetting or stomaching, serially diluted, plated on tryptic soy agar, and incubated for enumeration. Uninoculated FCS were processed in parallel.

Results: Following wet inoculation, *Salmonella* and STEC exhibited the highest D-values (time required for 90% reduction) on foam (92 to 111 h) and the lowest on wood (64 to 92 h) at 27°C. In contrast, LM persisted longer on wood (92 h) and lesser on SS (82 h). For dry inoculation, *Salmonella* persisted longer on foam (134 h) at 27°C whereas wood supported greater persistence of STEC and LM. Similar trends were observed at 21°C after wet inoculation, where all pathogens persisted longer on foam with D-values from 107 to 182 h. In contrast, the lowest D-values (43 to 47 h) at 21°C after wet inoculation were observed on wood for all pathogens. Dry inoculated FCS at 21°C showed similar findings. Overall, temperature, FCS type, and inoculation method impacted pathogen persistence in a dependent manner.

Significance: Surface type, temperature, and inoculation method influenced pathogen persistence, highlighting foam as high risk and wood as lower persistence surfaces.

P3-160: INVESTIGATION OF INDIGENOUS MICROBIAL POPULATIONS PRESENT ON WAXING ROLLER BRUSHES IN COMMERCIAL STONE FRUIT PACKINGHOUSES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Waxing roller brushes play a significant role in ensuring produce quality; however, their impact on microbial safety of fresh produce remains largely uninvestigated.

Purpose: To better evaluate the involvement of waxing roller brushes in the microbial safety of fresh produce, this project aims to characterize the microbial populations present on commercial waxing roller brushes throughout the harvesting season.

Methods: Three commercial produce packing facilities were sampled between July and September 2024. During each visit, 10 × 10 cm areas of waxing roller brushes were swabbed before and after cleaning and sanitation (n = 6 per facility per visit). Sponges were transported to the laboratory on ice and processed within 24 h to enumerate total aerobic bacteria, coliforms, and yeasts/molds using Plate Count Agar, CHROMagar ECC, and Dichloran Rose Bengal Chloramphenicol agar. In addition, 16S rRNA and ITS amplicon sequencing were employed to characterize the microbial communities present on the waxing roller brushes.

Results: At the beginning of the harvest season, APC levels on waxing roller brushes were 7.89 ± 0.46, 7.02 ± 1.35, and 7.49 ± 0.96 log CFU/area for facilities A, B, and C (before cleaning), respectively. After cleaning and sanitation, APC and mold/yeast levels remained unchanged in facilities A and B but were reduced in facility C. Similar trends were observed in August and September. A significant change in microbial richness and evenness (Shannon index, Kruskal–Wallis, $p < 0.05$) was detected only in facility B in August and in facility C in September, during which the relative abundances of Enterobacterales and Pseudomonadales dropped sharply after cleaning and sanitation.

Significance: Outcomes of this project highlight the importance of cleaning and sanitation programs applied to waxing roller brushes for protecting produce safety.

Award Application: Developing Scientist Award

P3-161: STEAM TENTING FOR SIMULTANEOUS MICROBIAL INACTIVATION AND WAX REMOVAL FROM PRODUCE POLISHING BRUSHES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Fresh produce is commonly waxed in packhouses to improve appearance and extend shelf life. However, wax polishing brushes have been reported to harbor the highest prevalence of *Listeria* spp., as wax and bacterial cells can become trapped within the brush bristles, creating a persistent contamination risk.

Purpose: This study evaluated the effectiveness of a newly developed steam tenting system designed to deliver uniform steam for efficient cleaning and sanitation of wax polishing brushes.

Methods: *Listeria innocua* ATCC 33090 (12.4 ± 0.3 CFU/mL) was used as a surrogate organism to assess microbial inactivation on polyester and polypropylene brushes, both with and without wax application (2 mg wax/g brush). The brushes were exposed to steam at 97°C for 90 seconds. The amount of wax removed from the brushes was also estimated by measuring weight change in brushes.

Results: Steam exposure at 97°C for 90 seconds resulted in no detectable survivors regardless of wax presence or brush type (polyester or polypropylene). About 90% of wax removal was observed within 1 min of steam tenting whereas commercial wax stripper applied on brushes for 10 mins could remove 72% wax from the brushes.

Significance: These findings demonstrate that steam tenting is an effective and durable sanitation strategy for eliminating microbial contaminants and removing wax from polishing brush surfaces, offering a practical alternative to traditional water- or chlorine-based cleaning methods.

P3-162: SIGNAL-DEPENDENT PHENOTYPIC SWITCHING OF SURFACE-ATTACHED ESCHERICHIA COLI DURING PRODUCE WASHING AND ITS MODULATION BY A FERMENTED NAVY BEAN EXTRACT

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Regarding the *E. coli* present on (or adhering to) the selected surface, we will adopt a contrasting approach. This involves specifically selecting the *curli* fimbriae, thereby localizing the bacteria to a single site and enhancing their survival; consequently, no results are expected to be observed in the regions situated downstream of the *E. coli*.

Purpose: This study aims to elucidate the experimental characteristics of organic *E. coli* (specifically regarding curli-related effects and functional activity) and to evaluate whether common white kidney bean extract (FBE) can modulate these factors to produce specific outcomes.

Methods: The survival of *E. coli* on lettuce surfaces was evaluated following treatment with either 0.85% NaCl or FBE (25–200 μg/mL). Planktonic and sessile *E. coli* populations were isolated separately, and the expression levels of the *csgD* and *acrB* genes were quantified using RT-qPCR. Survival rates were assessed following treatment with a low concentration of

ampicillin (8 μg/mL). Bacterial counts were measured in CFUs (n=3). In in vivo cells, the expression levels of *csgD* (2.8-fold) and *acrB* (2.7-fold) were maintained relative to those in beta cells ($p < 0.05$), while survival rates increased in the absence of ampicillin (8.9 log CFU/mL). Subsequently, when the biofilmed bacterial population was incubated with FBE (25–100 μg/mL) in the presence of ampicillin for 24 hours, the viable cell counts were observed to be 8.7, 8.5, and 1.8 log CFU/mL.

Significance: In cases of the colon presenting with surface lesions, tissue damage is driven by cellular and organ-level activity—specifically involving cellular tissue—rather than merely by mechanical forces. By identifying these determinants through the analysis of small-scale samples, this study addresses a gap in our understanding of the underlying sources of damage and offers mechanism-based strategies for mitigating associated risks.

P3-163: SURVIVAL AND INACTIVATION OF LISTERIA MONOCYTOGENES ON SOILED FOOD CONTACT SURFACES INFLUENCED BY SUBLETHAL STRESS

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Presentation type/Format: Technical

Category: Sanitation and Hygiene

Abstract:

Introduction: Exposure to sublethal sanitizer conditions may alter *Listeria monocytogenes* (*Listeria*) persistence and tolerance to antimicrobial interventions on food contact surfaces (FCS).

Purpose: This study evaluated the effects of prior sublethal oxidative (O) and acid stress (AS) on *Listeria* survival on soiled FCS, and its subsequent inactivation by lactic acid (LA; 2% v/v), peracetic acid (PAA; 120 ppm and 375 ppm), and/or ultraviolet light (UV-C; 1457 mJ/cm^2).

Methods: A 4-strain *Listeria* cocktail was subjected to sublethal oxidative (PAA, 30 s.) or acid

(LA, 15 s.) stress; sterile DI water served as the control (C). Cells were quenched with Dey/Engley broth, washed and resuspended in buffer. Stainless steel, PVC, HDPE, Teflon, and Nylon coupons (7 cm^2) were soiled with a soil-water-blueberry slurry, air-dried, and spot-inoculated (~8 logs). Coupons were kept in an environmental chamber simulating 24-h cyclic temperature and relative humidity typical of Georgia weather conditions. Coupons (n=6 per material) were sampled at 0, 8, 24, 48, 72, and 96 h post-inoculation (hpi). At 96 hpi, remaining coupons were treated with LA, PAA, UV-C, or their combinations. Survivors were enumerated on TSA and MOX and analyzed using regression and ANOVA.

Results: Time, stress, and their interaction significantly influenced *Listeria* populations ($p < 0.05$), but FCS material did not ($p > 0.05$). Initial populations averaged 8.15, 7.26, and 7.65 log CFU/coupon for C, O, and AS cells, respectively. AS declined at -0.007 log CFU/coupon·h^{-1} versus -0.017 log CFU/coupon·h^{-1} for O. At 96 hpi, *Listeria* averaged 6.30, 5.42, and 6.50 log CFU/coupon for C, O, and AS, respectively. Sanitizer efficacy was stress-dependent ($p < 0.05$). UV-C reduced *Listeria* in AS- and O- to 0.52% and 1.70% survivors, respectively, compared to >70% for C. All LA- and PAA-based treatments reduced populations to <1%.

Significance: UV efficacy depends strongly on the physiological state of *Listeria* cells.

Award Application: Developing Scientist Award

P3-164: SURVIVAL AND EFFICACY OF SANITATION STRATEGIES AGAINST CRONOBACTER SAKAZAKII IN POWDERED INFANT FORMULA AND NONFAT DRY MILK POWDER

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Effective sanitation is critical for controlling *Cronobacter sakazakii* in low-moisture foods (LMF) such as powdered infant formula (PIF) and nonfat dry milk (NFDM), which pose serious risks to neonates and infants.

Purpose: This study evaluated the survival of *C. sakazakii* in PIF and NFDM and assessed the efficacy of common sanitation practices.

Methods: Coupons inoculated with *C. sakazakii* strains (ATCC 894 and ATCC 29544, 0.5 mL) or matrix-culture slurries (1.42 g) were stored at 25°C and 30% relative humidity and enumerated over 28 days. Two sanitation strategies, isopropyl alcohol (IPA) and thermal treatments, were evaluated across three treatment groups: cleaning alone (scraping), sanitization alone (IPA treatment with 1 min contact time or oven treatment at 90°C for 20–60

min or 135°C for 3–9 min), and a combined method (cleaning + sanitization). Coupons were sampled on days 0, 1, and 5 for IPA and on day 1 for thermal treatment. Log reductions were compared using ANOVA and Tukey's test.

Results: *Cronobacter sakazakii* strains ATCC 894 and 29544 in PIF exhibited greater survivability during storage ($p<0.05$; 7.7 ± 0.5 and 7.1 ± 0.5 log CFU/cm^2, respectively) than pure cultures (6.3 ± 0.5 and 5.9 ± 0.5 log CFU/cm^2, respectively) and showed comparable survivability to NFDM slurries (6.4 ± 0.3 and 6.3 ± 0.4 log CFU/cm^2, respectively). Thermal treatment was more effective for matrix-culture slurries than for pure cultures. The efficacy of IPA treatment was vice versa. The combined method achieved the greatest reductions ($p<0.05$), ranging from 3.3 ± 0.1 to 8.1 ± 0.3 log CFU/cm^2 for IPA and 2.0 ± 0.6 to 4.7 ± 0.2 log CFU/cm^2 for thermal treatment.

Significance: Although thermal treatment outperformed IPA in the presence of food matrices, residual food reduced overall sanitation efficacy, emphasizing the importance of thorough cleaning before sanitization of production tools and surfaces.

Award Application: Developing Scientist Award

P3-165: THICKNESS AND UNIFORMITY OF SALMONELLA CULTURE ON DRY SURFACE INFLUENCE THEIR DEATH KINETICS FROM UV-C TREATMENTS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Surface sanitation is vital in healthcare and food processing. While UV-C irradiation is effective, its efficacy is often hindered by *tailing*, a non-linear inactivation behavior where a fraction of the population survives. The roles of bacterial load and spatial distribution in this phenomenon remain unconfirmed.

Purpose: This study evaluated how bacterial concentration, spatial distribution, and UV-C wavelength (254nm vs 222nm) influence light penetration, disinfection efficacy, and inactivation kinetics.

Methods: *Salmonella* Enteritidis PT30 was inoculated onto glass surfaces (15×15mm^2) using spot or spread methods at concentrations of 3.7–7.7 log CFU/cm^2. Distribution was visualized via Gram-stained microscopy and SEM. UV-C transmittance through bacterial layers was measured to quantify shielding. Samples were exposed to 254 nm and 222 nm UV-C (0–140 J/cm^2; 0–10 min). Survival was enumerated by plate counting, and kinetics were analyzed using biphasic mathematical modeling.

Results: Spread inoculation created uniform layers, while spot inoculation produced dense clusters. The penetration depth was determined to be 2.7 layers for 254 nm and 3.0 layers for 222 nm, providing a quantitative measure of intercellular shielding. 254 nm UV-C

consistently achieved higher log-reductions than 222 nm. Log-linear inactivation occurred at low- culture densities (<3.7 log), but significant tailing appeared at high densities (>7.65 log), especially with spot inoculation. The biphasic model identified a rapid initial phase followed by a slow phase (R^2>0.81 and *RMSE*<0.41).

Conclusion: UV-C efficacy is governed by bacterial load, spatial uniformity, and wavelength. Aggregation significantly limits UV penetration through intercellular shielding.

Significance: This study provides direct evidence of shielding as a primary mechanism for tailing, informing the optimization of disinfection protocols in critical industries.

Award Application: Developing Scientist Award

P3-166: CONTINUOUS WAVE (CW) LASERS ARE A RAPID, TARGETED THERMAL SURFACE SANITIZATION INTERVENTION

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Lasers are a precise and targeted thermal source for surface modification, but have not been assessed for microbial surface sanitization in food processing.

Purpose: To assess the efficacy of a near-infrared laser for microbial surface sanitization.

Methods: Sterilized stainless steel surfaces (304, 2B finish, *l* × *w* × *h*: 3.5 × 2.5 × 1 cm) were spot inoculated (20 µL) with *Bacillus spizizenii* ATCC 6633 spores or *Enterococcus faecium* NRRL B-2354, then air dried for 30 min. Inoculated surfaces were chilled (4°C, 24 h) until treatment with a CW fiber laser focused to a spot (1.66 mm), delivered to the surface by a galvo-style optical scanner using the following parameters: (i) 800W, 800 mm/s (1.89 s) or (ii) 200W, 100 mm/s (9.34 s) following a (iii) unidirectional or (iv) bidirectional scan path. Surfaces were massaged for 1 min in 10 mL PBS within Whirl-Pak bags, serial diluted onto BHI agar, then incubated at 37 and 35°C for 24 h for *E. faecium* and *B. spizizenii*, respectively. Treatments

were performed in triplicate. ANOVA and Tukey tests were performed. Non-fat dry milk (3:1 NFDM:water) was spread (1 g) on additional surfaces and equilibrated at 20°C for 5 h.

Results: Peak surface temperatures were estimated at 300–400°C. Method (ii) achieved more microbial reduction. Specifically, the highest microbial reductions were 7.2 ± 0.1 and 5.9 ± 0.6 log CFU/cm^2 for *E. faecium* and *B. spizizenii*, respectively (p<0.05). Similarly, a unidirectional scan path significantly increased *E. faecium* reduction by 4.0 ± 0.1 log CFU/cm^2, in comparison bidirectionality (p<0.05). However, unidirectionality during method (i) significantly decreased *B. spizizenii* reductions by 1.8 ± 0.4 log CFU/cm^2 (p<0.05). Treatments mildly altered surface coloration. No conditions removed surface-adhered NFDM despite repeated passes.

Significance: Optimizing laser scan paths and thermal profiles would improve surface sanitization and reduce surface modifications by limiting the peak temperature.

P3-167: ULTRAVIOLET INACTIVATION (UV-C) OF VIRAL DROPLETS ON FOOD CONTACT SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Respiratory viruses are frequently present in air as aerosols and can deposit as droplets on food-contact surfaces in industrial environments. The presence of virus-laden droplets on these surfaces poses a significant health risk to both consumers and workers.

Purpose: This study evaluates the effectiveness of UV-C treatment for inactivating virus droplets on food-contact surfaces.

Methods: Bacteriophage MS2, commonly used as a viral surrogate with an initial concentration of approximately 10^{10} PFU·mL^{-1}, was used in this study. To simulate realistic respiratory droplet conditions, MS2 was suspended in a custom-modified artificial saliva solution. Stainless steel (316L) and silicone rubber coupons (0.5-inch diameter) were used as food-contact surfaces. Four droplets (5 µL each) of the MS2-containing artificial saliva were deposited onto each coupon. The coupons were placed in a biosafety cabinet until the droplets were completely dried, leaving a uniform residue. Surface disinfection was performed using a 279 nm UV-LED source, and viral inactivation was quantified by spot plate assays using the appropriate *Escherichia coli* host cells.

Results: The initial viral load on the coupons prior to treatment was approximately 6 log PFU·mL^{-1}. The surface coupons were exposed to UV-C doses of 32, 64, 96, and 128 mJ·cm^{-2}. At the highest applied dose, maximum log reductions of 3.40 ± 0.15 log PFU·mL^{-1} and 3.09 ± 0.44 log PFU·mL^{-1} were achieved on stainless steel and silicone rubber surfaces, respectively. All UV-C doses resulted in statistically significant reductions compared to the untreated control, demonstrating effective viral inactivation. The inactivation kinetics followed a log-linear model, with adjusted R^2 values exceeding 0.92 for both stainless steel and silicone rubber coupons.

Significance: This research demonstrates the feasibility of applying UV-C based surface disinfection solutions to achieve effective viral inactivation under real-world conditions.

Award Application: Developing Scientist Award

P3-168: ANTIMICROBIAL EFFICACY OF PLASMA-ACTIVATED WATER AGAINST LISTERIA INNOCUA ON FOOD-CONTACT SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Clean-in-place (CIP) practices using chemical sanitizers may leave harmful residues on the food-contact surfaces, raising concerns about food safety. These residual chemicals can contaminate the food product and ultimately can affect human health upon consumption. Plasma-activated water (PAW) offers an eco-friendly alternative for sanitizing food-contact surfaces without leaving hazardous residues.

Purpose: To assess the effect of PAW on sanitizing stainless steel surfaces for reducing *Listeria innocua*–non-pathogenic surrogate of *Listeria monocytogenes*.

Methods: Sterilized stainless-steel coupons (4cm×2.5cm×0.1cm) were inoculated with approx. 7.5 log CFU/coupon of *L. innocua* and air-dried (16 hr at 4^{o}C) prior to treatment. Coupons were immersed and agitated in 25 mL of PAW for 3, 5, and 7 min. PAW was generated in batch mode using a high-voltage atmospheric cold plasma system at 50 and 60 kV, with plasma exposure times of 2, 4, and 6 min. Following treatment, coupons were analyzed for survival microbial populations. Reactive species such as NO_3^-, NO_2^-, H_2O_2, and O_3 in PAW were quantified to establish correlations with antimicrobial efficacy.

Results: Increasing the voltage and exposure time during PAW generation, along with longer coupon soaking times, resulted in significantly enhanced microbial reductions. PAW produced at 60 kV for 6 min achieved complete inactivation (p<0.05) of *L. innocua* across all soaking times. In contrast, PAW generated at 50 kV for 6 min resulted in residual microbial loads of 2.15, 0.84, and 0 log CFU/coupon after 3, 5, and 7 min of soaking, respectively. This difference correlated well with the higher concentrations of reactive species (NO_3- and H_2O_2) generated at 60 kV PAW compared to lower-voltage PAW, as further supported by the lower pH (3.6) and increased electrical conductivity (230 µS/cm).

Significance: Overall, this study demonstrates that PAW is an environmentally friendly CIP approach for food-contact surfaces.

P3-169: DISRUPTION OF FUNGAL STRESS ADAPTATION PATHWAYS BY A FERMENTED NAVY BEAN EXTRACT UNDER SANITATION-RELEVANT CONDITIONS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: During sanitation processes, spoilage molds are frequently exposed to sublethal stresses, enabling their survival and regrowth following treatment. These responses are generally associated with the maintenance of cell wall integrity and the activation of stress signaling pathways, supporting the persistence of molds after sanitation.

Purpose: Investigate whether fermented navy bean extract (FBE) disrupts fungal cell wall homeostasis and stress adaptation mechanisms—specifically by modulating the expression of the key regulatory genes *Fks1* and *HOG1*—and thereby limits the post-sanitation recovery of post-harvest spoilage fungi.

Methods: *Aspergillus niger* and *Fusarium oxysporum* were treated with FBE at concentrations ranging from 100 to 400 μg/mL. Transcriptional responses related to cell wall integrity and stress adaptation were assessed through qRT-PCR analysis of *Fks1* and *HOG1*. Morphological changes following FBE exposure were observed using light microscopy.

Results: Treatment with 100 μg/mL FBE completely inhibited the mycelial growth of both

fungal species, compared to the untreated control group. In *A. niger*, *Fks1* expression increased at concentrations of 200–400 μg/mL, and significant upregulation of HOG1 was observed at 200 μg/mL. *F. oxysporum* exposed to FBE exhibited distinct morphological changes including hyphal fragmentation, structural irregularities, and an increased accumulation of spores and cellular debris. These changes indicate that cell wall-associated and stress response pathways were continuously activated under FBE exposure.

Significance: These findings demonstrate that FBE limits the recovery capacity of spoilage molds following sanitation, indicating that it acts as more than just a growth inhibitor. By influencing fungal responses related to cell wall maintenance and stress adaptation, FBE can reduce the persistence of spoilage molds after sanitation and support improved hygiene strategies in food-handling environments.

P3-170: EVALUATION OF 222 NM FAR-UVC LIGHT FOR INACTIVATING BACTERIA ON READY-TO-EAT DELI HAM IN VARIOUS PACKAGING TYPES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Far-UVC irradiation at 222 nm has been found effective as a non-thermal approach to reducing microbial contamination on food surfaces. However, its efficacy when applied through common food-packaging materials has not been well characterized.

Purpose: This study evaluated how effectively 222 nm far-UVC treatment could inactivate bacteria on ready-to-eat (RTE) deli ham, whether packaged in materials with varying levels of far-UVC transmittance, or unpackaged.

Methods: The team first used a UV-visible spectrophotometer to measure the absorbance at 222 nm of common food-packaging materials including low-density polyethylene (LDPE), polyvinyl chloride (PVC), polypropylene, and polyethylene. LDPE was selected as the most far-UVC transmissive of the materials, and PVC as the least transmissive. Thin RTE ham slices inoculated with *Escherichia coli* O157:H7 or *Listeria monocytogenes* and packaged in LDPE or PVC were then treated using a 222 nm far-UVC lamp for 0, 1, 3, 5, or 7 minutes, with unpackaged RTE ham serving as the control. Surviving bacterial populations were reported as log CFU per gram.

Results: Treatment effectiveness was strongly influenced by the packaging material. Samples packaged in LDPE underwent measurable microbial reductions after far-UVC exposure. After 7 minutes of treatment, reductions were approximately 1.8 log CFU/g in the case of *L. monocytogenes* and 1.3 log CFU/g in the case of *E. coli* O157:H7. Such reductions were not statistically different to those observed in the unpackaged control samples. In contrast, samples packaged in PVC exhibited little or no microbial inactivation after the same treatment.

Significance: These findings highlight that transmittance of packaging materials critically determines whether far-UVC decontamination of packaged food will be effective. When paired with far-UVC-transmissive material such as LDPE, 222 nm far-UVC has potential as an effective post-packaging intervention to enhance the safety of deli meats.

Award Application: Developing Scientist Award

P3-171: EVALUATION OF THE MICROBIAL-INACTIVATION EFFICACY OF A 222-NM FAR-UVC LAMP-MOUNTED CONVEYOR-BELT SYSTEM AGAINST ESCHERICHIA COLI K-12 AND LISTERIA INNOCUA ON FOOD-CONTACT SURFACES

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: In the food and agricultural industries, microbial safety is critically important, notably on the conveyor belts that are widely used for transporting products. Far-UVC light at 222 nm is emerging as a promising intervention for surface disinfection.

Purpose: The team designed and built a proof-of-concept conveyor-belt system equipped with multiple 222-nm Far-UVC lamps and tested its efficacy against *Escherichia coli* K-12 and *Listeria innocua* contamination.

Methods: The conveyor-belt system's multiple 222-nm Far-UVC lamps were positioned above, alongside, and beneath its belt (59 inches in length). As stainless steel is widely used

for food-contact applications, 5 × 5 cm stainless-steel coupons were used as the target objects. Each coupon was inoculated with approximately 8 log CFU/mL of *Escherichia coli* K-12 and *Listeria innocua* and air-dried prior to treatment. As an inoculated coupon was conveyed through the system, Far-UVC treatment was conducted at one of three speeds: 13 cm/sec, 9.5 cm/sec, or 6 cm/sec. Microbial count was quantified using a plate-counting method and expressed as CFU/coupon.

Results: Reductions for *E. coli* K-12 ranged from approximately 1.5 to 2.5 log CFU/coupon across the high, medium, and low conveyor speeds, with slower speeds yielding better inactivation results. For *L. innocua*, reductions ranged from approximately 1 to 2 log CFU/coupon, again with slower belt speeds resulting in better outcomes.

Significance: This proof-of-concept study demonstrates that a conveyor-belt system incorporating 222 nm far-UVC lamps can effectively achieve microbial inactivation. The length of the conveyor system and its belt speed were identified as key parameters influencing disinfection efficacy. As such, it offers a robust model for enhancing food safety in continuous-processing environments.

P3-172: EFFICACY OF ACIDIFIED OIL EMULSION AGAINST ENTEROCOCCUS FAECIUM BY TEMPERATURE AND LACTIC ACID CONCENTRATION

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Wet sanitation can inadvertently increase the risk of microbial harborage in dry facilities. Acidified oil emulsions are one method of low-moisture sanitization, for which *Enterococcus faecium* NRRL B-2354 has been used as a conservative surrogate for *Salmonella*. The systems with certain organic acids, however, can be pungent given temperature elevation.

Purpose: Assess the antimicrobial efficacy of lactic acid-acidified oil emulsions.

Methods: Customer discovery interviews were conducted with 12 experts in dry sanitation. Water-in-oil emulsions were formulated with 1.5% sorbitan monooleate, 3% water, and varying levels of lactic acid (50, 125, 200mM). Lawn-based inoculum of *E. faecium* was acclimated at 33% ERH (22°C, 24h) on SS surfaces. The survival cell numbers after desiccation (*N*) were 7.7±0.1 log CFU/coupon. Antimicrobial assay was performed as a 10min immersion test (10mL) at varying temperatures (22, 45, 70°C). Treated specimens were transferred to TSB (30mL) for enumeration/detection. When a specimen was undetected by enrichment (37°C, 24h), the microbial log reduction (MLR) was interpreted as *N*. When a specimen was undetected by plating on TSA (the detection limit (DL) was 2.5 log) but remained positive

upon enrichment, the MLR was interpreted as *N*–DL.

Results: Interviews indicated a need for a cost-effective, low-flammability dry sanitizer, and prompted OleAcis formulation with lactic acid. The surfactant concentration was selected based on preliminary stability test where the 200mM acidified emulsion held at 70°C for 1h did not separate. With a 10min treatment, non-acidified oil and emulsion controls produced little to no MLR (<0.2) at all test temperatures, except the emulsion at 70°C showing an MLR of 6.1. With 50, 125, and 200mM acidified emulsions, the respective MLRs were 0.1, 0.2, and 0.4 at 22°C; 2.9, 5.3, and 6.1 at 45°C; and all >7.7 at 70°C.

Significance: Lactic acid-acidified oil emulsions are effective against desiccated *E. faecium*.

P3-173: EFFICACY OF CLEANING AND SANITIZING TREATMENTS ON SOILED FOOD CONTACT SURFACES AGAINST SALMONELLA ENTERICA

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Soiled food contact surfaces (FCSs) in fresh produce facilities challenge sanitation programs, as organic residues can shield pathogens and reduce sanitizer efficacy.

Purpose: This study evaluated the efficacy of liquid and foam-based detergents combined with commonly used sanitizers to reduce *Salmonella* on artificially soiled FCSs using microbial enumeration and ATP bioluminescence.

Methods: Polypropylene interlocking belt (IB) and polyvinyl chloride (PVC) roller coupons were artificially soiled with a sterile 10% (w/v) soil slurry, inoculated with ~10 log CFU of a *Salmonella* cocktail consisting of five produce-related outbreak strains adapted to 80 ppm rifampicin, and allowed to dry for 1 h in a biosafety cabinet. FCSs were cleaned using detergent applied either as a liquid with 30 s manual brushing or as foam without brushing, then rinsed with water and sanitized with free chlorine or peroxyacetic acid (350 ppm) or no sanitizer. A control group received no treatment. Coupons were neutralized in 100 mL Dey-Engley broth with 0.1% (v/v) Tween 80 and spiral-plated onto tryptic soy agar with rifampicin (TSAR). Plates were incubated at 37°C and enumerated after 24 h. ATP bioluminescence was

measured before and after treatment. Nine samples per treatment were analyzed using a mixed model with ANOVA.

Results: All treatments significantly reduced *Salmonella* relative to the untreated control ($p<0.001$). Foam-based combinations achieved the highest reductions (3.57–4.32 log CFU/coupon), while liquid detergent-based treatments were moderately effective (3.48–4.16 log CFU/coupon). No material-specific differences were observed ($p>0.05$), and ATP results followed similar trends.

Significance: Foam-based detergent cleaning improved sanitation efficacy over liquid detergent, while eliminating the need for manual brushing.

Award Application: Developing Scientist Award

P3-174: THE HYGIENE CONDITIONS OF CONVENTIONAL VS. MODIFIED BLUEBERRY MACHINE HARVESTERS

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Presentation type/Format: Poster

Category: Sanitation and Hygiene

Abstract:

Introduction: Consumption of fresh berries sometimes poses potential food safety challenges. Harvesting equipment represents a critical control point for berry contamination.

Purpose: This study evaluated the effects of conventional vs. modified OTR harvester type, surface site, and pre- vs. post-cleaning sanitation status on microbial contamination levels.

Methods: A total of 144 surface swab samples were collected from 9 sites on conventional (n = 4) and modified harvesters (n = 4) at two berry farms after 9 hours of operation and following cleaning and sanitation. Samples were analyzed for total aerobes (TA), yeasts and molds (YM), total coliforms (TC), and the presence of enterococci (EC) and fecal coliforms (FC).

Results: The surface of modified harvesters exhibited significantly higher TA, YM, and TC counts than that of conventional harvesters ($P \leq 0.05$). However, for both harvester types, cleaning reduced average TA counts to below 2 log CFU/cm^2. Among site-specific factors, catch plates, as well as the horizontal and vertical conveyor belts, recorded significantly higher TA and YM microbial counts than other sampled sites. EC and FC were detected in 4–9% of samples from conventional harvesters and 18–20% from modified harvesters. Detection of EC decreased from 26% (uncleaned) to 4% (cleaned), whereas FC remained at 11% regardless of cleaning status.

Significance: The study highlights modified harvester designs, while improving fruit quality, introduce complex surfaces that challenge equipment hygiene. These findings emphasize the need for equipment-specific cleaning and sanitation strategies targeting critical contamination sites such as catch plates and conveyor systems.

Award Application: Developing Scientist Award

P3-175: SANITIZER CHEMISTRY IMPACTS GROWTH AND CROSS-CONTAMINATION RISK OF SALMONELLA ENTERICA SEROVAR TYPHIMURIUM IN THE SANITATION BUCKET SYSTEM

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Presentation type/Format: Technical

Category: Retail and Food Service Safety

Abstract:

Introduction: Despite routine use of cloth-and-bucket with sanitizers for food contact surfaces in retail environments, limited evidence is available on the effectiveness of these systems.

Purpose: To assess the risk of *Salmonella enterica* serovar Typhimurium survival, growth, and subsequent cross-contamination in sanitizer bucket systems using various sanitizers and cloth materials.

Methods: A *S. enterica* serovar Typhimurium overnight culture (~16h, tryptic soy broth,

36±1°C, 200rpm) was prepared with soil load (bovine serum albumin, yeast extract, mucin) and ~10^6 CFU/ml was inoculated onto Formica boards in 5×10µL aliquots. Three sanitizers (chlorine-, quaternary ammonium-, and acid/anionic surfactant-based) were diluted per food contact surface protocols with yeast extract soil load and applied via cloth (non-woven, cotton, or microfiber). Following contact time, inoculation zones were swabbed and 4mL of bucket contents transferred to 36mL neutralizing buffer. Boards were sterilized and the protocol repeated for cross-contamination. Samples were serially diluted in PBS, filtered onto tryptic soy agar, and incubated ~24h. Each sample was tested in triplicate in three independent experimental runs.

Results: A quaternary ammonium-based sanitizer had the greatest efficacy on contaminated boards (3.67±0.76 $\log_{10}$ CFU/mL) and the least cross-contamination onto sterile boards (below limit of detection ($\log_{10}$<0.05; *P*<0.05)). Bacterial load in the sanitation bucket significantly increased after the cloth was exposed to each board (*P*<0.05). Soil load decreased chlorine ppm and increased acid and anionic surfactant-based sanitizer pH, reducing their efficacy (*P*<0.05). Statistical analysis was conducted using SAS version 9.4 (SAS Institute).

Significance: This study emphasizes the risk of cross-contamination in retail food systems when utilizing the sanitation bucket system. Sanitizer chemistry is critical to product selection for efficacy of food contact surface sanitation.

Award Application: Developing Scientist Award

P3-176: FOOD SAFETY AND MICROBIAL QUALITY IN RURAL RETAIL ESTABLISHMENTS

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Previous research suggests that food quality and safety at the retail level can vary based on store size, infrastructure, and available resources, particularly in urban environments. However, limited research has examined microbial food quality and safety in rural retail settings, especially in low-income communities.

Purpose: This study evaluated whether food retailers in low-income rural areas pose a greater risk of reduced microbial food quality or safety compared with retailers in higher-income suburban areas.

Methods: Ready-to-eat (RTE) and perishable foods were collected from three supermarkets in the greater Houston area and three supermarkets in rural counties between June and December 2025, using USDA rural–urban classifications and the Food Access Research Atlas. Foods were sampled in triplicate and included mixed salad greens, grapes, strawberries, sliced deli meat, ground beef, milk, and cheese, yielding 144 samples. Culture-based microbiological methods were used to measure aerobic plate counts (APC), coliforms, *Escherichia coli*, yeasts and molds, and *Salmonella* spp.

Results: *Salmonella* spp. and *E. coli* were not detected in any samples. Mean APC levels in milk were 3.43 log CFU/mL from rural stores and 3.79 log CFU/mL from non-rural stores (p = 0.78). Salad greens showed APC levels of 7.03 log CFU/g from rural stores and 6.08 log CFU/g from non-rural stores (p = 0.68). Deli meat showed the largest difference, with rural stores averaging 4.57 log CFU/g compared with 2.29 log CFU/g in non-rural stores (p = 0.22). Yeasts and molds were detected in fruit and cheese samples from both store types.

Significance: Overall, statistical analysis indicated no significant differences in microbial food quality indicators between rural low-income and suburban high-income retail environments ($p > 0.05$), differing from what has been observed in urban areas of low income, low food access.

Award Application: Developing Scientist Award

P3-177: LEAFY GREENS AT RETAIL: HANDLING PRACTICES AND FOOD SAFETY IMPLICATIONS

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Leafy green vegetables are widely consumed for their nutritional benefits but are highly susceptible to contamination by foodborne pathogens. Contamination at the point of sale is particularly concerning as it represents the final stage before consumption, since leafy greens are often eaten raw.

Purpose: This study aimed to assess retail handling practices for leafy greens and evaluate their potential impact through observational assessments and microbiological analysis.

Methods: A cross-sectional observational study was conducted at 5 retail establishments selling fresh leafy greens in Oklahoma County. Handling practices were evaluated using a standardized checklist focusing on display conditions, hygiene practices, temperature control, and handling behaviors. Leafy green samples were collected aseptically from retail displays and analyzed using standard microbiological methods.

Results: Variability in handling practices was observed across grocery stores and farmers' market. Certain practices indicated increased contamination risk, including water dripping

between shelves, storage with root vegetables, missing date labels, and improper misting. A total of 14 leafy green samples (curly mustard, collard greens, lettuce, kale and turnip greens) were collected, where indicator microorganisms were detected at varying levels. Coliform counts ranged from 1–360 CFU/mL, highest in collard greens. Generic *E. coli* was detected in low levels, highest in curly mustard (70 CFU/mL). Aerobic bacteria ranged from 2.14–3.98 log CFU/g. Streptococci colonies (1,738 and 2,480) were observed in undiluted kale samples. Pathogenic *E. coli* O157:H7 and *Salmonella* were not detected. Positive correlation was observed between handling practices and increased microbial load.

Significance: These findings highlight the importance of handling practices and support targeted food safety interventions, and employee training to reduce contamination risk at the point of sale.

Award Application: Developing Scientist Award

P3-178: BEHAVIORAL BARRIERS SUSTAINING REACTIVE FOOD SAFETY CULTURE IN HOSPITAL CATERING: A COM-B ANALYSIS

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Barriers related to capability, opportunity, and motivation play are central in food safety in high-risk environments characterized by a reactive food safety culture (FS-Culture).

Purpose: To analyze behavioral barriers in capability, opportunity and motivation that sustain a reactive FS-Culture in a hospital catering in Brazil.

Methods: A qualitative study was conducted in a hospital catering using semi-structured interviews (7 questions) guided by the COM-B model. Data were collected through audio-recorded interviews, transcribed, and analyzed using content analysis with deductive content analysis. Ethical approval was obtained from the Brazilian Ethics Committee (79676724.3.0000.5440).

Results: We interviewed 28 participants (19 food handlers; 9 managers). Three categories of barriers and 16 cores of meaning were organized: 1) Capability (reliance on experiential learning; inconsistent sanitization practices; improper chlorine use; failures in temperature

control; cross-contamination risks; gaps in supervisory and interpersonal skills), 2) Opportunity (inadequate infrastructure; limited space and equipment; unstable material supply; high staff turnover and understaffing; emotional overload; resistance to change; insufficient interventions), and 3) Motivational (fluctuating engagement with food safety; reinforcement of practices mainly during inspection; weak perceived connection between tasks and consumers health). The interaction between Capability and Opportunity barriers undermined Motivational and reinforced a reactive FS-Culture.

Significance: The findings highlight deeply rooted beliefs and habits that sustain inconsistent food safety practices and perpetuate a reactive FS-Culture. The COM-B framework proved to be a valuable structured approach for identifying and understanding behavioral barriers to food safety in hospital foodservice environments.

P3-179: THE LEVELS OF RESIDUES ON HARVESTED SPROUTS GROWN FROM ASCAROSIDE #18-TREATED SEEDS DURING THE SPROUTING PROCESS

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Previous experiments have confirmed that treating sprouting seeds with ascaroside (ascr) #18 can control the populations of foodborne pathogens on harvested sprouts. Continuous exposure to ascr during sprouting sometimes increased the efficacy of the treatment. However, the levels of ascr residue on harvested sprouts have not been determined.

Purpose: This study measured the residues of ascr #18 and ascr #9, a metabolite of ascr #18, on sprouts during the sprouting process.

Methods: Sprouts grown from 1 mM ascr #18 (in 1% ethanol) treated alfalfa, fenugreek, lettuce, radish, and spinach seeds on 1% water agar with or without added ascr #18 were used as experimental materials. On sprouting days 0, 4, and 7, samples were collected and flash-frozen on dry ice before being ground with a pestle. Next, ascr was extracted and analyzed using liquid chromatography-mass spectrometry. Sprouts grown from seeds treated with 1% ethanol and water served as controls. Each sample unit included 3 replications and data was subjected to ANOVA and LSD at a significance level of $p<0.05$.

Results: Spinach and lettuce seedlings had higher levels of the tested residues, while alfalfa sprouts had the lowest levels of the residues. On all 5 types of sprouts/seedlings, those

grown on water agar with 1 mM ascr #18 had higher ascr residues, exceeding the levels of both controls (p<0.05). However, samples from water agar without added ascr and 2 types of control sprouts had similar levels of tested residues. On individual sprouting days 4 and 7, similar phenomena were observed.

Significance: The study suggests that ascr residue accumulation is affected by ascr exposure, sprouting time, and seed type. It is encouraging that sprouts grown on water agar without added ascr had similar levels of ascr #18 and #9 residues to the 2 types of controls. These results suggest that ascr treatment could be used as a viable intervention to control pathogen growth on vegetable sprouts and seedlings.

Award Application: Developing Scientist Award

P3-180: FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF HOSPITAL FOODSERVICE MANAGERS: A CROSS-SECTIONAL STUDY IN PAKISTAN

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Hospital foodservices are critical for protecting vulnerable populations from foodborne illness, and foodservice managers play a key role in maintaining food safety standards.

Purpose: This study assessed food safety knowledge, attitudes, self-reported practices, and waste management practices among hospital foodservice managers and examined factors associated with safe food handling.

Methods: A cross-sectional survey was conducted among foodservice managers from 50 registered hospitals in Lahore, Pakistan, using a structured questionnaire. Food safety knowledge, attitudes, practices, and waste management were evaluated. Data were analyzed using descriptive statistics, chi-square tests, and Spearman correlation analysis.

Results: Overall, 68% of respondents demonstrated satisfactory food safety knowledge, and 72% reported positive attitudes toward food safety. However, only 54% reported consistently safe food-handling practices. Knowledge gaps were most pronounced in temperature control (41% correct responses), handling of leftovers (46%), freezing and thawing practices (49%),

and produce washing (52%). Food safety knowledge was positively correlated with self-reported practices ($r = 0.42$, $p < 0.01$) but showed a weaker association with attitudes ($r = 0.18$, $p > 0.05$). Managers who had received formal food safety or HACCP training scored significantly higher on knowledge assessments than those without training (74% vs. 59%, $p < 0.05$). Only 57% of respondents reported consistent waste segregation practices, and 44% identified logistical constraints in waste disposal.

Significance: These findings demonstrate that while baseline food safety knowledge among hospital foodservice managers is moderate, critical practice gaps remain. Targeted, role-specific training and stronger oversight of food handling and waste management procedures are necessary to enhance food protection in healthcare foodservice settings.

P3-181: EFFECT OF STORAGE TEMPERATURE ON THE GROWTH OF BACILLUS CEREUS IN COOKED RICE

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: *Bacillus cereus* is a common cause of foodborne illness, often associated with improper storage of cooked rice. Since it is found in soil, its spores contaminate raw rice and resist cooking. If held at room temperature for long periods, spores can germinate and grow to hazardous levels, producing emetic and diarrheal toxins. Consumers refer to the associated symptoms as “Fried Rice Syndrome”.

Purpose: This study quantified the growth of *B. cereus* ATCC 14579 and DSMZ 4312 (from vegetative cells and spores) in cooked rice under common storage conditions to assess health risks.

Methods: Cooked long-grain rice was inoculated separately with a cocktail of *B. cereus* vegetative cells (4.8 ± 0.4 log CFU/g) or spores (4.2 ± 0.1 log CFU/g). Rice was stored at refrigeration (4°C), room (25°C), or a "keep warm” temperature (57°C). Bacterial populations were enumerated at 0, 4, 8, 12, 24, and 48 h on selective media. Experiments were performed in triplicate, and statistical significance between means was determined using Tukey's HSD.

Results: At room temperature, both vegetative cells and spores demonstrated significant proliferation ($p < 0.05$), increasing from 4.6 ± 0.6 log CFU/g to 8.7 ± 0.3 log CFU/g, and from 4.2 ± 0.1 log CFU/g to 8.4 ± 0.2 log CFU/g, respectively. Refrigeration effectively inhibited growth, causing a slight decline in both populations. The warm-holding temperature of the rice cooker effectively controlled the pathogen by reducing vegetative cells and preventing spore germination and outgrowth; nevertheless, safety can be compromised if the temperature cannot be verified or held steady.

Significance: Storing cooked rice at ambient temperatures or using unmonitored 'keep warm' settings creates a substantial risk of *B. cereus* growth. To prevent foodborne illness, strict temperature control via proper refrigeration (□4°C) or verified hot holding (□57°C) is essential.

Award Application: Developing Scientist Award

P3-182: STANDARD 9 IN ACTION: LONGITUDINAL INSPECTION ANALYTICS IMPROVE FOOD SAFETY (2021–2025)

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Food safety oversight in university settings is complex due to high-volume, multi-facility operations. Using FDA Program Standard 9, we evaluated whether interventions (training, signage, and increased inspection frequency) were associated with improved inspection outcomes.

Methods: Longitudinal data from 249 routine inspections (Fall 2021–Fall 2025) across dining halls, restaurant/catering operations, retail food stores, and childcare facilities were analyzed. To ensure valid comparisons, sites were aggregated by facility and calendar year (accounting for name changes). Outcomes included overall inspection scores (0–100) and counts of five high-risk practices. One-way ANOVAs with Tukey's post hoc tests compared outcomes by year.

Results: Overall, scores differed by year, $F(4,244)=6.00$, $p<.001$, increasing from 88.77 (2021) to 94.16 (2025). Scores in 2024 and 2025 were higher than 2021 ($p=.012$, $p=.003$) and 2022 ($p=.016$, $p=.003$). Time/temperature abuse also differed, $F(4,245)=13.39$, $p<.001$, declining from 3.35 (2021) to 0.73 (2025); 2023–2025 were lower than 2021 ($p=.016$, $p<.001$, $p<.001$). No significant changes were observed for food from unsafe sources (0.88 to 0.37), inadequate cooking (□0.08), poor personal hygiene (1.00 to 0.56), or contaminated equipment (2.73 to

3.18) (all $p>.05$).

Significance: Standard 9 trend monitoring translated inspection data into actionable guidance and demonstrated measurable improvement in inspection scores and time/temperature control in a campus food system. This framework offers a scalable model for other universities and school food service programs.

P3-183: ROWANBERRY EXTRACT AS AN EFFECTIVE NATURAL ANTIMICROBIAL SOLUTION FOR REFRIGERATED HUMMUS

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Due to its high-water activity and moderate pH, hummus has been associated with multiple foodborne outbreaks over the past decade. Controlling pathogenic and spoilage microorganisms in this product remains challenging, particularly when consumer demand naturally derived preservation strategies. Plant-based extracts with antimicrobial properties offer a promising approach to maintaining product safety and quality throughout refrigerated shelf life.

Purpose: The aim of this study is to evaluate the effectiveness of Rowanberry extract at 0.4% in controlling microbial quality in hummus during refrigerated storage for 90 days.

Methods: Fresh hummus (pH 5.05, a_w 0.95) was prepared with either 0.4% sterile water (negative control) or 0.4% Rowanberry extract. Samples were aliquoted and inoculated with approximately 3 log CFU/g of *Escherichia coli* or *Listeria innocua*, alongside uninoculated samples for shelf-life assessment, and stored under refrigeration (4°C). Triplicates of each treatment were enumerated weekly for viable counts. *E. coli* was plated on MacConkey agar, *L. innocua* on Modified Oxford agar, and uninoculated samples on tryptic soy agar to monitor total aerobic plate counts. Differences between treatments were evaluated using a two-tailed *t*-test, with statistical significance determined at $p < 0.05$.

Results: By day 28, Control exhibited approximately 2 log CFU/g growth in APC and one log CFU/g increase in *L. innocua*. *E. coli* showed no change during this period. In contrast,

hummus samples containing 0.4% Rowanberry extract remained microbiologically static through day 28 across all organisms evaluated.

Significance: These findings demonstrate that 0.4% Rowanberry extract is a promising intervention to control microbial growth and maintain the microbial quality and safety of hummus during refrigerated storage.

P3-184: GAP ANALYSIS FOR KENTUCKY'S RETAIL FOOD INSPECTION PROGRAM: REGIONAL STATISTICS AND COMPARISONS

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Presentation type/Format: Poster

Category: Retail and Food Service Safety

Abstract:

Introduction: Kentucky Department for Public Health (KDPH) has identified a need to quantify gaps within the regulatory retail food inspection program, noting discrepancies in establishment inspections and permit fees.

Purpose: The aim of this study is to analyze gaps in missed inspections and uncollected fees to better meet state and federal regulatory requirements while addressing the burden of inspections.

Methods: In 2022, Kentucky had a total of 23,566 active food establishments included in this study. The inspection gap was computed by identifying the number of active establishments not receiving an inspection. The fee gap was calculated by identifying the number of establishments that received a regular inspection but did not pay a permit fee. Chi-squared tests of independence were performed to determine proportional differences in these gaps across regions. Regional forums presenting the gaps were conducted with local health departments (LHDs) to discuss barriers, identify opportunities for improvement, and provide context.

Results: In the sample of 23,566 active establishments, 21,904 (92.9%) received a regular inspection and 18,903 (80.2%) paid a permit fee for 2022. When examining the inspection

gap across KDPH's five regional areas of operation, a chi-squared test indicated statistically significant differences in the proportion of uninspected establishments by region ($\chi^2=641.1$, $p<0.001$). The chi-squared results for the fee gap in KDPH's five regional areas of operation demonstrated statistically significant differences in the proportion of establishments not paying an inspection fee by region ($\chi^2=68.1$, $p<0.001$).

Significance: Analyzing these gaps and contextualizing results through open-forum collaborations with local inspectors allowed KDPH to implement LHD trainings according to the specific needs of each region. The results from this study directly impact inspector performance and consistency to advance food protection across the Commonwealth.

P3-185: CULTURE DEPENDENT VS. CULTURE INDEPENDENT EVALUATION OF BACTERIAL COMMUNITIES ASSOCIATED WITH FISH STORED AT 0°C

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Introduction: Bacterial communities associated with fish change throughout storage. Different methodologies for assessing these communities may alter interpretation of seafood decomposition progression. Comparing microbial and molecular methods for evaluating bacterial communities provides insight into determining practical and reliable options to evaluate decomposition.

Purpose: This study compared bacterial community composition from fish stored at 0°C, using culture dependent and independent 16S sequencing.

Methods: Red snapper and Spanish mackerel were caught via hook and line in the Gulf of America and expired on ice. Whole gutted fish were stored at 0°C for 42 days. Triplicate samples were collected weekly for weeks 1-3 and every 3rd day for weeks 3-6, and DNA was analyzed using 16S rRNA amplicon sequencing with 26F1 and 534R1 primers (culture independent). Tissues samples were homogenized (1:10 with artificial seawater), plated on TSA, and incubated at 4°C for 2 weeks or 30°C for 3 days. DNA from up to 48 colonies from each sampling point was purified for 16S rRNA Sanger sequencing using 27F and 1492R

primers (culture dependent).

Results: Isolates from mackerel demonstrated countable growth by day 24 and day 7, when grown at 4 and 30℃, respectively. By day 24 *Pseudomonas* dominated the community at both temperatures. Isolates from snapper demonstrated countable growth by day 14 when grown at 4 or 30℃ and *Pseudomonas* dominated the community at both temperatures. Culture independent sequencing demonstrated diverse communities, initially, then diversity declined. with a majority of *Pseudomonas* present on day 21 for snapper and day 28 for mackerel.

Significance: Utilizing different methodologies to evaluate bacterial community composition during temperature-controlled storage provides key insights into method suitability for monitoring decomposition. Overall, the culture independent method detected more bacterial community diversity than the culture dependent method.

P3-186: ISOLATION AND CHARACTERIZATION OF VAGOCOCCUS LUTRAE IN HEAT-PROCESSED RTE SEAFOOD: AN EMERGING HUMAN PATHOGEN AND FOOD SAFETY THREAT

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Introduction: Isolation of *Vagococcus lutrae* from human patients and fresh seafood has increased since the first human case report. However, its alleged presumptive availability in RTE seafood remains unexplored to date.

Purpose: This study explored the prevalence of *V. lutrae* in RTE seafood.

Methods: Cooked, RTE, and canned seafood products (n=29) were acquired from local restaurants and retail stores, which contained shellfish and fish products. Using a two-step enrichment method, RTE seafood underwent nourishment alone or nourishment followed by heating (80°C, 20 min) before being cultured on TCBS agar. Bacterial colonies were streaked

for purification before 16S rRNA bacterial identification. Using parafilm-wrapped plates and anaerobic jar, *V. lutrae* was analyzed for oxygen-dependent growth. A turbidimetric assay was used to assess the viability of *V. lutrae* in response to different salinity concentrations (0.5–20%) and a thermal challenge (63°C, 8 h). Subsequently, commercial urea tablets and an agar diffusion test were used to assess bacterial antibiotic sensitivity and urease activity, respectively.

Results: A total of 86 bacterial isolates were recovered from seafood products after enrichment alone (62 regular strains) or enrichment coupled with heat treatment (24 heat-resistant strains). Of these isolates (regular/heat-resistant strains) of black (0/1), green (24/4), or yellow (38/19), one black colony isolate (1.2%) was confirmed as *V. lutrae,* which was recovered from RTE shrimp cocktail (3.5%). This strain tolerated various oxygen levels, salinity (0.5–20.5%) concentrations, and high temperatures (63°C and 80°C). Further analyses revealed that it was gentamicin-resistant ($P<0.05$) and urease-negative.

Significance: The present study highlights the need to investigate *V. lutrae*'s mechanism of heat resistance, that the bacterium may react differently to TCBS, and that the food processing and safety inspection regime requires a *V. lutrae*-inclusive transformation.

Award Application: Developing Scientist Award

P3-187: MODELING THE ANTIBIOTIC RESISTANCE BURDEN OF VIBRIO ALGINOLYTICUS FROM ONE-HEALTH PERSPECTIVE

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Presentation type/Format: Technical

Category: Seafood

Abstract:

Introduction: *Vibrio alginolyticus* (Valg) is a distinctive One-Health pathogen passing repeatedly among humans and other ecosystems.

Purpose: This study aimed at modelling the resistance burden of Valg including wide antibiotic classes.

Methods: We modelled 3434 Valg antibiogram data sourced from One-Health samples using ARRI (antibiotic resistance risk index) metrics for cross-class/territorial comparisons of Valg's resistance burden. Where ARRI is the quotient of the summation of Valg's resistant cases to all antibiotics assayed for a specific sample/territory and the overall Valg-resistant instances for all samples/territories in the study multiplied by the number of antibiotics assayed for the sample/territory to which Valg was resistant.

Results: The findings revealed that aquatic reservoirs had the heaviest Valg antibiotic resistance burden. The pattern of Valg resistance burden was human (ARRI=0.47) < water (ARRI = 31.40) < shellfish (ARRI = 59.43); implying higher burden in seafood-borne and waterborne *V. alginolyticus* samples. Also, China (ARRI = 12.46) and Tunisia (ARRI =28.02) were Valg resistance hotspots among nations. While Africa (ARRI = 76.52) and Asia (ARRI =127.16) were hotspots, South America (ARRI =0.44) North America (ARRI =1.70), and Europe (ARRI

=16.92) were identified as cold-spots among regions for Valg resistance burden.

Significance: The findings underscore that seafood- and aquatic-borne Valg resistance burden pose food safety and human health risks. With high Valg ARRI in shellfish and water, there is a demand for integrated resistance surveillance, antibiotic stewardship, and regulatory attention to aquacultural antibiotic uses especially in Asia and Africa.

P3-188: ASSESSMENT OF MICROBIAL QUALITY AND SAFETY OF WILD-CAUGHT SHRIMP AND FARM-RAISED SHRIMP SOLD IN TEXAS

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Introduction: Shrimp consumption is on the rise in the U.S. This demand is met by imported, farm-raised shrimp, where antibiotics are commonly used for disease prevention, resulting in antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) in the final product.

Purpose: This pilot study aims to conduct a comparative analysis of the quality and safety of wild-caught (WC) shrimp vs farm-raised (FR) shrimp available in retail markets around Houston, TX, as well as determine the prevalence of ARB and ARGs.

Methods: Raw WC and FR shrimp samples were collected between September to December 2025 in retail markets in the greater Houston, TX area. Culture-based microbiological and biochemical methods were used to enumerate APCs and coliforms as well as isolate potential pathogens.

Results: APCs and coliform levels in shrimp samples (n=24) ranged from 2.23 to 4.45 log CFU/g, and <1.95 to 2.54 log CFU/g, respectively. The prevalence of some targeted bacteria in

shrimp were as follows: *Salmonella* (0%), *Pseudomonas aeruginosa* (73%–77%), *Enterococcus* (92%–100%), and *Escherichia coli* (0%). *Enterococcus and* APCs for farmed-raised shrimp were higher (p □ 0.05) than those for wild-caught shrimp. Biolog GENIII was used for phenotypic characterization of presumptive *Salmonella* isolates and identified the following *Enterobacteriaceae*: *Serratia marcescens ss marcescens, Morganella morganii,* and *Morganella morganii ss morganii*, with farmed-raised shrimp also having *Hafnia alvei* and *E. coli.* Ongoing work will collect additional shrimp samples (to reach an n=30) and perform antimicrobial susceptibility testing (AST) to determine resistance profiles and use real-time PCR to detect ARGs in all samples.

Significance: Results to date indicate that WC shrimp have less bacterial contamination than FR shrimp. Continued work with these samples will provide preliminary data to inform whether wild-caught shrimp carry less ARB and/or ARGs as compared to farm-raised shrimp.

Award Application: Developing Scientist Award

P3-189: CHARACTERIZATION OF GULF OF AMERICA MOON JELLYFISH (AURELIA AURITA) AND ITS APPLICATION IN PREVENTING SHRIMP SPOILAGE

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Presentation type/Format: Poster

Category: Seafood

Abstract:

Purpose: To evaluate the antimicrobial effects of moon jellyfish for delaying spoilage of wild-caught shrimp from the U.S. Gulf Coast.

Introduction: Microbial spoilage is a major challenge in the shrimp industry. Although chemical preservatives are commonly used to reduce microbial load, they raise safety concerns for producers and consumers. Jellyfish have demonstrated antibacterial potential as food-derived additives. This study investigated the antimicrobial effects of moon jellyfish, an abundant species in the U.S. Gulf Coast, for delaying shrimp spoilage.

Methods: Moon jellyfish (approximately 4 kg) were collected from the Mississippi Gulf Coast and freeze-dried for proximate composition analysis following AOAC methods and amino acid profiling using an amino acid analyzer (LA8080, Hitachi, Japan). Lyophilized jellyfish powder was mixed with freshly harvested shrimp at 1, 2, or 3% (w/w). Total bacterial count,

psychrotrophic count, *Pseudomonas* count, hydrogen sulfide-producing bacteria count, and Enterobacteriaceae count were measured on days 0, 3, 7, 10, and 14 at 4, 24, or 37°C. Untreated shrimp served as the negative control, and shrimp treated with 1.25% sodium metabisulfite (SMS) served as the positive control. Data were analyzed using one-way ANOVA ($p < 0.05$) and are presented as mean ± SD (n = 3).

Results: Moon jellyfish consisted primarily of water (97.727 ± 0.037%), protein (0.259 ± 0.008%), lipid (0.113 ± 0.015%), and ash (1.735 ± 0.011%). Proline was the most abundant amino acid (3.072 ± 0.0869 nmol/g), while valine was the least abundant (0.060 ± 0.0005 nmol/g). All jellyfish treatments significantly reduced bacterial growth compared to the negative control ($p < 0.05$). The antimicrobial effect was dose-dependent, and the 3% treatment was comparable to SMS, with no significant differences observed.

Significance: Moon jellyfish effectively delayed microbial spoilage of shrimp and may serve as a natural alternative to chemical preservatives.

P3-190: EFFECTIVE BIOCONTROL OF E. COLI O157:H7 BY AN ACTIVE PACKAGING FILM INCORPORATED WITH LIPOSOME-ENCAPSULATED CAM-21 BACTERIOPHAGE

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: Nanoliposomes are a promising way to encapsulate and deliver bacteriophages. The incorporation of bacteriophages in active packaging has been widely explored to address challenges associated with foodborne disease outbreaks related to contaminated food.

Purpose: The objective of this study is to develop a packaging film incorporated with CAM-21 bacteriophage, encapsulated in nanoliposome, to mitigate food contamination by *Escherichia coli* O157:H7.

Methods: Liposomes were prepared by dissolving soy-phosphatidylcholine, cholesterol and rhamnolipid in chloroform, and forming a thin film via rotary evaporation. CAM-21 was added and the solution shaken (50 rpm, 37°C, 2 h) to detach the film, yielding a milky suspension that was extruded through membranes. A biodegradable carboxymethyl chitosan and kappa-carrageenan film solution was prepared, and CAM-21-incorporated liposomes were added at a 2% v/v concentration. Particle size, zeta potential, transmission electron microscopic (TEM) morphology, encapsulation efficiency, and antimicrobial activity were assessed.

Results: Dynamic light scattering results showed well-formed vesicles with Z-average sizes of

approximately 320 nm and 440 nm, along with good size uniformity (PDI = 0.22), indicating stable liposomal dispersions. TEM analysis confirmed the successful formation of liposomes and proper encapsulation of the bacteriophage within the liposomal structures. The encapsulation efficiency was about 82%, confirming efficient entrapment of the bacteriophage. The films containing CAM-21 exhibited effective reduction in bacterial counts.

Significance: The impact of this research lies in the application of liposome-encapsulated bacteriophages in biocomposite films to control *E. coli* O157:H7 contamination in food.

Award Application: Developing Scientist Award

P3-191: CONTROL OF SHIGA-TOXIGENIC ESCHERICHIA COLI IN FRESH-CUT STRAWBERRIES BY A NOVEL EDIBLE COATING ENRICHED WITH ONION SCALE EXTRACT NANOCAPSULES

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: Fresh-cut strawberries are highly prone to contamination by enterotoxigenic *E. coli* O157:H7, which has been associated with numerous outbreaks of diarrheal diseases posing significant risk to human health.

Purpose: The study aims to develop novel edible CMC-based coatings enriched with encapsulated onion scale extract (OSE) for inhibiting growth of *E. coli* O157:H7 and extending shelf life of fresh-cut strawberry stored at chilling temperature.

Methods: OSE was encapsulated with maltodextrin (MD) at different extract-wall ratios (1:5, 1:10, and 1:20 w/w) using emulsification method. The most efficient MD-OSE capsules were selected for activating CMC coatings that were further characterized (viscosity, zeta potential, color, total phenolic content, antioxidant/antimicrobial activity) prior to application. Fresh-cut strawberries artificially contaminated with *E. coli* O157:H7 (~5.0 log CFU/mL) were coated with CMC-MD-OSE solution to investigate its impact on growth behavior and quality parameters.

Results: Nanocapsules with 1:20 extract-wall ratio exhibited highest yield (96.19±0.21%),

encapsulation efficiency (93.16±1.87%), zeta potential (-35.22±0.90 mV), water solubility (96.24±0.56%), smallest particle size (344 nm), PDI (0.2), MC (7.45±1.80%), cytotoxicity against Caco-2 cells, potent antimicrobial activity, in addition to an improved viscosity. *E. coli* O157:H7 population counts were significantly ($P < 0.05$) reduced by 3 log units after 6 days at 4^{o}C in active-coated strawberry that displayed significantly ($P < 0.05$) lower water loss, harder texture, maintained pH/total soluble solids/total phenolic content with better color/visual appearance compared to uncoated samples.

Significance: This is the first research on CMC-MD-OSE formulation as a cheap, effective postharvest intervention to control pathogen proliferation, and a promising alternative to the prevailing fruit glazing practice for storage and/or display of minimally processed fresh-cut fruits.

Award Application: Developing Scientist Award

P3-192: PHAGE-BASED PRIMARY PACKAGING ELIMINATES SALMONELLA INFANTIS IN FRESH POULTRY MEAT WITHOUT AFFECTING QUALITY INDICATORS AND THE MICROBIOTA

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: Multidrug-resistant *Salmonella* Infantis (SI) is a major food safety challenge in fresh poultry meat, with an urge for new strategies to control it.

Purpose: To evaluate the effect of a sprayable active edible coating (AEC) based on alginate with a SI specific three-bacteriophage cocktail on the reduction of SI on chicken breast, as well as its impact on quality indicators and the microbiota.

Methods: A total of 77 grams of fresh chicken breasts were artificially inoculated with SI at 4 log CFU/mL of SI of rinsate. Three groups of 12 samples were spray treated with: i) 1.5 mL of water, ii) 1.5 mL of phage cocktail, and iii) 1.5 mL of AEC. All groups were stored at 4°C for 48 h, then SI was quantified using a qPCR SureCount kit. Quality indicators were monitored during refrigerated aerobic storage for 15 days, including pH, color, and Aerobic Plate Counts (APC). DNA extraction was performed using the DNA-soil kit (QIAGEN), and the microbiota

was profiled by 16S rRNA V3–V4 sequencing with ASV inference and genus-level comparisons at days 0 and 14. Two replicates were conducted.

Results: AEC produced a significant reduction of 1.64 log CFU/g *Salmonella* at 48 h versus the untreated control (4.15 log CFU/g) and the phage cocktail (1.97 log CFU/g) ($p<0.05$), whereas the phage cocktail showed an earlier effect that diminished by 48 h. The pH remained stable across storage with no significant treatment effect. Color coordinates showed only modest, non-significant shifts between control and AEC. APC increased as expected in all groups, exceeding 7.56±0.05 log CFU/g by day 12, indicating no shelf-life extension. At day 12, 16S all profiles converged to *Pseudomonas*-dominated communities on all three tested groups.

Significance: Spraying the AEC designed here reduced SI on chicken breast without changing pH or color and without affecting the microbiota, suggesting a promising new mitigation strategy to reduce *Salmonella* in poultry meat.

Award Application: Developing Scientist Award

P3-193: SUSTAINABLE AND SMART ANTIMICROBIAL PACKAGING: PECTINASE-RESPONSIVE BACTERIOPHAGE DELIVERY VIA CA^{2+}-CROSSLINKED PECTIN FILMS

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: The rise of antibiotic-resistant pathogens, notably methicillin-resistant *Staphylococcus aureus*, necessitates the development of alternative antimicrobial strategies, such as bacteriophages (phages).

Purpose: This study introduces an innovative approach using pectin-based packaging materials designed for the controlled release of phages triggered by bacterial enzymes in the presence of pathogens, specifically targeting *S. aureus* to combat bacterial contamination.

Methods: The films were fabricated via solution casting by incorporating 30% (w/w) glycerol (relative to solid content) and 10^9 PFU/mL of phages, such as SAC, into a 1.5% (w/v) pectin solution. Subsequently, the films were crosslinked by immersion in calcium chloride solutions at various concentrations. The morphology of films was characterized using scanning electron microscopy, and their moisture resistance was assessed in relation to the degree of crosslinking upon exposure to water. Finally, the antimicrobial efficacy was evaluated in various solution to investigate the influence of bacterial pectinase on the enzyme-triggered release mechanism.

Results: At a multiplicity of infection of 1, SAC exhibited rapid antibacterial kinetics, inhibiting *S. aureus* within 1 h and sustaining bactericidal efficacy for 24 h. The SAC exhibited robust stability across temperatures (-18 to 60°C) and pH levels (5 to 10), with genomic analysis confirming the absence of antibiotic resistance or virulence genes. Calcium crosslinked pectin films showed the formation of a dense polymer matrix enhanced the moisture properties. In soy milk, the phage-contained film reduced bacterial counts by 8.90-log CFU/mL without added pectinase, and by 10.47-log CFU/mL when pectinase was added, compared to group without phage treatment.

Significance: These results highlight the potential of SAC-contained pectin films as a novel enzyme-responsive system for targeted antibacterial activity in food packaging applications.

Award Application: Developing Scientist Award

P3-194: COPPER-INFUSED PACKAGING: A SUSTAINED-RELEASE MECHANISM TO CONTROL THE GROWTH OF LISTERIA MONOCYTOGENES ON READY-TO-EAT MEATS DURING REFRIGERATED STORAGE

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Category: Packaging

Abstract:

Introduction: Sustained antimicrobial-release mechanisms are the next frontier to ensure food safety during prolonged storage of foods. Packaging infused with antimicrobials such as copper offer a passive and continuous intervention to control the growth of *Listeria monocytogenes* (LM) on ready-to-eat (RTE) meat surface.

Purpose: The objective of the study was to evaluate the antimicrobial efficacy of copper-infused packaging against *L. monocytogenes*, spoilage and product quality of no-antimicrobial added, RTE meat in vacuum-package during refrigerated storage.

Methods: Commercially manufactured ham without any antimicrobials and antilisteria compounds was used for this study. LM 4b, 1/2a, and 1/2c were co-inoculated on ham surface to obtain low (3 log CFU/g) and high (6 log CFU/g) inoculum levels. Individually inoculated ham slices were vacuum packaged in plastic bags (1) with no copper (control); (2) containing 75 mg/m² copper and (3) with 100 mg/m² copper. All samples were stored at 4°C for up to 12 weeks. Microbiological analyses (n=10/treatment) were conducted weekly for LM and monthly for spoilage organisms, including aerobic, anaerobic, psychrotrophic, and lactic acid bacteria. Viable colonies were counted and reported as log CFU/g. Experiment was repeated in two independent trials. LM growth kinetics were quantified by fitting population data to the modified Gompertz model. Meat quality, color (Δ□*) and texture puncture force analysis, was analyzed on day 0, month 1, 2, and 3.

Results: Growth models indicated that copper-infused packaging reduces LM growth rates across both trials, by approximately 29–39% and 24–40% for the 75 and 100 mg/m² copper treatments, respectively, compared with the control, while showing minimal effects on lag phase and no consistent reduction end of shelf-life populations.

Significance: Copper-infused packaging can be used as a sustained release mechanism as an additional hurdle to control LM in RTE foods during storage.

Award Application: Developing Scientist Award

P3-195: OPTIMIZING THE DELIVERY OF VIBRIO BACTERIOPHAGES THROUGH CALCIUM-MEDIATED CONTROLLED RELEASE SYSTEMS IN ALGINATE FILMS FOR ENHANCED SCALLOP SAFETY

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: Rising seawater temperatures driven by climate change have accelerating the emergence of multidrug-resistant *Vibrio parahaemolyticus*, necessitating advanced bioactive delivery platforms that provide sustained antimicrobial activity without compromising food matrix integrity.

Purpose: This study investigated modulating the release rates of a novel lytic phage, VPy01, through strategic calcium cross-linking in alginate-based packaging for the *Vibrio* biocontrol in scallops.

Methods: Phage VPy01 (1.54×10^{11} PFU/mL) was characterized and immobilized in 2% (w/v) sodium alginate films (10^8 PFU/film). Films were cross-linked with four calcium salts ($CaCl_2$, $CaCO_3$, hydroxyapatite, and $CaSO_4$) at a 0.5 molar ratio. Phage release rates were quantified in PBS (200 rpm, 24 h), and optimal SA-$CaCl_2$ films were evaluated for mechanical and barrier properties (n = 6). Biocontrol efficacy was validated using scallop slices (3.5 ± 0.5 g, n = 6)

inoculated with 5×10^6 CFU/mL of *V. parahaemolyticus* at 5°C. Weight loss and hardness were monitored over 72 h; significance was determined via ANOVA ($p < 0.05$).

Results: VPy01 release was significantly differentiated by calcium source; $CaCl_2$ mediated immediate release within 3 h, while $CaCO_3$, hydroxyapatite, and $CaSO_4$ exhibited prolonged release up to 12 h. SA-$CaCl_2$ films showed superior swelling ratios (41.3 ± 2.9%) and oxygen barrier capacity (6.01 ± 0.30 $cm^3 \cdot \mu m/m^2 \cdot d \cdot k \cdot Pa$). In scallops, the system achieved a 1.8-log CFU/scallop reduction in 24 h ($p < 0.05$). Furthermore, the film halved the weight loss rate (reduced to approximately 20% compared to 47.2% in control) and preserved scallop hardness over 72 h by preventing moisture evaporation.

Significance: Selecting specific divalent cations enables precise modulation of phage release rates, establishing a versatile biopreservation platform. Such customizable systems provide a viable, antibiotic-free approach for mitigating public health risks in the seafood supply chains.

Award Application: Developing Scientist Award

P3-196: ANTIMICROBIAL PERFORMANCE AND UV-BLOCKING EFFICACY OF TIO_2-LOADED ELECTROSPUN PULLULAN/PVA NANOFIBER MATS FOR FOOD PACKAGING APPLICATIONS

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Presentation type/Format: Poster

Category: Packaging

Abstract:

Introduction: Fresh, ready-to-eat foods are easily contaminated by pathogens such as *E. coli*, *Salmonella*, and *Listeria*, and UV exposure further degrades product quality, making it imperative to use antimicrobial packaging with effective UV-blocking properties.

Purpose: This study developed electrospun pullulan/PVA nanofiber mats with TiO_2 nanoparticles to provide antibacterial and UV-blocking properties for food packaging.

Methods: Pullulan and PVA solutions containing different concentrations of TiO_2 nanoparticles were electrospun to produce nanofiber mats. Antimicrobial activity was evaluated against *E. coli*, *Salmonella* spp., and *Listeria* spp. using a direct contact method (n = 3). UV-visible spectra were recorded between 290–400 nm, and the ultraviolet protection factor (UPF) was calculated following AS/NZS 4399 guidelines. Mechanical properties were measured using tensile testing to determine Young's modulus, tensile strength, and elongation at break (n = 5).

Results: TiO_2-loaded nanofiber mats significantly reduced bacterial populations compared with control samples. Reductions of approximately 3.2 log CFU/g for *E. coli*, 3.5 log CFU/

g for *Salmonella* spp., and 3.0 log CFU/g for *Listeria* spp. were observed after 24 h of contact. The nanofiber mats also demonstrated strong UV-blocking performance, with UPF values exceeding 50, effectively blocking both UVA and UVB radiation. Mechanical testing showed that the control nanofiber mats exhibited a Young's modulus of 151 ± 20 MPa, tensile strength of 7.86 ± 0.37 MPa, and elongation at break of 156 ± 12%, while TiO_2 incorporation slightly decreased tensile strength and elasticity but maintained acceptable mechanical integrity for packaging applications.

Significance: TiO_2-enhanced pullulan/PVA nanofiber mats can be used as food packaging to reduce microbial growth and protect foods from harmful UV exposure, improving safety and shelf life.

Award Application: Developing Scientist Award

P3-197: APPLICATION OF CHEMICAL AND PHYSICAL INTERVENTIONS IN ENHANCING MICROBIAL FOOD SAFETY AND EXTENDING SHELF LIFE OF ROOT VEGETABLES

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Presentation type/Format: Technical

Category: Packaging

Abstract:

Introduction: Root vegetables including carrots and ginseng, harvested from soils are vulnerable to microbial contaminants such as foodborne pathogens and spoilage microorganisms, which lead to health risk concerns and reduced shelf life. Fresh baby carrots are usually consumed as a component of salad or as “finger food” without heat treatment. Therefore, effective antimicrobial measures and suitable packaging technology are necessary.

Purpose: In this study, multiple interventions were used to extend the shelf life of fresh baby carrots and ginseng roots.

Methods: Combining antimicrobial treatments (antimicrobial solution washing, antimicrobial edible coating, antimicrobial gas and their combinations) with food packaging (air, vacuum, skin and MAP) were investigated. The efficacy of each treatment or combination (three samples per treatment/combination) was evaluated by assessing the reduction in microbial populations (*E. coli*, *Listeria*, *Salmonella*, total aerobic bacteria [TPC], and yeasts and molds [YMC]). The quality characteristic values (color, texture, weight loss, etc.) were also monitored.

Results: Both antimicrobial solution washing and antimicrobial gas treatment contributed to the microbial reduction, and their combination with pouch and skin tray packaging showed

the most antimicrobial effectiveness and quality maintenance. For example, the combined treatment reduced the populations of TPC and YMC from 4 to < 1 log CFU/g and *E. coli* from 7 to < 1 log CFU/g after 3 days and no growth was observed through 11-week storage at 4°C. There were no significant differences in texture and weight loss between the treated and control samples. The optimal treatments were applied to fresh ginseng roots and extended their shelf life beyond 12 months at 4°C.

Significance: This study demonstrates that the combined interventions can extend the shelf life of fresh root vegetables. This approach will be of value to farmers to expand markets for their fresh products.

P3-198: EVALUATING LARGE LANGUAGE MODELS FOR STRUCTURED EXTRACTION OF FOOD RECALL ANNOUNCEMENTS

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Presentation type/Format: Technical

Category: Data Management and Analytics

Abstract:

Introduction: Food recall announcements are essential in communicating food safety risks to the public. However, their unstructured formats limit direct integration into automated food safety analytics and surveillance systems.

Purpose: This study investigates whether large language models (LLMs) can reliably transform food recall announcements into structured semantic representations under zero-shot and few-shot settings, and how their performance compares with supervised extraction approaches.

Methods: We curated a food recall dataset of over 6,500 records, annotated by human experts with product and hazard information as the gold standard. LLMs were prompted to generate structured outputs capturing product and hazard attributes for each record. We evaluated multiple commercial LLMs (GPTs, Geminis) using direct prompting, 2-stage reflection, static and dynamic few-shot learning, and Retrieval-Augmented Generation (RAG). Model performance was compared against supervised benchmarks (TF-IDF with logistic regression, RoBERTa) using macro- and micro-F1 scores.

Results: Zero-shot prompting with LLMs yielded poor performance with only minimal improvements from 2-stage reflection across LLM versions. In the presence of severe class imbalance, incorporating RAG with balanced static few-shot prompts substantially improved the macro-F1 score. Gemini-3-Flash with balanced few-shot prompting and RAG achieved the best-performing Macro-F1 of 0.748 and a Micro-F1 of 0.856, compared to 0.720 and 0.807 for RoBERTa-large, respectively. This shows that appropriately augmented LLMs can exceed task-specific supervised models with minimal supervision.

Significance: This work highlights the practical application of LLMs for structured information extraction in food safety applications. By reducing reliance on extensive manual annotation and model training, LLM-based approaches offer a cost-effective, low-supervision, and scalable solution for rapid food safety analytics and surveillance.

P3-199: MINING TRENDS IN SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) PREVALENCE DATA IN BEEF PRODUCTS USING USDA-FSIS SURVEILLANCE DATA

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: Shiga toxin-producing *Escherichia coli* (STEC), including O157:H7 and non-O157 serogroups, remain important food safety hazards in beef products. USDA-FSIS surveillance data provide insight into long-term prevalence trends and regional patterns relevant to risk management.

Purpose: To analyze FSIS beef sampling data from 2013–2025 to evaluate annual and regional prevalence trends of *E. coli* O157:H7 and non-O157 STEC and assess differences by product type.

Methods: FSIS beef sampling records were analyzed in R. Samples were grouped by U.S. Census region and product type (raw-ground vs. raw-intact beef). Annual prevalence was calculated as weighted pooled proportions and summarized by region and year using compact tabular formats. Zero prevalence values were reported as <0.0001%.

Results: Non-O157 STEC prevalence was consistently higher than *E. coli* O157:H7 across all regions and years, accounting for 96.27% of STEC-positive samples nationwide compared to

3.73% for O157:H7. *E. coli* O157:H7 prevalence remained low, frequently below 0.01%, with no detections in some years (2013 and 2020–2025). Regional differences in non-O157 STEC prevalence were observed, with the Midwest (0.28%), South (0.48%), and Northeast (0.32%) generally higher than the West (0.20%). Raw ground beef showed higher prevalence than raw-intact products. Temporal trends indicated gradual regional variation without major shifts, with annual non-O157 STEC prevalence generally ranging from 0.05% to 0.25%.

Significance: FSIS surveillance data confirm persistently low *E. coli* O157:H7 prevalence and higher non-O157 STEC prevalence in beef products, supporting the value of long-term surveillance for food safety decision-making.

Award Application: Developing Scientist Award

P3-200: DEVELOPMENT OF A POWER BI-BASED REAL-TIME DATA MANAGEMENT AND VISUALIZATION TOOL FOR SALMONELLA BIOMAPPING IN THE SECONDARY PROCESSING OPERATIONS OF COMMERCIAL FACILITIES

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: Timely interpretation of microbiological data is critical for food safety, yet results are often underutilized due to delayed reporting and limited integration tools.

Purpose: To develop and validate a Power BI-based dashboard for real-time biomapping and interpretation of *Salmonella* detection and quantification data in poultry secondary processing.

Methods: A Power BI dashboard integrated qualitative and quantitative *Salmonella* data (log_{10} CFU/mL) from secondary processing operations. Filters included plant, product type, day, and sampling unit (1,800-lb combo). Automated summaries covered prevalence, count distributions, and combo-level aggregation. The system was tested using a one-year dataset of 2,881 poultry part samples from 10 plants, summarized into 288 combos. Visualizations included prevalence summaries, load distributions, heterogeneity, and plant-specific profiles.

Results: Of 2,881 samples, 598 were positive (20.8% prevalence). Most samples were non-

detects (79.2%), 15.6% were >0–1 $\log_{10}$ CFU/mL, 4.9% □1 $\log_{10}$ CFU/mL, and 0.24% exceeded 3 $\log_{10}$ CFU/mL. Among positives, 74.7% were low-level (>0–1 $\log_{10}$ CFU/mL), median 0.63 $\log_{10}$ CFU/mL, with 75% below ~1.0 $\log_{10}$ CFU/mL. At combo level, mean prevalence was 20.2%, but contamination was heterogeneous: ~⅓ combos negative, ~⅓ □25% positive, and ~⅓ □25% positive. Plant profiles ranged from low-burden (□89–97% non-detects) to high-burden plants with frequent low-level positives, often in second shift.

Significance: This Power BI tool shows how real-time visualization can transform lab data into actionable food safety intelligence, improving process control, corrective actions, and risk-based decision-making in poultry processing.

P3-201: SLICE THICKNESS DETERMINES DEHYDRATION KINETICS AND SALMONELLA INACTIVATION IN BEEF JERKY UNDER SMALL-SCALE PROCESSING CONDITIONS

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Presentation type/Format: Poster

Category: Low-Water Activity Foods

Abstract:

Introduction: Beef jerky is a ready-to-eat, shelf-stable meat product whose safety relies on achieving validated *Salmonella* lethality while controlling moisture loss during dehydration.

Purpose: To evaluate the effects of product thickness, drying temperature, and surface moisture treatment on *Salmonella* inactivation during beef jerky processing using a home-style dehydrator.

Methods: Beef eye of round was sliced to three thicknesses (0.38, 0.64, and 0.95 cm), subjected to two surface moisture treatments (direct immersion or spice-mix marinade), and dried at 68 or 71°C. Water activity (a_w) was measured hourly, and *Salmonella* populations were enumerated during 6 h of drying and after 7 and 14 days of ambient storage. Three independent trials were conducted with two technical replicates. The data were subjected to analysis of variance (ANOVA) followed by the Tukey's test ($p < 0.05$) and microbial modeling

conducted using R software.

Results: Slice thickness significantly influenced dehydration kinetics and time to reach a_w ≤ 0.85 (p = 0.0144), whereas drying temperature (p = 0.87) and surface moisture treatment (p = 0.24) did not. After 6 h, all treatments achieved >6-log CFU/g reductions, exceeding USDA-FSIS lethality benchmarks for jerky. However, viable *Salmonella* populations (~3 log CFU/g) were recovered after 14 days of storage, indicating desiccation tolerance rather than post-process growth. Inactivation curves exhibited pronounced shoulder and tailing behavior. Of five primary models evaluated, the Weibull model with a residual population best described inactivation kinetics (APZ = 94.05%, adjusted R^2 = 0.96, RMSE = 0.26).

Significance: Product thickness and moisture loss kinetics are critical factors for validating dehydrated meat processes and ensuring reliable regulatory lethality against *Salmonella*.

Award Application: Developing Scientist Award

P3-202: BASELINE CAMPYLOBACTER CONCENTRATIONS ACROSS PROCESSING STAGES AND PART TYPES IN COMMERCIAL POULTRY OPERATIONS

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: *Campylobacter* contamination in poultry products remains a major food safety concern. Poultry processing establishments play a critical role in mitigating contamination; however, limited information exists on concentration changes under commercial operations.

Purpose: Establish baseline *Campylobacter* concentrations across processing stages, part types, and post-chill treatments in commercial operations.

Methods: Routine surveillance data from a U.S. poultry integrator were analyzed, comprising 14,186 records from 31 processing plants collected between 2018 and 2024. Rinsate samples were obtained from six stages: Pre-scalder, Post-scalder, Hot rehang, Post-pick, Post-chill, and Parts. *Campylobacter* concentrations (log CFU/mL) were enumerated on Campy-Cefex agar after incubation at 42°C for 48 h (Limit of Detection=1 CFU/mL). A general linear model was used to estimate concentrations by stage, part type, and post-chill dip treatment, with plant as a blocking factor using PROC GLM in SAS 9.4. Tukey-adjusted post-hoc comparisons

were performed (α = 0.05).

Results: *Campylobacter* concentrations were highest at Pre-scalder (3.88 log CFU/mL) and decreased after scalding (Post-scalder: 1.26 log CFU/mL; $P < 0.01$). Levels rebounded at Post-pick (3.80 log CFU/mL; $P < 0.01$) before a reduction at Hot rehang (1.00 log CFU/mL; $P < 0.01$). Post-chill carcasses and parts further reduced to <0.02 CFU/mL, with >94.8% of samples below LOD. Parts (nugget, thigh, leg, breast, wing, drum, fillet, tender) generally had very low concentrations (<0.03 log CFU/mL). Post-chill dipping reduced contamination from 0.14 to 0.07 log CFU/mL ($P < 0.01$), with the greatest effect on nuggets (0.30 to 0.05 log CFU/mL; $P < 0.01$).

Significance: This result provides baseline data on *Campylobacter* concentration trends during commercial processing, supporting future risk assessments and offering information for future research on intervention effectiveness under real-world conditions.

Award Application: Developing Scientist Award

P3-203: IDENTIFYING KEY PROCESSING FEATURES FOR SALMONELLA PERFORMANCE IN FINISHED RAW CHICKEN PRODUCTS USING ADVANCED STATISTICAL ANALYTICS TOOLS

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: To explore how Artificial Intelligence (AI) and advanced analytics may improve poultry safety, we applied Statistical Process Control (SPC), random forest, and follow-up statistics to identify processing features that may associate with *Salmonella* outcomes in commercial poultry processing data.

Purpose: We aim to identify processing features that are critical for *Salmonella* performance in finished products to inform control strategies.

Methods: Three analytical methods were applied to evaluate the association between processing features at various stages and *Salmonella* outcomes. A total of 2441 *Salmonella* samples of finished products collected over 209 days were included in the analysis. We used SPC rules to identify potential out-of-control features from August 2022 to March 2023 in data from one commercial processing facility. Random forest analysis was conducted to rank the relative importance of features to *Salmonella* outcomes. Single factor statistics were used to investigate the directionality (more risky or less risky) of the association.

Results: After analyzing 13 processing features, the three most important features were second intervention spray bar peracetic acid (PAA), pre-chill PAA, and chiller pH. Second intervention spray bar PAA was important for chicken parts and ground chicken, as process failure (specifically, a day with at least one PAA measurement below the company-reported minimum) was associated with 2.4 times higher odds (95% CI: 1.0–5.9) of a *Salmonella*-positive result on that process day than on other days. Process failure in pre-chill PAA (day below company limits) was associated with 2.0 times higher odds (95% CI: 1.0–4.5) of a *Salmonella*-positive result.

Significance: The overall ranking for the relative importance of processing features as well as follow-up analyses provide a framework for establishing management priorities and designing foods safety control strategies at key stages throughout poultry processing.

Award Application: Developing Scientist Award

P3-204: AI-ASSISTED QUALITY ASSURANCE FOR MICROBIOLOGICAL METHOD PERFORMANCE METRICS THROUGH MODEL CONTEXT PROTOCOL INTEGRATION

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Presentation type/Format: Poster

Category: Data Management and Analytics

Abstract:

Introduction: Complex statistical KPIs for method validation and verification are prone to calculation errors when performed manually, while microbiologists require interactive guidance to properly interpret performance metrics across different study designs and matrices.

Purpose: Develop and evaluate an AI-assisted training system that ensures accurate KPI calculations through Model Context Protocol while providing contextualized guidance for interpreting method performance data.

Methods: A training and statistical evaluation platform was connected to LLM tools (e.g.,

ChatGPT) using Model Context Protocol (MCP), enabling AI to execute validated calculation functions while maintaining conversational interaction. The system verifies inputs, calls algorithms for 15+ KPIs including Probability of Detection, relative accuracy, sensitivity, specificity, and uncertainty estimates, validates outputs against regulatory benchmarks, and provides explanations. MCP architecture separates reliable computation from interactive guidance, preventing hallucinated calculations. Framework was tested using worked examples from ISO 16140 validation and verification protocols covering qualitative and quantitative methods.

Results: The system achieved 100% calculation accuracy when tested against FDA BAM and published validation examples. The AI identified data quality issues, guided users through study design selection for culture-based and molecular methods, explained statistical outputs, and flagged results requiring attention. Users transitioned from conceptual understanding to practical application significantly faster than traditional documentation-based learning.

Significance: This establishes a reliable framework for AI-assisted microbiological training, ensuring calculation integrity while democratizing access to complex statistical methods and improving confidence in method performance assessments across laboratories.

P3-205: GROWTH OF B. CEREUS AND S. AUREUS IN STEAMED SHREDDED WHEAT

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: *Bacillus cereus* and *Staphylococcus aureus* can contaminate wheat and if allowed to grow can produce heat stable toxins that may survive the subsequent baking process. For some baked products, the wheat is steamed and shredded before baking. This creates a high-water activity material that could allow pathogens to grow.

Purpose: Steamed, shredded wheat inoculated with *B. cereus* and/or *S. aureus* or co-inoculated were held to assess the growth and determine the maximum safe holding time of the wheat (<3 log increase).

Methods: Wheat berries were steamed and shredded prior to inoculation with low levels of *B. cereus* and *S. aureus* cocktails. Three trials were performed: *B. cereus* or *S. aureus* alone, or co-inoculation of both organisms. The inoculated wheat was held at 30°C with sampling every two hours from hour 0–12 and at hour 25. Samples were enumerated on MYP and BP agar and incubated at 37°C for 24 hours.

Results: The initial concentration for individual inoculations of *B. cereus* and *S. aureus* was 3.51±0.06 and 2.65±0.02 log CFU/g, respectively. At hours 12 and 25, the enumeration of *B.*

cereus was 6.79±0.30 and 8.39±0.04 log CFU/g and *S. aureus* was 5.32±0.09, and 8.00±0.02 log CFU/g. For the co-inoculation trial, the initial concentration was 2.07±0.30 and 2.23±0.16 log CFU/g for *B. cereus* and *S. aureus*. At hours 12 and 25, the average concentration was 6.02±0.09, and 7.70±0.18 log CFU/g for *B. cereus* and 5.36±0.1, and 7.71±0.12 log CFU/g for *S. aureus*.

Significance: Steamed shredded wheat at 30°C results in >3 log CFU/g of growth of *B. cereus* at 12 hours and *S. aureus* at 25 hours individually and at hour 12 for both organisms for the co-inoculation. Therefore, the wheat must be baked within 12 hours of steaming to manage the growth and toxin formation of the two organisms studied.

P3-206: DECODING THE SPOILAGE SUSPECTS OF QUESO FRESCO USING SHOTGUN METAGENOMICS

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: While traditional cultural methods are typically the first step in addressing a spoilage problem, they may result in an incomplete representation of the microorganisms causing the issue due to cultural bias or non-culturable organisms. With the advent of sequencing technologies such as shotgun metagenomics, a culture-independent, non-targeted approach can determine relative populations in a sample and help to identify the microorganisms likely to cause premature spoilage in a product over time.

Purpose: The objective of this study was to evaluate the microbial profile in a queso fresco product not meeting shelf life.

Methods: Shotgun metagenomics was employed using nanopore technology from Oxford Nanopore, in combination with their GridION® NGS/HTS instrument and library preparation/ sequencing reagents. Samples (10–15) from two different production days (1 kg or 10 oz queso fresco samples) were composited at the beginning, middle, and end of shelf life (30 days, 60 days, and 120 days). Relative microbial populations were assessed at each pull time using shotgun metagenomics.

Results: Shotgun metagenomics showed a significant difference in the proportion of *Streptococcus thermophilus,* a common fermentation strain, between sample sizes/production

days at the beginning of shelf life. The 10 oz sample size showed a steep decrease in *S. thermophilus* by the middle of shelf life which persisted through the end of shelf life, while the 1 kg sample size showed a more gradual decrease. By the end of shelf life, both sample sizes exhibited a spoiled microbial profile with a higher proportion of *Leuconostoc,* a common psychrotrophic bacteria likely contributing to the premature spoilage of the product.

Significance: Traditional cultural methods could not pinpoint the root cause of the spoilage. By employing a culture-independent, non-biased molecular approach with shotgun metagenomics, the causative spoilage agent(s) were able to be identified.

P3-207: TOWARDS UNDERSTANDING BIOFILM DEVELOPMENT AND CONTROL IN FOOD SYSTEM: METABOLOMIC CHANGES IN BURKHOLDERIA CEPACIA BIOFILMS AFTER TREATMENT WITH ANTIMICROBIAL COMBINATION

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: *Burkholderia cepacia* forms persistent biofilms on food contact surfaces, where metabolic adaptation contributes to its antimicrobial tolerance. Untargeted metabolomics using liquid chromatography-mass spectrometry can profile intracellular changes linked to biofilm resilience.

Purpose: To determine how a model enzyme–antimicrobial combination (α-amylase with meropenem) triggers important changes in intracellular metabolism in *B. cepacia* biofilm.

Methods: *B. cepacia* biofilms were developed on stainless-steel coupons, incubated for 48h at 37°C in yeast extract dextrose calcium carbonate broth. Per our recent publication, coupons were treated with sublethal antibiofilm combination of α-amylase (1250 µg/mL) and meropenem (4 µg/mL) for 24h, with untreated biofilms as controls. Intracellular metabolites were extracted using chilled methanol–water and was analyzed using LC-MS targeting metabolites <1.5 kDa. Data were processed using the software MS-DIAL and analyzed using MetaboAnalyst. Group differences were assessed using principal component analysis, ANOVA, and partial least squares discriminant analysis, and pathway analysis was done using

the KEGG.

Results: A total of 348 metabolic features were detected, of which 87 matched IDs in the database, HMDB. Fourteen metabolites were significantly upregulated, including uridine, cytidine, phenylalanine, and tryptophan (log_2 fold-change ≥ 1.5, p <0.05), whereas 15 were significantly downregulated including, cGMP, cAMP, glutamic acid, and NAD (log_2 FC ≤ -1.5, p <0.05). PLS-DA and PCA plots showed clear separation between the treated and untreated groups. Pathway analysis showed that the D-amino acid metabolism was the most significantly impacted ($p = 2.38 \times 10^4$).

Significance: The sublethal antibiofilm combination significantly affected the biofilm metabolome, highlighting D-amino acid metabolism as a potential target for biofilm control and emphasizing the need to develop food-compatible antimicrobial strategies.

Award Application: Developing Scientist Award

P3-208: UNDERSTANDING HOW PRESERVATION STRATEGIES RESHAPE SPOILAGE MICROBIAL DYNAMICS IN SPINACH JUICE

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Thermal and chemical preservation strategies are commonly applied to slow spoilage in ready-to-eat (RTE) vegetable products. These interventions can reshape the product's microbiota affecting spoilage dynamics. This study investigated how preservation treatments influence microbial dynamics and spoilage microbiota in spinach juice during refrigerated and mild abuse storage.

Methods: Fresh spinach juice was prepared and divided into untreated (UJ), thermally treated (72°C, 1 min; TJ), and chemically treated (gallic acid, 8mg/mL; CJ) samples, which were stored at 4 and 15°C for 7 days. Microbial growth was monitored using culture-based enumeration on TSA, and 16S rRNA sequencing was performed identify and classify bacterial species.

Results: Thermally (n=14) and chemically (n=14) treated samples resulted in a 2.8 and a 3.2

log reduction, respectively, compared to UJ (n=14). During storage at 4°C and 15°C, UJ showed a 1 log increase (from 7.1 to 8.1 log CFU/mL) and a 1.4 log increase (from 7.1 to 8.8 CFU/mL), respectively. Less microbial growth and diversity were seen for treated samples during the storage period at both temperatures. When 16S rRNA amplicon sequencing was examined, higher abundance of Proteobacteria and Bacteroidota were observed in UJ. As it relates to specific pathogenic and/or spoilage microbial species, thermal and chemical treatments reduced and/or inhibited *Aeromonas*, *Enterobacter*, Enterobacteriaceae, *Exiguobacterium, Erwinia,* and *Lactococcus*, in the culture-dependent approach. Distinct post-treatment microbial ecosystems emerged, with *Bacillus* dominating TJ and *Pseudomonas* prevailing in CJ.

Significance: Overall, this study demonstrates that spoilage in preserved spinach juice is driven by treatment-specific microbial communities. These findings highlight the importance of considering post-treatment microbial ecology when designing preservation strategies to further extend shelf life and improve the safety of RTE vegetable products.

P3-209: MICROBIAL DYNAMICS OF VACUUM-PACKED BEEF PATTIES: EFFECTS OF STORAGE TEMPERATURE AND SEASONALITY

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Microbial spoilage of vacuum-packed beef is influenced by storage conditions and raw material variability, which may differ across production periods/seasons and affect shelf life and consequently product quality.

Purpose: This study investigated microbial dynamics of vacuum-packed beef during refrigerated storage, evaluating the effects of production period/season and storage temperature through culture-based and molecular approaches.

Methods: Two batches of commercial vacuum-packed beef patties were sampled in both late spring and autumn-winter, stored at two refrigeration temperatures (1°C and 8°C). Samples were taken after 0, 3, 7, 14, and 21 days of storage for microbiological analysis, pH measurement, and basic sensorial analysis. In addition, 16S rRNA gene amplicon sequencing was performed at each sampling point to complement culture-based analyses.

Results: All batches had a commercial shelf life of 14 days. In the autumn batches, overall

microbial counts were generally lower at the start of storage (4.91 ± 0.02 log CFU/g) compared to the spring batch (5.97 ± 0.23 log CFU/g). The evolution of pH during storage differed between seasons, with more pronounced changes in the autumn batches, while sensory analysis indicated progressive quality deterioration. According to amplicon sequencing, by day 14, the dominant spoilage-associated lactic acid bacteria were *Dellaglioa algida* and species of *Latilactobacillus* and *Lactococcus* genera, while the overall microbial diversity decreased. Storage at 8°C accelerated microbial proliferation without altering community structure, and 16S rRNA sequencing confirmed culture-based temporal trends.

Significance: This research highlights how production period and storage temperature shape microbial succession in vacuum-packed beef and underscores the utility of direct, sequence-based analytical approaches in thoroughly characterizing microbial dynamics.

P3-210: WITHDRAWN

WITHDRAWN

P3-211: MICROBIOLOGICAL COMPARISON OF PORK LOIN PRESERVATION BETWEEN TWO SLAUGHTERHOUSES IN QUEBEC

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Several factors specific to slaughterhouses can affect the diversity and abundance of microorganisms on pork meat at the end of processing, thereby impacting its shelf life.

Purpose: The objective of this study was to evaluate, using conventional microbiological methods, the shelf life of vacuum-packaged pork loins originating from two slaughterhouses with different intrinsic characteristics.

Methods: For each slaughterhouse (A and B), 25 vacuum-packaged pork loins were stored at 0.5°C. Five loins per slaughterhouse were sampled on days 0, 35, 59, 65, and 72. Surface

sampling was performed using a sponge, which was subsequently homogenized in 40 mL of 1x PBS. Aliquots of 10 µL (spot plating), 100 µL (spread plating), and 1 mL (coliform plating only) were plated on the following media under the corresponding incubation conditions for microbial enumeration: PCA at 30°C and 7°C, MRS at 25°C and 7°C under anaerobic conditions, *Listeria* selective agar base at 37°C, and 3M™ Petrifilm™ EC/CC at 35°C.

Results: Storage time had a significant effect on microbial loads of pork loins (two-way ANOVA, $p < 0.0001$), while no overall effect of slaughterhouse was observed. Coliform levels were influenced by both storage time and slaughterhouse, with a significant interaction (two-way ANOVA, $p = 0.0016$), showing higher counts in slaughterhouse A from day 65 (Šídák, $p < 0.0001$). *L. monocytogenes* was detected only at day 35 in slaughterhouse A. From day 35 to 65, MRS and PCA counts did not significantly differ (Šídák, $p > 0.05$), indicating dominance of psychrotrophic lactic acid bacteria in both slaughterhouses.

Significance: Vacuum storage at 0.5°C promotes the dominance of psychrotrophic lactic acid bacteria regardless of intrinsic slaughterhouse characteristics studied, contributing to microbiota stabilization and supporting shelf life and food safety. However, such slaughterhouse-specific factors may influence the occurrence of pathogenic or fecal indicator bacteria.

Award Application: Developing Scientist Award

P3-212: CHARACTERIZATION OF LEUCONOSTOC SPOILAGE STRAINS FROM AN INDUSTRIAL SWEETENED DAIRY PRODUCTION FACILITY IN MEXICO

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Presentation type/Format: Poster

Category: Microbial Food Spoilage

Abstract:

Introduction: Food spoilage causes economic losses and contributes to food waste, yet high-value sectors such as sweetened dairy products remain understudied. A Mexican manufacturer of sweetened dairy products experienced spoilage in ice cream base (ICB) formulations associated with *Leuconostoc* spp.

Purpose: This study aimed to evaluate spoilage capacity of *Leuconostoc* strains and their persistance in food environment.

Methods: A total of 29 *Leuconostoc* spp. isolates (15 from 2021 and 14 from 2023) were genotyped using RAPD analysis and hierarchical clustering (Ward's method). The 2021 isolates were further assessed for spoilage potential by inoculating 6 log CFU/mL into ICB and monitoring bacterial concentration, viscosity, and pH after incubation at 10°C for 10 days. Finally, five representative strains were tested for susceptibility to nisin (6 ppm) and

Lactiplus® (0.5%) in preservative-free ICB under the same incubation conditions, with free-preservatives and uninoculated controls. All experiments were conducted in triplicate and data were analyzed using ANOVA and Tukey's test (JMP 11).

Results: RAPD analysis revealed two main phylogenetic groups, containing 10 and 19 isolates each from both sampling years, suggesting the persistence and possible adaptation of *Leuconostoc* spp. to the plant environment. Spoilage capacity differed significantly among strains ($p<0.0001$), and no direct correlation was observed between bacterial growth, viscosity increase and acidification. All evaluated strains exhibited partial resistance to nisin, producing spoilage levels similar to the preservative-free control. In contrast, although Lactiplus® did not completely prevent spoilage, it showed greater efficacy, reducing product acidification by approximately 7% compared to nisin.

Significance: These findings improve understanding of spoilage mechanisms in dairy matrices traditionally considered stable and provide a basis for designing more effective control strategies of preservation.

P3-213: A FOOD-TRIGGERED MICRONEEDLE PLATFORM FOR REAL-TIME SPOILAGE ASSESSMENT OF PACKAGED FISH

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Presentation type/Format: Technical

Category: Microbial Food Spoilage

Abstract:

Introduction: Consumers lack access to real-time food quality information, contributing to premature disposal of edible foods and increased food waste.

Purpose: The objective of this study was to develop and evaluate a food-activated microneedle sensor for in situ spoilage monitoring of packaged foods.

Methods: Microneedle arrays were fabricated from gelatin (15% w/v) and dehydrated using a freeze–thaw protocol to achieve mechanical rigidity during storage. Anthocyanins extracted from red cabbage were incorporated as pH-responsive indicators. Mechanical performance was evaluated by compressive testing (100–700 gf) and penetration through polyethylene films (14, 38, and 76 μm; n = 10 uses per patch). Spoilage sensing was assessed using raw fish samples stored at 4°C for up to 10 days. Sensor color change was quantified using image analysis and correlated with measured food pH.

Results: Optimized, dehydrated microneedles exhibited significantly lower height reductions of 12.8 ± 5.2% at 100 gf compared to 43.64% ± 9.03 in their gelled counterparts (n = 6, $p < 0.0001$). Sensor color shifted from purple to blue in these patches as fish spoiled across four

day timepoints, showing significant decreases in red colour intensity values (sRGB) in arbitrary units from 87.1 ± 4.2 on Day 2 to 66.6 ± 4.9 on Day 3 (n = 8, $p < 0.0001$). This change correlated to pH changes of 6.67 to 7.18, indicative of the onset of spoilage. The sensor also displayed rapid, real-time classification by correctly identifying fish spoilage (pH = 7.20) within 45 minutes based on a significant shift in sRGB from 84.8 ± 3.2 at 30 mins to 67.8 ± 3.2 at 45 mins (n = 6, $p < 0.0001$).

Significance: This platform enables low-cost, consumer-level spoilage monitoring within sealed packaging, supporting informed food safety decisions and reducing avoidable food waste.

Award Application: Developing Scientist Award

MP-01: HEALTH CANADA'S INNOVATIVE APPROACHES TO FOOD SAFETY RISK COMMUNICATIONS

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Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: Health Canada promotes safe food-handling practices to reduce the risk of consumer foodborne illness.

Purpose: Design and implement innovative food safety education initiatives for diverse audiences.

Methods: Over a three-year period, Health Canada researched, created, and launched three targeted projects, each for a specific audience. These initiatives were driven by a comprehensive needs analysis, designed to engage these new audiences through innovative, behavior-changing strategies. Each project leveraged trusted sources, including educators and health professionals, to effectively connect with and influence target consumer groups.

Results: The resulting projects were published: in 2024, the *Safe Recipe Style Guide: Shaping Food Safety Behaviours* (SRSG) for dietitians, recipe writers, chefs; in 2025, the *Food Safety Activity Kits* (FSAK) for educators to use with children in grades 4 to 6 (ages 9 to 13) to promote hands-on learning about safe food practices; in 2026, the online course *Food Safety: Empowering You to Take Action* for health professionals and educators, to support the delivery

of consistent, accurate food safety messaging.These projects are in the early stages of implementation. Assessing the effectiveness of behavior change strategies is a critical next step. Upcoming surveys will measure shifts in consumer knowledge and safe food-handling practices, providing valuable insights into the impact of these innovative initiatives.

Significance: Engaging stakeholders across the food system, amplifies reach and impact of Health Canada’s food safety messages through trusted messengers. Broad engagement increases consumers’ likelihood of seeing, understanding, and applying safe food handling practices at home.

MP-02: FREE GLOBAL RESOURCE: IPWA KEY WELFARE INDICATOR GUIDES FOR POULTRY

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Andy Vance, IPWA

Presentation type/Format: Technical

Category: Communication, Outreach and Education

Abstract:

Introduction: The poultry sector requires consistent, science-based tools to evaluate and improve animal welfare across diverse production systems worldwide.

Purpose: The objective of this project was to develop globally applicable Key Welfare Indicator (KWI) Guides that provide standardized welfare assessment metrics for broilers, layers, and turkeys.

Methods: The International Poultry Welfare Alliance (IPWA) convened an international group of poultry scientists, veterinarians, welfare specialists, and industry experts to identify practical welfare indicators based on scientific literature and existing welfare frameworks. Indicators were evaluated for scientific validity, practicality for on-farm measurement, and relevance across production systems. Selected indicators were documented with standardized definitions, measurement protocols, monitoring frequency, and suggested corrective actions. The guides were then translated by IPWA members into multiple languages to support global accessibility and adoption.

Results: The resulting KWI Guides establish standardized welfare indicators and measurement protocols for broilers, layers, and turkeys. Each guide provides clear methods for routine welfare monitoring and management response on farms. Industry partners,

researchers, and educators have begun using the guides to benchmark welfare practices and harmonize welfare data. Early adoption demonstrates their usefulness in creating a common scientific framework and improving comparability of welfare metrics across organizations and regions.

Significance: The IPWA KWI Guides provide practical, science-based tools that support responsible poultry production and improved animal health management. Standardized welfare measurement strengthens transparency and contributes to food protection and public health goals.

MP-03: AN INTRODUCTORY COLLEGIATE COURSE TO ADDRESS THE WORKFORCE SHORTAGE OF FOOD SAFETY AUDITORS, INSPECTORS, AND ASSESSORS

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Jerry Wojtala, International Food Protection Training Institute

Presentation type/Format: Marketplace

Category: Communication, Outreach and Education

Abstract:

Introduction: A significant global workforce shortage exists in food safety auditing, inspections, and assessment (AIA) occupations and collegiate students are often unaware of these careers. Higher education is an underutilized system that can impart the knowledge and skills necessary for future AIA professionals, but curriculum that can be easily utilized by educators is scarce.

Purpose: To create a turnkey curriculum, introducing the concepts and careers of AIA, that can be adopted and implemented by any higher education establishment.

Methods: A project team of five academic institutions and one industry partner produced a list of food safety topics for the course. Topics were then ranked by stakeholders from industry, academic, and regulatory agencies using the Delphi method. The final topic list was used to create the introductory curriculum of instructional e-learning modules, videos, case studies, scenario-based simulations, and a suggested list of guest speakers.

Results: Fifteen modules will be piloted during the Fall 2026 academic year. Twelve members of the academic community expressed interest in piloting the curriculum at their respective institutions, and additional educators are able to opt in to use the curriculum in future semesters. The curriculum is supported by a flexible delivery model and can be fully asynchronous or a hybrid (asynchronous modules with synchronous discussion) solution. Assessment methods include: knowledge checks and scenario-based food safety AIA activities. The curriculum will be housed on the SCORM Cloud; educators can pull content from the cloud and upload materials to their own Learning Management System.

Significance: The creation and distribution of this curriculum addresses a gap in workforce development for food safety AIA education by raising awareness among university students of existing career opportunities and providing them with foundational knowledge for success in these roles.

MP-05: A ONE HEALTH, PAN-EUROPEAN NETWORK STRENGTHENING SURVEILLANCE, CLINICAL MANAGEMENT, AND CONTROL OF ZOONOTIC HEPEVIRUSES

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Presentation type/Format: Poster

Category: Viruses and Parasites

Abstract:

Introduction: Zoonotic Hepeviruses, notably *Paslahepevirus balayani* (hepatitis E virus, HEV), are a largely underestimated public health issue. HEV is considered a priority foodborne pathogen, but surveillance in farms, wildlife, and food products is far from harmonized. The identification of *Rocahepevirus ratti* (ratHEV) as a cause of hepatitis, and its widespread detection in rodent populations, reveals an additional potential hazard.

Purpose: ONWARD (One Health Zoonotic Hepevirus Network; COST Action CA24140; hev-onward-eu) was created to close the scientific, clinical and policy gaps that limit capacity to respond to zoonotic Hepeviruses. It brings together experts spanning virology, food safety, veterinary medicine, hepatology, epidemiology, ecology and public health, enabling coordinated development of diagnostic tools, integrated surveillance approaches, clinical research frameworks, risk-assessment strategies and public-health engagement.

Methods: The network will harmonize diagnostics for HEV and ratHEV, define surveillance indicators, and map key transmission routes across food and environmental sectors. Shared guidelines, training resources and collaborative platforms will be developed to enhance cross-sectoral capacity and support a unified One Health response.

Results: Within its first year, ONWARD has established dissemination activities and created

synergistic contacts, with several international and European stakeholder bodies. Collaborative activities are underway in key areas, and workshops and training networks have been scheduled.

Significance: ONWARD establishes the first coordinated framework dedicated to zoonotic Hepeviruses. Its outputs will support future strategic directions, reinforce outbreak preparedness, and form the foundations for long-term, integrated human–animal–food surveillance.

MP-05: SENSOR-ENABLED DECISION SUPPORT SYSTEM FOR THE MITIGATION OF SALMONELLA IN END-TO-END POULTRY SUPPLY CHAIN

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Mahmoud Almasri, University of Missouri

Vijay Anand, Kennesaw State University

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Paula Penagos, University of Missouri

Prakash Dadi, University of Missouri St. Louis

Kalyan Gudigandla, University of Missouri St. Louis

Tatijana Fisher, Lincoln University

Presentation type/Format: Technical

Category: Meat, Poultry and Eggs

Abstract:

Introduction: *Salmonella* contamination in the poultry supply chain remains a persistent food safety challenge, driven in part by fragmented data systems and siloed-mitigation practices which must be addressed to strengthen poultry food safety.

Purpose: (1) Design and validate an integrated decision-support system capable of mitigating *Salmonella* risks across the poultry supply chain by combining descriptive, predictive, and prescriptive analytics within a One Health data environment. (2) Improving

testing efficiency, reducing operating costs, and supporting data-driven decisions that balance food safety, economic performance, and sustainability.

Methods: The Sensor-enabled Decision Support System (SENS-D) integrates multi-sector data spanning animal health, human health, and environmental factors. Descriptive analytics leverage GIS-based visualization for real-time monitoring and early warning of *Salmonella* risks. Predictive analytics were developed using laboratory experiments to model *Salmonella* growth and shelf-life dynamics under varying time–temperature conditions. Prescriptive analytics employ mixed-integer nonlinear programming models to optimize strategic sensor placement and sensor-enabled intelligent distribution, including processing, distribution, and product diversion decisions.

Results: Experimental and modeling studies demonstrated that SENS-D significantly improves testing efficiency and reduces operational costs up to 60% compared with current practices. Mathematical models predicted shelf-life >85% accuracy. The integrated analytics framework enables proactive risk identification, optimized sensor deployment, and informed distribution decisions that jointly enhance safety, efficiency, and sustainability across the supply chain.

Significance: SENS-D represents a novel, end-to-end, cloud-based decision-support solution for pathogen control in poultry, advancing One Health principles and national food safety goals.

MP-06: NAVIGATING HIGH-PRESSURE PROCESSING (HPP): A RESEARCH-BASED TOOLKIT FOR FOOD ENTREPRENEURS

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Presentation type/Format: Marketplace

Category: Food Processing Technologies

Abstract:

Introduction: High-pressure processing (HPP) is increasingly used by food entrepreneurs as a nonthermal, cold pasteurization technology that enhances food safety while maintaining fresh-like quality. As adoption grows, entrepreneurs working with HPP tollers or service providers often encounter challenges related to packaging compatibility, process validation, regulatory requirements, and shelf-life determination. To address these barriers, a practical toolkit was developed to support the successful use of HPP across a range of food products.

Purpose: The purpose of this toolkit is to provide food entrepreneurs with clear, science-based guidance for effectively utilizing HPP. The roadmap highlights critical decision points throughout product development, from formulation and packaging selection to process validation and market readiness.

Methods and Results: The toolkit integrates key technical and regulatory considerations into accessible resources designed for small- and mid-scale food businesses. Core elements include an overview of HPP principles, guidance on HPP-compatible packaging, and structured approaches for conducting process validation studies to ensure microbial safety. Shelf-life study design is also addressed, with emphasis on factors influencing product quality, safety, and labeling claims. Practical tools such as checklists, decision trees, and fact

sheets help entrepreneurs communicate effectively with HPP service providers and navigate common challenges associated with cold pasteurization.

Significance: This toolkit addresses the growing need for accessible, research-based HPP guidance for food entrepreneurs. By translating complex technical and regulatory concepts into practical tools, it lowers barriers to adoption and supports the safe, effective commercialization of HPP-treated foods.

MP-07: JUMPSTART TO PRODUCE SAFETY: TAILORED TECHNICAL ASSISTANCE INCREASES ADOPTION OF FOOD SAFETY PRACTICES ON SMALL AND UNDERSERVED FARMS

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Robson Machado, University of Maine Cooperative Extension

Presentation type/Format: Marketplace

Category: Produce

Abstract:

Introduction: Small and medium-sized produce growers face challenges meeting rising food safety expectations. Many need tailored technical assistance to adopt effective produce safety practices.

Purpose: This four-year project aimed to increase food safety knowledge, confidence, and adoption of science-based practices among underserved small and medium-sized farms in New Hampshire and Maine through personalized, on-farm support.

Methods: The project provided individualized technical assistance to 60 farms over three active years. Most of these farms were classified as “Not Covered” or “Qualified Exempt” under the FSMA Produce Safety Rule. Each farm received an on-farm produce-safety risk assessment that focused on Worker Health and Hygiene, Agricultural Water, and Packinghouse Activities. Following the assessment, farms received a confidential Jumpstart report outlining feasible improvements. To serve as lasting, low-cost educational tools, micro-learning videos featuring participating growers were created.

Results: Many participants implemented meaningful changes aligned with FSMA recommendations. Twenty-eight New Hampshire farms completed the program (two ceased operations).

Specific areas of improvement included:

- Twelve farms improved Worker Health and Hygiene by adding handwashing stations, signage, or training.
- Thirteen farms strengthened their wash-pack operations through the use of Standard Operating Procedures (SOPs), improved cleaning documentation, or designated clean areas.
- Thirteen farms improved agricultural chemical management, often by adopting or correctly measuring food-safe sanitizers.
- Six NH growers began routine water testing.
- Seven growers shared their improvements in short micro-learning videos.

Significance: Targeted, individual technical assistance helps farms adopt produce safety practices. This approach is crucial for reducing contamination risks and building long-term regional capacity via sustainable, farmer-focused educational resources.

MP-08: THE BUYER'S HANDBOOK FOR AI IN FOOD

Primary Author:
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Angela Anandappa, Alliance for Advanced Sanitation

Presentation type/Format: Marketplace

Category: Data Management and Analytics

Abstract:

Introduction: Artificial Intelligence (AI) solutions have surged in popularity and number. While assessing their relevance is not difficult, the feasibility and necessary data conditions for use are not well understood, causing confusion. Most aspects of AI are obscure and not already understood by those who are not solution developers. Machine Learning (ML) research has determined when solutions are feasible, but these results are not communicated in a manner that is useful to potential end users in making sound food safety operations and research decisions.

Purpose: This project developed guidelines and decision methods for assessing AI tools on whether they are possible (whether the technology could actually exist as described), and feasible in context (whether the available data and complexity of the scenario permit the technology to work as expected). This work aimed to provide more clarity about the plausibility of AI tools.

Methods: Research in the ML field was reviewed both within and outside the food safety context, classifying by problem type, data characteristics, and final performance, among other features. These were used to describe the current state of AI and its capabilities, considered in relation to problem types that are encountered in food. The findings were summarized in user-friendly documents and tools with varying scopes and degrees of granularity.

Results: Decision-making resources were made available. These allowed experts in other areas to learn key information about the capabilities of AI, serving as ML-aware supplements to subject-specific knowledge, explaining what problems were valid targets for AI solutions, and what solutions were plausible for a given problem.

Significance: Using the results of ML research presented in a format applied directly, using language relevant to experts outside of the ML field, these resources grant the ability to make informed decisions regarding AI technologies and make sound investments possible.

MP-09: AI-POWERED FOOD TESTING: DRIVING OPERATIONAL EXCELLENCE THROUGH INTELLIGENT DATA ANALYTICS

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Abdul Sami, Directorate of Research and Development, Punjab Food Authority

Muhammad Adnan haider, Directorate of Research and Development, Punjab Food Authority, Lahore, Pakistan

Presentation type/Format: Technical

Category: Laboratory and Detection Methods

Abstract:

Introduction: The rapid evolution of Artificial Intelligence (AI) and data analytics is reshaping the landscape of food testing laboratories, enabling smarter decision-making and higher levels of operational performance. These technologies are redefining traditional laboratory practices by enhancing analytical accuracy, reducing human error, and enabling faster, evidence-based decision support systems for food safety management.

Purpose: This presentation explores how AI-powered analytical systems can transform laboratory workflows improving precision, efficiency, and reliability across the entire testing process.

Methods: Key applications include automated interpretation of analytical data from instruments such as milk analyzers, chromatographs, and spectrophotometers; early detection of data anomalies; predictive modeling for contamination or adulteration risks; and

real-time optimization of laboratory operations.

Results: By integrating intelligent data analytics into quality management systems, laboratories can enhance compliance with ISO/IEC 17025, minimize measurement uncertainty, and streamline calibration and validation processes, ultimately ensuring more consistent, credible, and transparent testing outcomes.

Significance: The session will emphasize practical strategies for implementing AI technologies without compromising scientific integrity, traceability, or regulatory credibility. Case-based discussions will illustrate how data-driven insights support proactive risk management, resource optimization, and continuous improvement within accredited food testing environments. Participants will gain a forward-looking understanding of how AI and human expertise together can elevate laboratory performance standards, strengthen food safety assurance, and foster a culture of innovation and operational excellence.

MP-10: THE DEVELOPMENT OF A TRACEABILITY TRAINING PROGRAM FOR RETAILERS: A COURSE FOR UNDERSTANDING THE FOOD TRACEABILITY RULE

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Kathleen Nicholas, North Carolina State University

Kate Shumaker, The Ohio State University, Holmes County Extension Office

Reza Tahergorabi, North Carolina A&T State University

Tahl Zimmerman, High Point University

Benjamin Chapman, North Carolina State University

Presentation type/Format: Marketplace

Category: Retail and Food Service Safety

Abstract:

Introduction: The FDA's recently published Food Traceability Rule (FTR) under the Food Safety Modernization Act (FSMA), also known as FSMA 204, introduces complex-recordkeeping requirements for entities handling items on the Food Traceability List. This creates a critical need for rapid education among retail food establishments that were previously exempt from such regulations.

Purpose: This course offers a specialized curriculum designed to help retail food operators, particularly small businesses, understand the FTR and implement practices to adhere to the

new legislation.

Methods: The course was developed by food safety personnel at NC State University, The Ohio State University, High Point University, and North Carolina A&T State University, with feedback incorporated from the FDA and other stakeholders. The course consists of a two-part educational intervention: Part 1 is an asynchronous, module-based course delivered via an online learning management system that focuses on foundational concepts, while Part 2 is an interactive live session. In Part 2, participants work through case studies designed to familiarize them with components of the FTR they might encounter in their own establishments. Lastly, participants draft their own Food Traceability Plan.

Results: Course participants will learn to identify which foods within their inventory are subject to the rule, and will master the requirements for providing data to the FDA. Through dual-format training, participants can master concepts in easily digestible components, making the requirements more approachable. In addition, by incorporating experiential and observational learning, participants can deepen their understanding by reflecting on practical insights gained through activities**,** enabling them to learn from their peers.

Significance: Successful implementation of these traceability practices across the retail sector is vital for reducing the impact of foodborne illnesses associated with high-risk foods.

DEVELOPING SCIENTIST COMPETITORS

Abdelrahim, Dalia, *Alexandria University* (P3-191)

Acosta, Anna, *The Pennsylvania State University* (P3-039)

Adesope, Seth, *University of Arkansas* (P2-011, T2-06)

Adeyanju, Adenike, *University of Massachusetts Amherst* (P2-246)

Aduloju, Precious, *West Virginia University* (P1-132)

Afrane, Douglas Adjei, *Rutgers University* (P1-140)

Ajasa, Maryam Oluwafunmilayo, *Iowa State University* (P2-075)

Ajayan, Amritha, *University of Minnesota* (T8-08)

Ajayi, Boluwatife, *Texas A&M University* (T11-11)

Akao, Victor, *Florida Agricultural and Mechanical University* (P2-113)

Akoto, Esther Y., *Iowa State University* (P3-102)

Ali, Mostafa, *The Ohio State University* (T4-07)

Almalysha, Mohammed, *University of Missouri* (T7-10)

Andersen, Zoe, *Washington State University* (P2-144, P2-145)

Appiah Darkwah, Andrew, *Prairie View A&M University* (P3-188)

Appolon, Charles Bency, *University of Georgia* (P3-163)

Arinzechukwu, Chinaza, *University of Guelph* (T13-02)

Arshad, Adina, *University of Massachusetts Amherst* (P3-032)

Arthur, Vera, *University of Arkansas* (P3-164)

Arul Arasan, Tamil Selvan, *Michigan State University* (P2-130)

Bachschmidt, Thomas, *Laval University* (P3-211)

Bains, Kirat Khushwinder, *University of Arizona* (P2-186)

Baker, Jakob, *Cornell University* (P1-025)

Balyatanda, Suhan Bheemaiah, *Kansas State University* (P3-110)

Bandara, Adheesha, *University of Missouri* (P1-192, P1-194)

Barmon, Milon, *Emory University* (P1-234)

Barnett-Neefs, Cecil, *University of Illinois Urbana-Champaign* (P2-077)

Barron-Montenegro, Rocio, *Pontificia Universidad Catolica* (P1-035, P1-036, P3-192)

Bashir Taas, Kamran, *University of Missouri-Columbia* (T10-02)

Basnet, Ashesh, *Tennessee State University* (P1-124, P2-165)

Beaulieu, Rosie, *Laval University* (P2-025)

Berry, Bethany, *Johns Hopkins University* (P2-009)

Betiku, Eniola, *University of Arkansas* (T11-07)

Bhadauria, Aditi, *University of Georgia* (P3-145)

Bharathan, Greeshma, *Auburn University* (P3-144)

Bhatt, Swara, *University of Connecticut* (P3-027)

Bhattarai, Samiksha, *University of Maryland* (P2-151)

Biswas, Priya, *University of Nebraska-Lincoln* (T11-12)

Black, Micah T., *Auburn University* (P3-128)

Bose, Jestin, *Tennessee State University* (T10-11)

Boyapati, Tejaswi, *South Dakota State University* (T10-01)

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